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ORIGINAL PAPERS

DISTRIBUTION AND QUALITATIVE AND QUANTITATIVE DETERMINATION OF PESTICIDE RESIDUES FROM A SAMPLE OF RED GRAPEFRUIT, AFTER TREATMENT WITH FUNGICIDE SOLUTIONS

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Abstract. *The Most citrus fruits are compromised each year due to post-harvest fungal infections. To reduce fungal infections, packing centers use fungicide solution mixtures to prevent infections that occur during harvest and then during storage. The most common fungal pathogens of citrus are commonly treated with the fungicides imazalil, Pyrimethanil due to their effectiveness in controlling these pathogens at low cost and ease of handling. However, little is known about how it alters tissues in citrus physiology. In this study we will investigate the behavior and retention of imazalil and pyrimethanil in the tissues of the red grapefruit fruit (peel and core before washing and after washing with hot water and simple dish detergent).*

This work demonstrates a viable approach for assessing the quality of citrus fruits, and we can even proceed to thoroughly wash each fruit before consumption, as we remove a quantity of residue.

This work demonstrates that the treatment affects the citrus tissues and the substances migrate throughout the pulp, although the treatment was applied to the peel.

Washing, which is the first step in food processing on a domestic and industrial scale, helps to reduce pesticide residues on the fruit surface. In this study, the effectiveness of washing the peel was also investigated.

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Although many chemicals are applied to citrus to prevent certain diseases or control pests, there is little literature available on how these treatments alter the physiology of the fruit. Usually, the packaging only analyzes the level of treatments on the products.

Keywords: pesticides, citrus fruits, fungicide treatment.

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Introduction

The citrus industry is a major contributor to the agricultural economy. It has been reported that nearly 90 million tons of citrus fruits are produced annually worldwide, with grapefruit, lemons and oranges being at the highest consumption rate.

Unfortunately, the citrus industry is exposed to many pest and disease threats that can drastically reduce citrus production each year.

These threats include preharvest diseases such as *Diplodia natalensis* and *Phomopsis citri*, postharvest diseases including *Penicillium digitatum* and *Penicillium italicum*, overripe fruit, insect damage, and a variety of physiological disorders.

The citrus season typically runs from early winter to late spring for most cultivars in both hemispheres; therefore, both have to rely on off-season product import and in-season product export to keep citrus products on the market.

Exporting fruit abroad takes a considerable amount of time, so the products must maintain their shelf life. Therefore, proper chemical application prior to shipment is crucial to the process.

There are many pre-harvest and post-harvest pathogens, most of which can be controlled with fungicides. *P. digitatum* and *P. italicum* are the most abundant post-harvest diseases. *P. digitatum* is the more virulent of the two and is expected to contribute nearly 50% of total post-harvest losses. *P. digitatum*, also called green mold, is the most widespread post-harvest pathogen of citrus, which is capable of infecting any citrus species in wounds caused during harvest. Wounds also occur as the fruit enters the packing warehouse as it is processed for market distribution. Not only do the fruits provide nutrients that allow *P. digitatum* to thrive, but the skin of the fruit also contains aromatic substances that induce germination. *P. italicum* , also known as blue mold, is similar to *P. digitatum* in many ways.

To reduce product losses due to *P. digitatum* and *P. italicum infections* , packinghouses use aqueous fungicide applications to prevent and reduce infections that occur during harvest. Imazalil, also known as enilconazole (1-[2-(2,4-dichlorophenyl)-2(2-propenyloxy)ethyl]-1H - imidazole, IMZ), is the fungicide most commonly used to treat *P. digitatum* and *P. italicum infections* due to its low cost, effectiveness and ease of application.

This paper focuses on the determination of the concentration of fungicidal analyte from the peel, from the core of the fruit and from the core homogenized with the peel, after this treatment.

Material and Methods

A laboratory sample consisting of 20 pieces of red grapefruit from the same batch, taken through the strategic national surveillance program, were used for the analysis, which were analyzed first of all according to reg 396/2005, respectively homogenization of the core together with the peel, then just the core, then just the shell.

Reference standards, standards, reagents and Quechers
REAGENTS

- Acetonitrile, HPLC grade, purity≥99.9%
- Methanol, HPLC grade, purity ≥99.9%
- Ammonium formate, 1 M
- Formic acid, purity > 95%
- Ultrapure water
- QuEChERS 5982-5650CH with pre-weighed saline mixture: 4 g MgSO 4 , 1g NaCl, 1 g sodium citrate dihydrate and 0.5 g sodium citrate se xa drate ;
- QuEChERS 5982-5056CH consisting of: 900mg MgSO4, 150mg PSA
- 5 M NaOH solution
- LC-MS/MS tuning solution
- Triphenylphosphate standard solution, concentration 1000 µg/ml - used as an internal standard;

The reagents used in this procedure must be of LC-MS/MS purity.

For this study, MR/MRC pesticides are used in powder form (Sigma Aldrich, Dr Erhenstorfer, CPA Chem), oily and solution concentrations of 1000 mg/L, according to the list of analyzed pesticide standards.

Extraction and purification

Extraction and purification by method modulation QuEChERS (SR EN 15662)

From the group matrices with high water content and high acid content , as classified according to Document N 0 DG/SANTE 1 2682:2019, belong to the category of citrus fruits (lemons, tangerines, tangerines, oranges, sa) and the category of small fruits and forest fruits (strawberries, blueberries, raspberries, black currants, red currants, white currants, grapes) and the category with a high water content, which includes: apples, tomatoes, peppers, eggplants, cucumbers, cabbage, etc.).

The extraction is performed as follows:

Weigh 10 g of the sample with an accuracy of ±0.1 g, on an analytical balance, in a 50 ml centrifuge tube , over which to add a ceramic stopper.

The pH correction is carried out with 5 M NaOH solution, until pH=4.5-5.5 (from close to close, homogenizing and checking the pH each time).

Add 50 µL internal standard TFP (10µg/ml) , vortex for 30 seconds.

Add 10 ml of acetonitrile and homogenize for 10 minutes.

Add the saline mixture (QuEChERS 5982-5650CH) , consisting of: 4 g of MgSO 4 , 1 g of NaCl, 1 g of sodium citrate dihydrate and 0.5 g of sodium citrate se xhy drate , after which it is vigorously stirred for one minute.

Centrifuge for 5 minutes at 5000 rpm, then keep in the freezer for an hour.

Take 6 ml of the supernatant and transfer it into the 15 ml centrifuge tubes, in which there is the saline mixture (QuEChERS 5982-5056CH) , consisting of: 900 mg MgSO4, 150 mg PSA. Homogenize energetically for one minute.

Centrifuge for 5 minutes at 5000 rpm.

It is filtered, through PTFE filters (0.2 µm), in 10 ml test tubes.

Take 500 µL of the obtained filtrate with a micropipette and add it to the 2 ml vial.

Add 500 µL mixture of mobile phases (APA : MEOH (50:50) +5 mM ammonium formate (5 ml of 1M ammonium formate/l of mixture) + 0.1% formic acid (1 ml of formic acid/l of mixture)).

Vortex for 1 minute.

This 2 ml vial , what it contains solution final, it is inserted into the autosampler and read sample by LC-MS/MS.

Analysis of pesticide residues in grapefruit is done using liquid chromatography coupled with mass spectrometry, LC –MS/MS.

The liquid chromatograph analysis system coupled with a mass spectrometer consists of an EXION LC liquid chromatograph (consisting of: Degasser, AD Autosampler, Controller, AD Column Oven, AD Pump, coupled with an AB SCIEX 4500 QTRAP mass spectrometer .

Results and Discussions

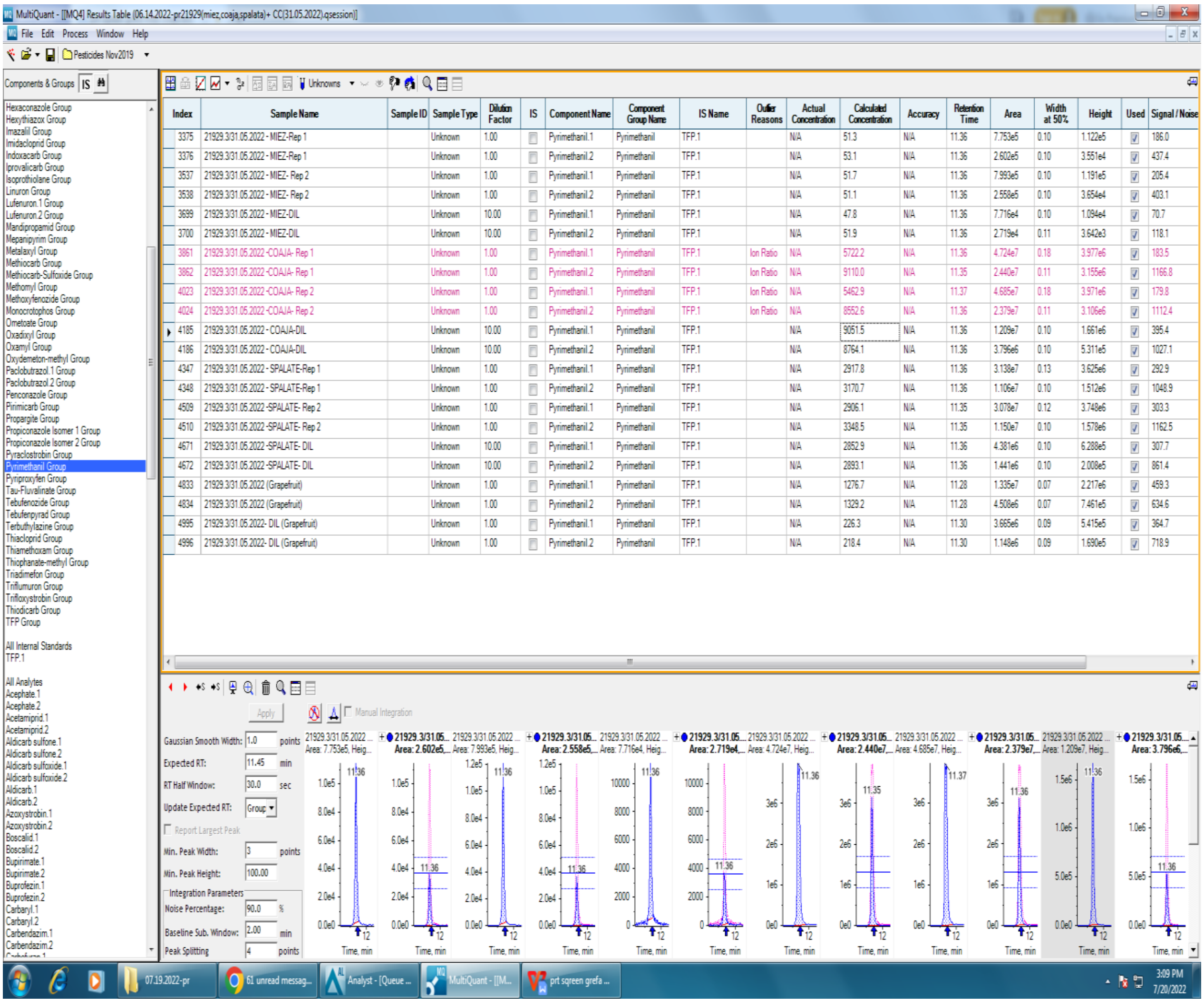
The grapefruit sample was analyzed according to figure no. 1 (mixture of peel and core; only core; only peel, mixture of peel and core after washing the fruit with hot water and dish detergent).

Four analytes were identified and quantified in this analysis, respectively Azoxystrobin, Carbendazim, Imazalil and Pyrimethanil.

	21929.3/3 1.05.2022 (Grapefruit)	21929.3/3 1.05.2022 DIL (Grapefruit)	21929.3/3 1.05.2022 – MIEZ-Rep 1	21929.3/3 1.05.2022 – MIEZ-Rep 2	21929.3/3 1.05.2022 – MIEZ-DIL		21929.3/3 1.05.2022 –COAJA-Rep 1	21929.3/3 1.05.2022 –COAJA-Rep 2	21929.3/3 1.05.2022 – COAJA-DIL		21929.3/3 1.05.2022 –SPALATE-Rep 1	21929.3/3 1.05.2022 –SPALATE-Rep 2	21929.3/3 1.05.2022 –SPALATE-DIL
1 Azoxystrobin.1	72.5		0.7	0.6			170.8	151.3			41.8	45.2	
2 Carbendazim.1	24.6		2.1	2.1			38.7	37.4			15.9	17.3	
3 Imazalil.1		2237.8	74.1	73.2					5324.9				1674.5
4 Pyrimethanil.1		2262.5	51.3	51.7					9051.5				2852.9

Figure 1. Distribution of detected analyte concentrations.

Figure 2. Result from the analysis equipment software for Pyrimethanil



Index	Sample Name	Sample ID	Sample Type	Dilution Factor	IS	Component Name	Component Group Name	IS Name	Outlier Reasons	Actual Concentration	Calculated Concentration	Accuracy	Retention Time	Area	Width at 50%	Height	Used	Signal / Noise
3375	21929.3/31.05.2022 - MIEZ-Rep 1		Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	51.3	N/A	11.36	7.753e5	0.10	1.122e5	✓	186.0
3376	21929.3/31.05.2022 - MIEZ-Rep 1		Unknown	1.00		Pyrimethanil.2	Pyrimethanil	TFP.1		N/A	53.1	N/A	11.36	2.602e5	0.10	3.551e4	✓	437.4
3537	21929.3/31.05.2022 - MIEZ- Rep 2		Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	51.7	N/A	11.36	7.993e5	0.10	1.191e5	✓	205.4
3538	21929.3/31.05.2022 - MIEZ- Rep 2		Unknown	1.00		Pyrimethanil.2	Pyrimethanil	TFP.1		N/A	51.1	N/A	11.36	2.558e5	0.10	3.654e4	✓	403.1
3699	21929.3/31.05.2022 - MIEZ-DIL		Unknown	10.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	47.8	N/A	11.36	7.716e4	0.10	1.094e4	✓	70.7
3700	21929.3/31.05.2022 - MIEZ-DIL		Unknown	10.00		Pyrimethanil.2	Pyrimethanil	TFP.1		N/A	51.9	N/A	11.36	2.719e4	0.11	3.642e3	✓	118.1
3861	21929.3/31.05.2022 -COAJA- Rep 1		Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TFP.1	Ion Ratio	N/A	5722.2	N/A	11.36	4.724e7	0.18	3.977e6	✓	183.5
3862	21929.3/31.05.2022 -COAJA- Rep 1		Unknown	1.00		Pyrimethanil.2	Pyrimethanil	TFP.1	Ion Ratio	N/A	9110.0	N/A	11.35	2.440e7	0.11	3.155e6	✓	1166.8
4023	21929.3/31.05.2022 -COAJA- Rep 2		Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TFP.1	Ion Ratio	N/A	5462.9	N/A	11.37	4.685e7	0.18	3.971e6	✓	179.8
4024	21929.3/31.05.2022 -COAJA- Rep 2		Unknown	1.00		Pyrimethanil.2	Pyrimethanil	TFP.1	Ion Ratio	N/A	8552.6	N/A	11.36	2.379e7	0.11	3.106e6	✓	1112.4
4185	21929.3/31.05.2022 - COAJA-DIL		Unknown	10.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	9051.5	N/A	11.36	1.209e7	0.10	1.661e6	✓	395.4
4186	21929.3/31.05.2022 - COAJA-DIL		Unknown	10.00		Pyrimethanil.2	Pyrimethanil	TFP.1		N/A	8764.1	N/A	11.36	3.796e6	0.10	5.311e5	✓	1027.1
4347	21929.3/31.05.2022 - SPALATE-Rep 1		Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	2917.8	N/A	11.36	3.138e7	0.13	3.625e6	✓	292.9
4348	21929.3/31.05.2022 - SPALATE-Rep 1		Unknown	1.00		Pyrimethanil.2	Pyrimethanil	TFP.1		N/A	3170.7	N/A	11.36	1.106e7	0.10	1.512e6	✓	1048.9
4509	21929.3/31.05.2022 -SPALATE- Rep 2		Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	2906.1	N/A	11.35	3.078e7	0.12	3.748e6	✓	303.3
4510	21929.3/31.05.2022 -SPALATE- Rep 2		Unknown	1.00		Pyrimethanil.2	Pyrimethanil	TFP.1		N/A	3348.5	N/A	11.35	1.150e7	0.10	1.578e6	✓	1162.5
4671	21929.3/31.05.2022 -SPALATE- DIL		Unknown	10.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	2852.9	N/A	11.36	4.381e6	0.10	6.288e5	✓	307.7
4672	21929.3/31.05.2022 -SPALATE- DIL		Unknown	10.00		Pyrimethanil.2	Pyrimethanil	TFP.1		N/A	2893.1	N/A	11.36	1.441e6	0.10	2.008e5	✓	861.4
4833	21929.3/31.05.2022 (Grapefruit)		Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	1276.7	N/A	11.28	1.335e7	0.07	2.217e6	✓	459.3
4834	21929.3/31.05.2022 (Grapefruit)		Unknown	1.00		Pyrimethanil.2	Pyrimethanil	TFP.1		N/A	1329.2	N/A	11.28	4.508e6	0.07	7.461e5	✓	634.6
4995	21929.3/31.05.2022- DIL (Grapefruit)		Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	226.3	N/A	11.30	3.665e6	0.09	5.415e5	✓	364.7
4996	21929.3/31.05.2022- DIL (Grapefruit)		Unknown	1.00		Pyrimethanil.2	Pyrimethanil	TFP.1		N/A	218.4	N/A	11.30	1.148e6	0.09	1.690e5	✓	718.9

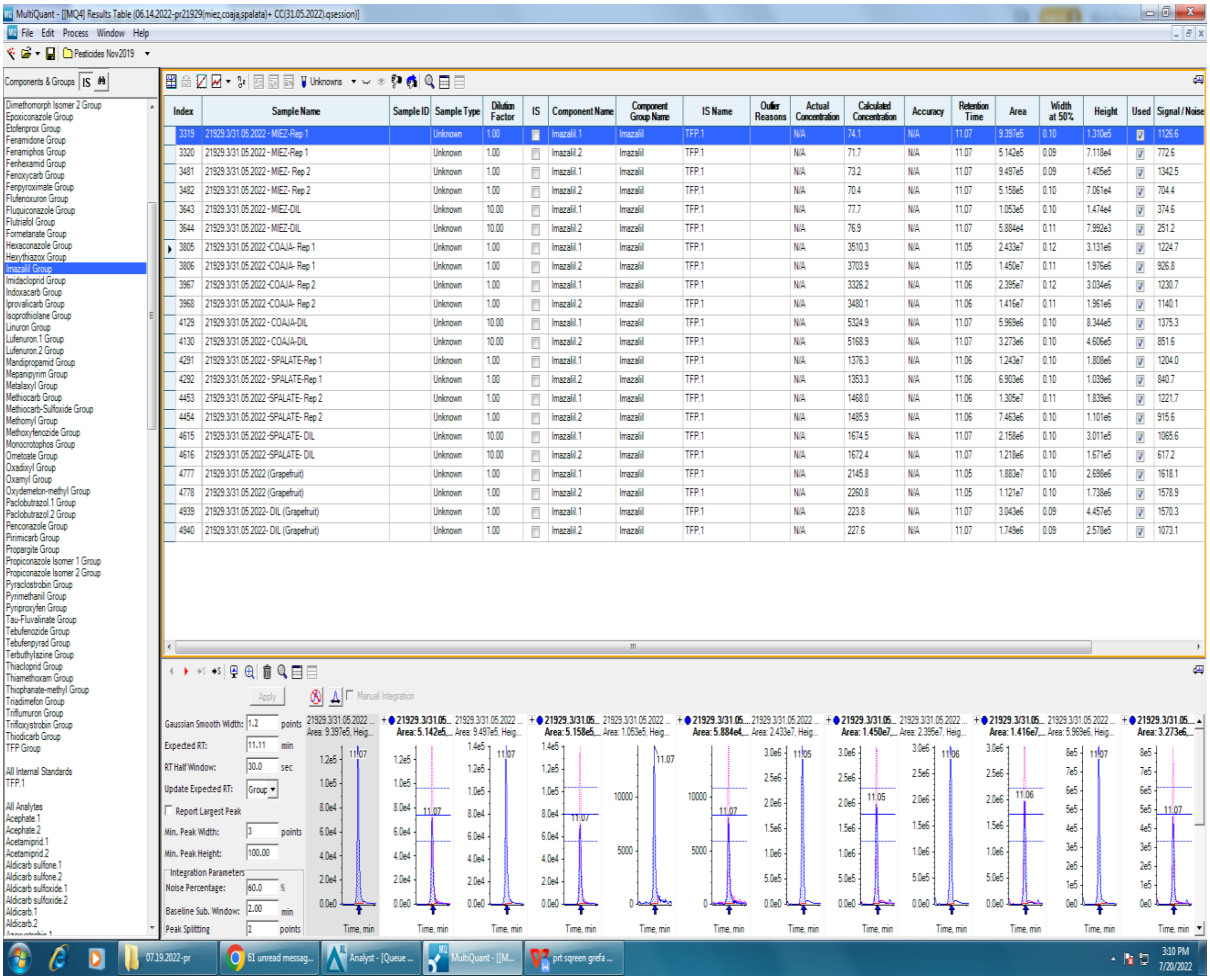


Figure 3 - Result from the analysis equipment software for Imazalil

Conclusions

This work demonstrates a viable approach for assessing the quality of citrus fruits, and we can even proceed to thoroughly wash each fruit before consumption, as we remove a quantity of residue.

This work demonstrates that the treatment affects the citrus tissues and the substances migrate throughout the pulp, although the treatment was applied to the peel.

Washing, which is the first step in food processing on a domestic and industrial scale, helps to reduce pesticide residues on the fruit surface. In this study, the effectiveness of washing the peel was also investigated. Although many chemicals are applied to citrus to prevent certain diseases or control pests, there is little literature available on how these treatments alter the physiology of the fruit. Usually, the packaging only analyzes the level of treatments on the products.

R E F E R E N C E S

[1]SR EN ISO 17025, 2018 General requirements for the competence of testing and calibration laboratories.

[2]European Regulation 396/2005 on establishing the maximum permissible limits for pesticide residues in and on fruits, vegetables, cereals and other products of plant origin, with subsequent additions.

[3]Document N0 DG/SANTE/11945/2015*Method validation and Quality control procedures for pesticide residues analysis in foood and feed.

[4]Document No. SANTE/12682/2019 Analytical Quality Control and Method Validation Procedures for Pesticide Residues Analysis in Food and Feed