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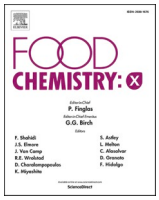

Effect of storage conditions on content of pesticide residues in sweet cherries

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ABSTRACT

Dynamics of pesticides decomposition in sweet cherry fruits at different technologies of long-term storage, ultra-low oxygen and modified atmosphere packing, and after post-harvesting application of 1-methylcyclopropen and ozone has been studied. We assumed that type of pesticide and fruit storage conditions may have a profound effect on pesticide residues content. Therefore, levels of residues after applying combinations of active ingredients including acetamiprid, boscalid, cyprodinil, fenhexamid, fenpyrazamine, fludioxonil, fluopyram, pyraclostrobin, pirimicarb, tebuconazole, thiacloprid, and trifloxystrobin were monitored. We found these contents below tolerated maximum residue limits when applied at recommended times and depended on period prior to withdrawal. Low contents of acetamiprid, boscalid, fenpyrazamine, thiacloprid, pirimicarb, and fludioxonil were found when fruit were stored in modified atmosphere packages. Ozone affected degradation of tebuconazole, pyraclostrobin, and cyprodinil. However, differences between storage regimens were not statistically significant ($p \geq 0.05$). Kinetic of degradation was studied with fruits stored after treatment with 1-methylcyclopropen and ozone.

1. Introduction

Control of pesticide residues in crops is generally related to maximum residue levels (MRL). These levels are set using field trial conditions for a particular pesticide at which the highest allowed residue levels expected under use according to good agricultural practice is reached (https://ec.europa.eu/food/plants/pesticides/maximum-residue-levels_en (European Commission)). Pesticides present in permitted amounts are important to maintain the quality of fruit during long-term storage. Their absence or decomposition under modern storage conditions can cause acceleration in decay of fruit quality. The understanding of the pesticide degradation in relation with other factors and the determination of pesticide residues thus appear very important both for the correct assessment of the food risks and optimization of the application techniques in order to create an efficient management (Mocanu et al., 2012).

Fruit treated with plant protection products must contain pesticide active ingredients at levels below MRL to avoid harm to human health. Simultaneously, the fruit has to be protected against rot and landfill diseases. The rate of decomposition of residues depends on a variety of conditions including climate during ripening, precipitation, processing, humidity, and the type of fruit, all being confirmed in previously published studies (Camara et al., 2017; Camara et al., 2020).

The Pesticides Directive provides information concerning active substances used in plant protection products, such as MRL in food, and emergency authorizations of plant protection products in EU member states (https://ec.europa.eu/food/plants/pesticides/eu-pesticides-database_en (European Commission)). With the continued development of plant protection products and the registration of newly authorized products containing different active substances, increasing demand for them and insufficient information require addressing the degradation of pesticides in fruit to ensure that the residue levels are below MRL. The ever-decreasing permitted levels of pesticides require the use of increasingly sensitive analytical methods. While the relatively rapid decline in pesticide residues occurs within the pre-harvest period due to various environmental factors, their drop in post-harvest time can be

Abbreviations: MAP, modified atmosphere packing; MCP, 1-methylcyclopropen; ULO, ultra-low oxygen.

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Table 1
Application schedule and treatment rates of pesticides used in pre-harvest treatment.

Days before harvest		28	21	14	7	3	1
Application dates	2018	May 22	May 29	June 5	June 12	June 15	June 18
	2019	May 21	May 28	June 4	June 11	June 14	June 17
Spray type		Active substances and application of the respective dosage					
T1				**Acetamiprid**(0.25 kg/ha)**Cyprodinil, Fludioxonil**(1 kg/ha) **Thiacloprid**(0.2 L/ha)	**Fluopyram, Tebuconazole**(0.6 L/ha) **Boscalid, Pyraclostrobin**(0.25 kg/ha)**Trifloxystrobin**(0.45 kg/ha)**Pirimicarb**(0.5 kg/ha)		**Fenpyrazamine** (1.2 kg/ha)
T2		**Thiacloprid**(0.2 L/ha)	**Acetamiprid**(0.25 kg/ha)**Cyprodinil, Fludioxonil**(1 kg/ha)	**Fluopyram, Tebuconazole**(0.6 L/ha) **Boscalid, Pyraclostrobin**(0.25 kg/ha)**Trifloxystrobin**(0.45 kg/ha)**Pirimicarb**(0.5 kg/ha)		**Fenpyrazamine** (1.2 kg/ha) **Fenhexamid**(1 L/ha)	
T3		**Acetamiprid**(0.25 kg/ha)**Trifloxystrobin** (0.45 kg/ha)	**Fluopyram, Tebuconazole**(0.6 L/ha)**Boscalid, Pyraclostrobin**(0.25 kg/ha)**Pirimicarb**(0.5 kg/ha)		**Fenpyrazamine**(1.2 kg/ha)**Fenhexamid**(1 L/ha) **Tebuconazole**(0.75 L/ha)**Cyprodinil, Fludioxonil**(1 kg/ha)		
T4		**Boscalid, Pyraclostrobin**(0.25 kg/ha)**Cyprodinil, Fludioxonil**(1 kg/ha) **Pirimicarb**(0.5 kg/ha)	**Trifloxystrobin**(0.45 kg/ha)**Thiacloprid** (0.2 L/ha)	**Fenpyrazamine**(1.2 kg/ha)**Fenhexamid**(1 L/ha) **Tebuconazole**(0.75 L/ha)			

slower and depends on the storage conditions (https://ec.europa.eu/food/plant/pesticides_en (European Commission)). Furthermore, storage time and conditions such as temperature and humidity have been shown to have a major effect on fruit susceptibility to pests, on the accumulation of pesticide residues, and on fruit quality (Shimshoni et al., 2019). Newly developed enhanced storage technologies and follow-up post-harvest protection, as well as technologies used to maintain high quality of fruit require an assessment of the impact of these technologies and products on the degradation of pesticide residues during the long-term storage (Yigit & Velioglu, 2020; Pandiselvam et al., 2020).

Our study aimed at monitoring the dynamics of degradation of the original active substances in selected pesticides applied on sweet cherries during storage using various long-term technologies including ultra-low oxygen (ULO) and modified atmosphere packing (MAP), as well as application of 1-methylcyclopropen (MCP) and ozone. Spraying plans were designed to include combinations of pesticides and their application periods. The results were compared with those obtained using an untreated control. Degradation kinetics of pesticides was determined under storage conditions including treatment with MCP and ozone. We observed decline in pesticide levels affected by different storage conditions and compared the storage conditions with respect to decomposition and/or preservation of all pesticides tested. Additionally, changes in quality of sweet cherry fruit affected by diseases and insects were observed during the storage and percentage of the damaged fruit evaluated. To our best knowledge, no complex study with respect to the number of pesticides and types of storage applied to stone fruits has been reported yet.

2. Materials and methods

2.1. Chemicals and standards

The pesticide standards that included acetamiprid, boscalid, cyprodinil, dimethoate, dithianon, fenhexamid, fenoxycarb, fenpyrazamine, fludioxonil, fluopyram, iprodione, pyraclostrobin, pirimicarb, tebuconazole, thiacloprid, and trifloxystrobin with a purity in the range of 99.4–100 % were purchased from Sigma Aldrich (Germany). The chemical structures selected compounds are shown in Figures S13-S14. Stock solutions were prepared in acetonitrile and stored at −20 °C. Deionized water for the preparation of the mobile phase was produced by Watek system (Germany). Ammonium formate for mass spectrometry was obtained from Fluka (Germany). Formic acid used in the mobile phase and acetonitrile in HPLC grade was obtained from Sigma-Aldrich (Germany). Methanol (HPLC grade) was from Merck (Germany).

2.2. Fruit

The fruit of the sweet cherry variety´Tamará were harvested from experimental planting at the Research and Breeding Institute of Pomology in Holovousy, Holovousy, Czech Republic. Field experiments were carried out in an orchard located in the Holovousy area. The 5 years old variety´Tamará was in a clip of 4.5 × 1.5 m in a cultivated tall spindle axe. Each variant comprised a total of 15–18 trees. The fruit were harvested in boxes at the time of their harvest maturity to ensure homogeneity of the samples.

2.3. Application of pesticides in sweet cherry planting

The model spraying plan for the application of products and the study of pesticide residues was created so that the products did not repeat in the respective variant, but at the same time were present in all variants. The list of applied preparations was based on the standard integrated protection designed for stone fruit that is commonly used for the treatment of stone fruit in the Research and Breeding Institute of Pomology in Holovousy Ltd. The combination and spraying plans corresponded to standard procedure recommended for protection of sweet cherries. (Lansky, 2005; Pistekova, 2015). The preparations were applied in experimental planting of cherries with an area of 300 × 50 m. All preparations were mixed to form a single solution containing all tested products that were then applied to cherries. Pesticide applications started approximately four weeks before the harvest taking into account climatic conditions and the expected ripening date. The experimental set was divided in 5 variants, in which the individual products were applied

as model pesticide mixtures commonly found on the market and used for sweet cherry treatment. Subsequent applications followed after each 7 days to protect fruit against harmful organisms. Combinations of active ingredients including acetamiprid, boscalid, cyprodinil, fenhexamid, fenpyrazamine, fludioxonil, fluopyram, pyraclostrobin, pirimicarb, tebuconazole, thiacloprid, and trifloxystrobin were tested in four variants marked as T1-4 differing with the spraying plan and active ingredients in applied preparation. Variant T1 did not include fenhexamid, variant T2 did not include thiacloprid, variant T3 did not include acetamiprid and thiacloprid, and variant T4 did not include acetamiprid, cyprodinil, and thiacloprid. Variant T5 was an untreated control. The pesticide application was carried out using the NP 400S tractor sprayer that was equipped with a spray boom of 200 cm. A total of 7 ALBUZ ATR nozzles at a distance of 25 cm were placed on the application frame to ensure even coverage of trees with spray liquid. Traveling speed of the tractor when applying pesticides spray was 1.5 km/h and the spraying occurred at the windless weather. This spraying plan was applied during years 2018 and 2019. The tested pesticide preparations, their active ingredients, and other characteristics are listed in supplementary Table S1. The individual variants, pesticide doses, combinations, and application dates are detailed in Table 1. All preparations were mixed to form a single solution containing all tested pesticides that were then applied to cherries.

2.4. Storage conditions

The storage experiments were divided in four subunits: The first part of the samples was placed in plastic boxes in MAP bags, the second part was treated with MCP before storage, and the third part was treated with ozone. All fruit included in these three treatments were stored at a temperature of 1.2–1.6 °C. The fourth part was stored in ULO boxes in an atmosphere containing 2% O_2 and 1% CO_2 at a temperature of 1.5–2 °C and a humidity of 99%. The fruit of the 5th variant, which was not treated, was stored in plastic boxes placed in the ULO box. The cherries were removed from storage after the normal storage time of 28 days and the residues determined. However, the normal storage time for the Tamará variety in a refrigerated warehouse was 14 days. When using storage in MCP packaging, the normal storage time is extended up to 30 days or even more depending on cherry variety.

Post-harvest treatment with MCP comprised a single application that effectively inhibited production of ethylene, thus preventing fruit ripening. The preparation containing 1.58 mg/m^3 MCP was dispersed on the first day after storage by means of a diffuser in the gas-tight closed space of the ULO box. The exposure time was 24 h after which the atmosphere containing MCP was ventilated. Then, the storage container was sealed again, and the storage continued in a normal atmosphere.

Treatment of the fruit before storage was also carried out using 0.2 ppm ozone for 8 h. Then, the ozone rich atmosphere was ventilated, the storage container resealed, and the storage continued in a normal atmosphere as required by the internal recommendations of the Research and Breeding Institute of Pomology in Holovousy, Ltd. Storage containers (MAP) specially designed for cherries is a method of packaging in which the atmospheric air in the container has been replaced by a modified atmosphere, usually carbon dioxide and temperature was decreased to 1.5 °C. This packaging slowed the aging and ripening process and also reduced the weight loss. At the same time, the stored cherries retained their taste, nutritional value, and fresh appearance even after long-term storage.

2.5. Sample preparation and analysis of pesticide residues

The cherries were pitted and cryogenic milled with dry ice before further treatment. A total of about 0.5 kg cherries was ground. Extraction was carried out according to the modified QuEChERS extraction of Czech technical standard procedure (CNS EN 15662.2018(Czech technical standard)). Sample with a weight of 10 g was homogenized in homogenizer Cutter R23 (Robot Coupe, France) and extracted with 10 mL acetonitrile in a vertical shaker GenoGrinder MiniG 1600 (SPEX® SamplePrep LLC, Metuchen, NJ 08840, USA) at 8,000 RPM for 5 min. Then, QuEChERS extraction kit salts (Agilent 5982–5650, USA) were added to the sample and shaken again in the vertical shaker at 8,000 RPM for 5 min. The extract was centrifuged at 3,000 RPM for 5 min. The supernatant (6 mL) was transferred in a 15 mL centrifuge tube that contained QuEChERS Dispersive Kit (Agilent 5982–5056, USA) for dispersive solid-phase extraction and shaken for 30 s. Afterwards, a 70 μL aliquot of the liquid was diluted with 930 μL 50% aqueous methanol.

Validation was carried out according to the European SANTE/11813/2017 guidelines (SANTE, 2017). Recovery, precision, linearity, matrix effect, and limit of quantitation (LOQ) were determined. Recovery and precision expressed in terms of repeatability (RSD) were evaluated using blank cherry samples spiked at 0.01, 0.1, and 1 mg/kg in 8 replicates at each concentration.

Multiresidue LC-MS/MS method was developed and optimized for monitoring the whole spectrum of the tested pesticides. HPLC 1260 Infinity HP Series coupled to mass spectrometric detector Triple Q MS 6490 (Agilent, USA) was used for determination of pesticides in extracts. All chromatographic separations were carried out using reversed phase Zorbax Eclipse XDB-C18 column (150 × 2.1 mm, 5 μm) kept at 40 °C. The mobile phase comprised part A (5 mmol/L aqueous ammonium formate) and B (5 mmol/L ammonium formate in methanol). Gradient elution followed a sequence 0–7.5 min 30% B in A; 7.5–11 min 100% B; 11–14.5 min 30% B in A. Injection volume was 10 μL and the flow rate 0.65 mL/min.

Identification/quantification of target analytes was performed using tandem quadrupole mass spectrometric analyser operated in a positive and negative electrospray (ESI+, ESI-) ionization modes using cell accelerator voltage 5 V, ion source voltage ESI 3,000 V, V-charging 1,500 V, gas temperature 200 °C, gas flow 14 L/min, nebulizer 413.7 kPa, sheath gas heater 400 °C, and sheath gas flow 11 L/min. The analyte concentrations were quantified using the average response factor from a series of calibration standards prepared in the matrix extract. Generated experimental data were evaluated using MassHunter Software version B.04.00 for Agilent LC-MS/MS. Each variant of fruit was measured in 4 replicates and the average value was listed. Optimized and tuned MS/MS transitions, specific fragments, collision energies, and polarity are summarised in Table S2.

2.6. Statistical analyses

Normality of the data and homogeneity of variances were tested using the Shapiro-Wilk and the Levenẽs tests, respectively. Two-way ANOVA test was carried out to compare the effects of storage and spray variant on the total antioxidant activity. ANOVA followed by the Tukey HSD test for data with a homoscedascity and the Kruskal-Wallis test for data, which did not reveal homoscedascity, were applied for pairwise comparisons between the storage variants or for the evaluation of the effect of the storage period length on the content of residues. All statistical calculations were carried out using Statistica v. 12 software (Dell Inc., Tulsa, U.S.A.). Multivariate redundancy analysis was performed using Canoco 5.0 software (ter Braak & Smilauer, 2002). Percent decrease in individual residues during 28 days after harvest was used as response data, while storage variant and spray variant were explanatory variables.

3. Results and discussion

3.1. Method validation

The calibration curve linearity requirement of $R^2 \geq 0.99$ was met for all tested analytes. The matrix effect was determined via comparison of calibration curves constructed from experiments in pure solvent and matrix-matched calibration. The values determined according to SANTE

Table 2
Validation data of analytical method used for analysis of pesticides in cherries.

Analyte	Recovery (%), mean ± RSD (%)			R^2	Matrix
	0.01 mg/kg	0.1 mg/kg	1 mg/kg		effects (%)
Acetamiprid	101 ± 2	106 ± 2	107 ± 4	0.9997	−1
Boscalid	102 ± 4	108 ± 3	103 ± 4	0.9992	0
Cyprodinil	102 ± 4	107 ± 5	106 ± 4	0.9995	2
Fenhexamid	65 ± 13	70 ± 14	66 ± 13	0.9986	−2
Fenpyrazamine	104 ± 6	108 ± 4	104 ± 3	0.9988	6
Fludioxonil	105 ± 7	113 ± 4	102 ± 4	0.9973	0
Fluopyram	107 ± 3	111 ± 2	104 ± 4	0.9986	−1
Pyraclostrobin	104 ± 3	110 ± 3	107 ± 4	0.9992	−1
Pirimicarb	102 ± 2	108 ± 3	107 ± 3	0.9995	−1
Tebuconazole	104 ± 8	108 ± 7	97 ± 10	0.9977	−3
Thiacloprid	101 ± 2	107 ± 3	107 ± 4	0.9997	−1
Trifloxystrobin	103 ± 4	109 ± 4	108 ± 5	0.9993	7

R^2 – coefficient of determination; n = 8.

were in the range of −3 to 7 %. A difference of ± 20% is according to SANTE acceptable (SANTE, 2017). The LOQ of 12 pesticides was set at a target value of 0.01 mg/kg while the determined LOQ values for all these analytes were much lower. Performance characteristics of the applied analytical method obtained in validation process are summarized in Table 2.

3.2. Determination of pesticide residues

Our current results confirmed that pesticide residues remain unchanged or decompose very slowly during the storage of fruit in a refrigerated warehouse. According to previously published studies, pesticide residues in food are affected by storage, handling, and processing that occur between the harvest of raw agricultural commodities and their consumption. The behaviour of residues during storage and processing can be rationalized in terms of physicochemical properties of pesticides and the nature of the process. For example, post-harvest ozone treatment had an effect on the degradation and toxicity of selected pesticides (Holland et al., 1994; Song et al., 2003; Velioglu et al., 2018; De Souza et al., 2018). The most important factor was the storage temperature since it affects kinetics of chemical reactions. For example, the average half-life time of residues on apples and lemons was ten times longer when stored at low temperature compared to the room temperature (Athanasopoulos and Pappas, 2000; Akyildiz et al., 2014; Ticha et al., 2008; Pérez et al., 1999; Alothman et al., 2010).

The effect of storage type in our study shown in Fig. 1a-f varied for each tested year. The levels of pesticide residues in cherries from 2018 harvest are presented in Table 3 while those from 2019 are shown in supplementary Table S3 and Figure S1. All samples were measured in four replicates and the mean values were given with 20% uncertainty. Results were evaluated in terms of measurement uncertainty that is even broader than just SD while it was to get really precise results that can be discussed and not overlapped by measurement of such low concentration levels. Regarding the evaluation of the active substance content, a statistically significant difference in acetamiprid residues was observed between the MAP and ULO storage regimens in 2018. The former resulted in lower content of residues as shown in Fig. 1a. Considering the active substance, a statistically significant difference was monitored for fenpyrazamine revealing the lowest content of residues after post-harvest ozone treatment compared to other storage variants (Fig. 1b). A statistically significant difference was noted for fludioxonil after the use of MCP and ozone. Lower residue levels were found for storage with ozone (Fig. 1c). Contents of fenhexamid residues were the smallest after treatment with MCP compared to ULO, MAP, and ozone. (Fig. 1d). Tebuconazole levels were reduced after ozone storage more than when MCP treatment was applied (Fig. 1e). Lower pyraclostrobin residues were observed for storage with ozone compared to storage in MAP packaging (Fig. 1f). No statistically significant differences were found between residue levels and type of storage for the active substances thiacloprid, pirimicarb, fluopyram, boscalid, cyprodinil, and trifloxystrobin (Supplementary Figures S2-S4).

Multifactorial analysis confirmed correctness of the results. The declining trend could be clearly seen for the active substances during post-harvest ozone treatment and during storage in MAP packaging (Fig. 2). Post-harvest ozone treatment preceding the storage leads to a reduction in the surface microflora on harvested and subsequently stored crops and to a decrease in the level of ethylene in the warehouses. This treatment then allows an extension of the shelf life and preservation of organoleptic properties of the crops (Amit et al., 2017; Glowacz et al., 2015).

Storage in MAP reduced the content of fludioxonil, fenpyrazamine,

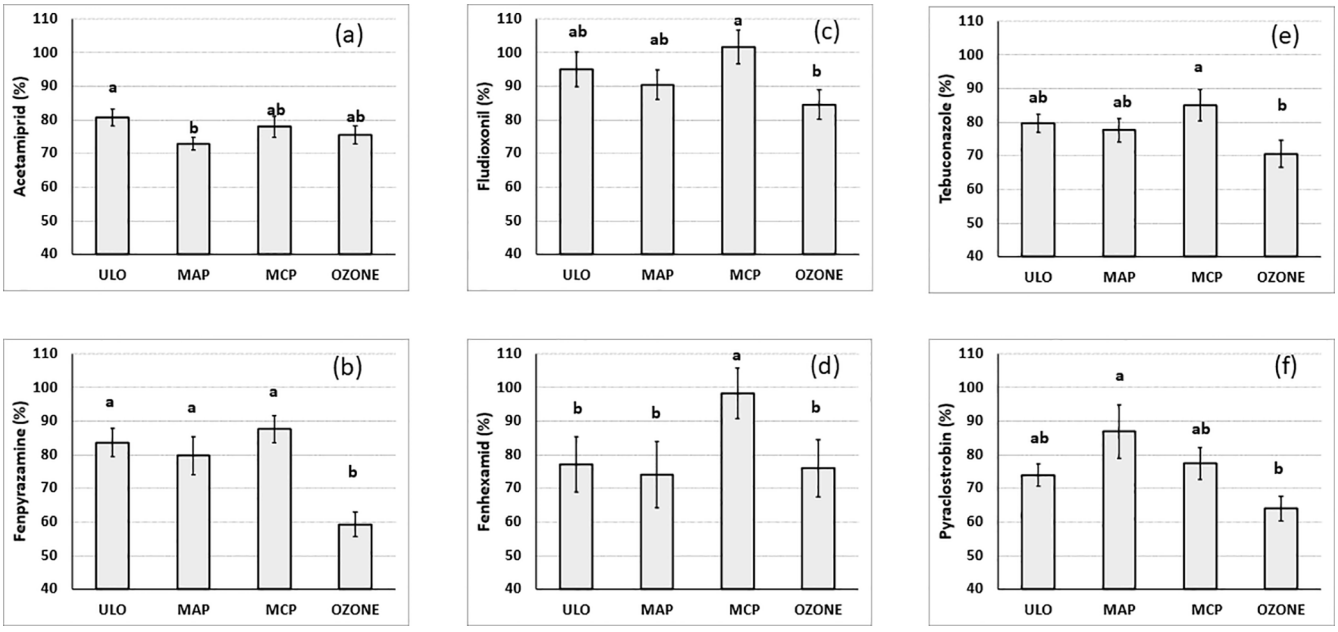

Fig. 1. Relativized content of active substances (a) acetamiprid, (b) fenpyrazamine, (c) fludioxonil, (d) fenhexamid, (e) tebuconazol, and (f) pyraclostrobin residues on the 28th day after the 2018 harvest for individual storage modes and combined spray variants.

Table 3
Content of residues of selected pesticides in ng/g determined in fresh and stored cherries in 2018.

Spray variant	Days of storage/ storage conditions	Acetamiprid	Thiacloprid	Pirimicarb	Fenpyrazamine	Fludioxonil	Fluopyram	Boscalid	Fenhexamid	Tebuconazole	Pyraclostrobin	Cyprodinil	Trifloxystrobin
T1	0	119.3 ± 23.9	90.6 ± 18.1	304.9 ± 61.0	644.6 ± 128.9	141.4 ± 28.3	63.8 ± 12.8	102.7 ± 20.5	–	36.0 ± 7.2	20.5 ± 4.1	249.8 ± 50.0	187.2 ± 37.4
	28/ULO	92.6 ± 18.5	60.5 ± 12.1	289.4 ± 57.9	659.1 ± 131.8	134.1 ± 26.8	55.6 ± 11.1	105.4 ± 21.1	–	27.6 ± 5.5	15.6 ± 3.1	164.4 ± 32.3	193.2 ± 38.6
	28/MAP	82.0 ± 16.4	49.8 ± 10.0	250.6 ± 50.1	663.7 ± 132.7	129.1 ± 25.8	50.3 ± 10.1	108.1 ± 21.6	–	28.3 ± 5.7	17.5 ± 3.5	141.5 ± 28.3	201.9 ± 40.4
	28/MCP	85.9 ± 17.2	55.1 ± 11.0	289.9 ± 58.0	433.8 ± 126.8	121.9 ± 24.4	52.7 ± 10.5	96.0 ± 19.2	–	26.8 ± 5.4	14.8 ± 3.0	152.0 ± 30.4	176.5 ± 35.3
	28/ozone	84.7 ± 16.9	51.8 ± 10.4	289.6 ± 57.9	473.5 ± 142.8	113.2 ± 22.6	53.1 ± 10.6	106.7 ± 21.3	–	23.0 ± 4.6	13.2 ± 2.6	118.3 ± 23.7	204.7 ± 41.0
T2	0	32.5 ± 6.5	19.2 ± 3.8	207.0 ± 41.4	109.3 ± 21.9	29.1 ± 5.8	112.3 ± 22.5	73.1 ± 14.6	212.3 ± 42.5	31.4 ± 6.3	<LOQ	44.7 ± 8.9	81.4 ± 16.3
	28/ULO	29.5 ± 5.9	14.6 ± 2.9	244.1 ± 48.8	84.8 ± 17.0	33.8 ± 6.8	110.7 ± 22.1	73.8 ± 14.8	234.5 ± 46.9	27.8 ± 5.6	<LOQ	32.3 ± 6.5	95.9 ± 19.2
	28/MAP	26.5 ± 5.3	13.2 ± 2.6	210.2 ± 42.0	76.1 ± 15.2	30.3 ± 6.1	100.3 ± 20.1	70.4 ± 14.1	222.3 ± 44.5	25.5 ± 5.1	<LOQ	26.2 ± 5.2	88.2 ± 17.6
	28/MCP	29.9 ± 6.0	15.5 ± 3.1	245.8 ± 49.2	86.8 ± 17.4	36.4 ± 7.3	112.3 ± 22.5	79.1 ± 15.8	260.7 ± 52.1	32.5 ± 6.5	<LOQ	36.4 ± 7.3	111.4 ± 22.3
	28/ozone	27.2 ± 5.4	12.2 ± 2.4	224.8 ± 45.0	59.2 ± 11.8	32.5 ± 6.5	105.3 ± 21.1	77.3 ± 15.5	255.5 ± 51.1	27.0 ± 5.4	<LOQ	25.7 ± 5.1	101.7 ± 20.4
T3	0	12.3 ± 2.5	–	117.4 ± 23.5	414.7 ± 82.9	284.8 ± 57.0	100.2 ± 20.0	56.7 ± 11.3	375.7 ± 75.1	247.9 ± 49.6	<LOQ	402.2 ± 80.4	46.1 ± 9.2
	28/ULO	10.2 ± 2.0	–	103.1 ± 20.6	338.3 ± 67.6	224.3 ± 44.9	84.2 ± 16.8	51.1 ± 10.2	324.5 ± 64.9	200.2 ± 40.0	<LOQ	28.4 ± 5.7	36.2 ± 7.2
	28/MAP	<LOQ	–	96.5 ± 19.3	325.7 ± 65.1	223.3 ± 44.7	77.6 ± 15.5	48.1 ± 9.6	311.3 ± 62.3	192.0 ± 38.4	<LOQ	260.2 ± 52.0	40.1 ± 8.0
	28/MCP	<LOQ	–	98.5 ± 19.7	400.7 ± 80.1	263.7 ± 52.7	79.4 ± 15.9	51.0 ± 10.2	394.7 ± 78.9	211.1 ± 42.2	<LOQ	295.6 ± 59.1	38.5 ± 7.7
	28/ozone	<LOQ	–	106.2 ± 21.2	267.9 ± 53.6	208.5 ± 41.7	85.3 ± 17.1	49.5 ± 9.9	284.3 ± 56.9	196.3 ± 39.3	<LOQ	276.4 ± 55.3	42.1 ± 8.4
T4	0	–	15.5 ± 3.1	45.9 ± 9.2	46.3 ± 9.3	32.0 ± 6.4	–	38.6 ± 7.7	67.4 ± 13.5	52.2 ± 10.4	<LOQ	10.1 ± 2.0	56.0 ± 11.2
	28/ULO	–	<LOQ	40.0 ± 8	33.8 ± 6.8	28.9 ± 5.8	–	36.3 ± 7.3	52.2 ± 10.4	37.6 ± 7.5	<LOQ	<LOQ	48.2 ± 9.6
	28/MAP	–	<LOQ	36.7 ± 7.3	31.4 ± 6.3	28.2 ± 5.6	–	32.8 ± 6.6	62.3 ± 12.5	38.0 ± 7.6	<LOQ	<LOQ	48.3 ± 9.7
	28/MCP	–	<LOQ	29.1 ± 5.8	35.0 ± 7.0	31.4 ± 6.3	–	34.6 ± 6.9	68.0 ± 13.6	36.9 ± 7.4	<LOQ	<LOQ	49.6 ± 9.9
	28/ozone	–	<LOQ	38.7 ± 7.7	20.2 ± 4.0	25.6 ± 5.1	–	30.3 ± 6.1	48.4 ± 9.8	29.7 ± 5.9	<LOQ	<LOQ	40.7 ± 8.2

n = 4, Measurement uncertainty 20%.

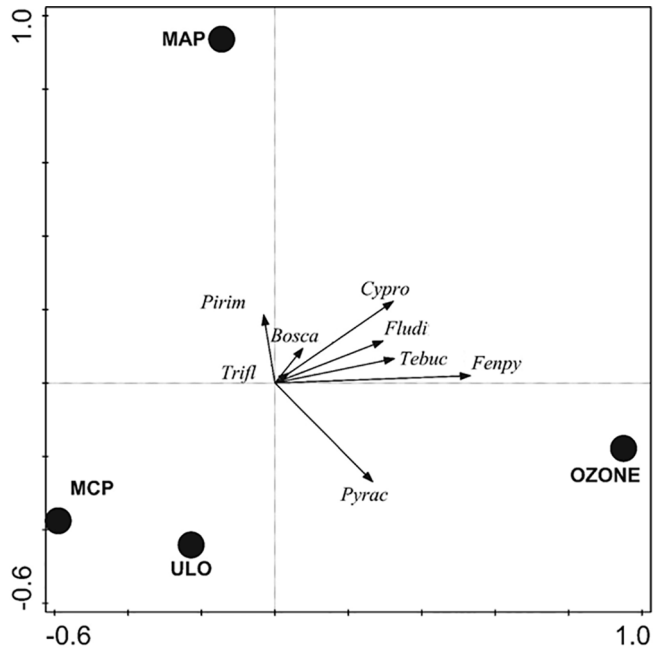

Fig. 2. Multifactorial analysis of the percentual decrease in the residues of pirimicarb, trifloxystrobin, pyraclostrobin, fenpyrazamine, fludioxonil, cyprodinil, tebuconazole, and boscalid during 28 days after the 2018 harvest for individual storage variants. The variation in all ordinate directions is significantly higher than random ($p = 0.014$; permutation test).

boscalid, and acetamiprid in the year 2019, but statistically significant differences in content were monitored only for fludioxonil. The highest content of its residues was determined in fruits treated with ozone, while lower levels were characteristic under MCP and in ULO storage conditions. A similar content gradient was detected for boscalid, although the differences between storage regimens were not statistically significant. Presence of thiacloprid, pirimicarb, and fluopyram was most significantly reduced in MCP-treated fruit, but differences between storage regimens were not statistically significant either. It is likely, that the chemical structure of all the compounds changes through their reaction with MCP.

Our results concerning pesticide residue detection and storage confirmed for the active substance fludioxonil (Switch) that pesticide residues were in 2019 higher for the variants T2–0.037 ± 0.007 mg/kg and T3–0.080 ± 0.016 mg/kg, but for variant T4 the values were 0.014 ± 0.003 mg/kg. Contents of the fludioxonil residue were reduced to values less than 0.01 mg/kg when MAP storage bags were used. Similar trend was observed for the active substance pyraclostrobin (Signum) where the residue level for T1 variants was close to the low-residue limit of 0.01 mg/kg. The values were reduced to less than 0.01 mg/kg when the cherries were treated with ozone or MCP after harvest. However, it should be kept in mind that Signum also contains a second active substance boscalid for which the degradation of residues to under the established limit of 0.01 mg/kg did not occur. The same situation applied to product Switch for which the other substance also exceeded the limit of quantification.

The differences in contents of the residues monitored in 2018 and 2019 were due to the unripe cherries resulting from different conditions during fruit ripening including the sunlight, precipitation, and temperature. The difference in the initial concentrations of the same pesticide applied under the same conditions, when also the spray dose was the same, led to different values mainly due to climatic conditions on the day of the first applications (amount of precipitation, number of sunny days). This also affected the biological degradation by natural enzymes, especially the ones enabling for example hydrolysis and other reactions. At the same time, each factor can affect the individual active substance differently. Yet, the residue levels have always been well below MRL for all active substances in all tested variants. During storage, pesticides contents usually remained preserved or only a slight decrease was monitored. The levels fell even below the limit of quantification for some substances and variants (Supplementary Figures S5–S9).

3.3. Kinetics of degradation

While reaction kinetics of the decomposition of the active substances was obviously affected by the external conditions including temperature, humidity, oxygen/ozone, and MCP, the question persists, whether the fruit itself affects the decomposition rate. Sweet cherries in storage conditions including MCP and ozone were analyzed in 2018 with respect to pesticide residues content after periods of 28 and 42 days and compared to their content in cherries stored for 14 days to monitor the kinetics of pesticides decomposition. First 14 days served for equilibration in the storage conditions and the content of pesticides at 14th day was taken as 100% level. The content of active substances was then determined at 28th and 42nd day of storage. The degradation rates using MCP and ozone storage conditions for spray variant T1 are shown as an

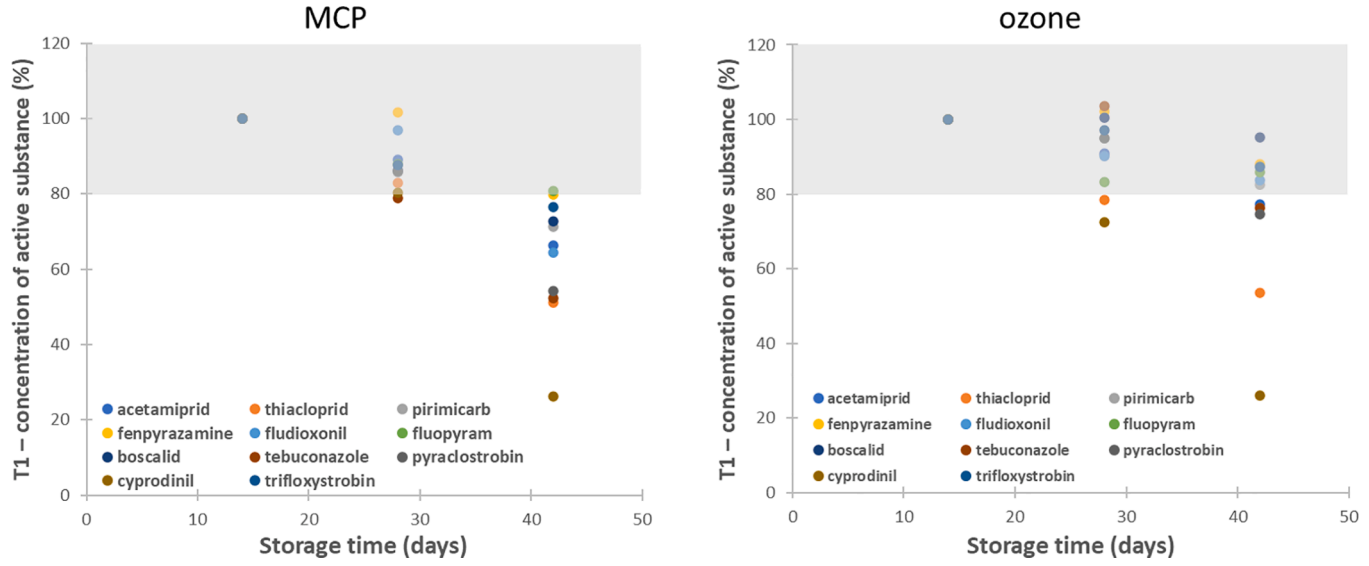

Fig. 3. Pesticides degradation in 42 days using MCP and ozone storage conditions for spray variant T1.

example in Fig. 3 (Tables S4, S5). Results for treatment variants T2-T4 are shown in Figures S10-S12. In all these figures the level of measurement uncertainty corresponding to ± 20% is marked. In no case, the potential increase in active substance content did not exceed this level and a decrease was evaluated in lower percentage values then 80%.

The time of pesticide application was one of the most important factors in the degradation of the active substance. The trends in residue degradation were found to be very similar regardless the storage technologies. In the T1 variant, fenpyrazamine in cherries stored with MCP decomposed in 28 days most significantly to 80 % of the original level. In contrast, degradation in ozone decreased the residue content to 85 % during the same time period that fits in the measurement uncertainty level. Fludioxonil decomposed to 60 % of the original value in MCP and to only 85 % in ozone. Similar trends were observed for the active substances boscalid and trifloxystrobin. Application of MCP caused a slight decrease in their level, while no decomposition was observed in ozone.

A decreasing trend was also noticed for contents of all active substances of the tested pesticides in the T2 treatment variant. The degradation of fenpyrazamine in variant T3 using MCP ended at 80 % of the original concentration and its course fluctuated during storage. In contrast, fenpyrazamine decreased in ozone to 50 % of the original level with fluctuations similar to those in MCP. Ozone also had a more pronounced effect on the degradation of fludioxonil compared to MCP where the degradation was slower. The course of decomposition of fludioxonil in the spray variant T4 was different using ozone and fluctuated around 80% of the original concentration during the time of storage while practically no degradation was monitored after application of MCP. The same trends occurred for boscalid and trifloxystrobin.

Generally, a faster degradation in ozone was observed for cyprodinil (T1, T2, and T4 to 30 %), thiacloprid (T1 to 40 %), tebuconazole (T2 to 40 % and T4 to 50 %). Pyraclostrobin with MCP degraded in T1 to 50 % while its contents in variants T2, T3, and T4 were less than limit of quantification. Substances most stable in ozone during the 42 days period were boscalid (T1 decrease to 95 %, T2 and T3 to 90 %, and T4 variant to 80 % original content, still within the measurement uncertainty level), trifloxystrobin (T1 to 80 %, T3 to 70 %, and T4 to 90 %), and fenhexamid (T3 to 70 % and T4 to 90 % original concentration).

Although a slight decrease in the concentration of the pesticides to less than MRL was characteristic after using ozone and MCP postharvest treatment, sufficient quantity of the active substance was preserved, and the fruit was protected from landfill diseases during the storage. Direct comparison enabled to confirm positive effect of application of the pesticides no matter which spraying variant was used and all variants except for control proved to be effective against harmful organisms and landfill diseases since no significant effect of them was observed. The loss of fruit due to diseases and insects was negligible in variants treated with pesticides preparations and even after 28 days of storage represented less than 2%. In contrast, a significant percentage of fruit amounting about 20% on average, was spoiled in the nontreated variant.

4. Conclusions

The application of pesticides on sweet cherries was carried out applying the manufacturers' recommendations and given protection periods for selected products. Based on the treatment with these plant protection products, the formation of rot and landfill diseases, as well as the weight loss using the tested storage types were minimized. Degradation of pesticides was affected first by the environmental climatic conditions and, after the harvest and storage, by additional factors that included storage conditions and ambient humidity. It also depended on the nature and chemical structure of the pesticide itself. We also found that our storage technologies affected degradation of residues of the active substances acetamiprid, fludioxonil, fenhexamide, tebuconazole, and pyraclostrobin ($p < 0.05$). The positive aspect of our research is the beneficial finding that all the tested fruit samples contained pesticide residues well below the MRL. Moreover, contents of some residues in the stored cherries were less than the limit of quantification. We speculate that in addition to the compounds and storage conditions such as MCP and ozone, the fruit itself or at least chemistry of its surface may also affect the decomposition of the pesticides. Confirmation of this idea will need more extensive measurements carried out with a wider variety of fruits.

CRediT authorship contribution statement

Aneta Bilkova: Writing – original draft, Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources. **Pavlina Knapova:** Formal analysis. **Pavol Suran:** Investigation, Methodology. **Jiri Kwiecien:** Data curation, Formal analysis. **Frantisek Svec:** Conceptualization, Funding acquisition, Resources, Supervision. **Hana Sklenarova:** Investigation, Methodology, Project administration.

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. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochx.2021.100185.

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Further reading

WHO. Principles and methods for the risk assessment of chemicals in food: Chapter 8: Maximum residue limits for pesticides and veterinary drugs. From: https://www.who.int/foodsafety/chem/residue_limits.pdf.