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

Corina Mihaela OPRITA (CIOARA)¹, Elena RADU², Ingrid Alina COMAN³, Natalia ROȘOIU⁴

Abstract. The Most citrus fruits are compromised each year due to postharvest 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 μ g/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 μ m), 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 μ L 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.

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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	1
3 Imazalil.1		2237.8	74.1	73.2	1	4		5324.9		-	1674.5
4 Pyrimethanil.1		2262.5	51.3	51.7		1		9051.5			2852.9
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Figure 1. Distribution of detected analyte concentrations. Figure 2. Result from the analysis equipment software for Pyrimethanil

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^ Index	x Sampl	e Name	Sample ID Sample Type	Dilution Factor	IS	Component Name	Component Group Name	IS Name	Outer Reasons	Actual Concentration	Calculated Concentration	Accuracy	Retention Time	Area	Width at 50%	Height	Used	Sigr
337	5 21929.3/31.05.2022 - MIEZ-R	lep 1	Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	51.3	N/A	11.36	7.753e5	0.10	1.122e5	V	186.
337	6 21929.3/31.05.2022 - MIEZ-R	lep 1	Unknown	1.00	٥	Pyrimethanil.2	Pyrimethanil	TFP.1		N/A	53.1	N/A	11.36	2.602e5	0.10	3.551e4	7	437
353	7 21929.3/31.05.2022 - MIEZ- F	Rep 2	Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	51.7	N/A	11.36	7.993e5	0.10	1.191e5	V	205
353	8 21929.3/31.05.2022 - MIEZ- F	Rep 2	Unknown	1.00	E	Pyrimethanil.2	Pyrimethanil	TEP.1		N/A	51.1	N/A	11.36	2.558e5	0.10	3.654e4	7	403
3695	9 21929.3/31.05.2022 - MIEZ-D	IL	Unknown	10.00	E	Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	47.8	N/A	11.36	7.716e4	0.10	1.094e4	V	70.7
370	0 21929.3/31.05.2022 - MIEZ-D	IL	Unknown	10.00	E	Pyrimethanil.2	Pyrimethanil	TFP.1		N/A	51.9	N/A	11.36	2.719e4	0.11	3.642e3	1	118
386	1 21929.3/31.05.2022 -COAJA-	Rep 1	Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TEP.1	Ion Ratio	N/A	5722.2	N/A	11.36	4.724e7	0.18	3.977e6	1	183
iroup 386	2 21929 3/31 05 2022 -COA/A-	Ren 1	Unknown	1.00		Pyrimethanil.2	Pyrimethanil	TEP 1	Ion Ratio	N/A	9110.0	N/A	11.35	2.440e7	011	3 155e6	1	116
/02	3 21929.3/31.05.2022 -COAJA-	Ren 2	Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TEP.1	Ion Ratio	N/A	5462.9	N/A	11.37	4.685e7	0.18	3.971e6		179
412			Unknown	1.00		Pyrimethanil.2	Pyrimethanil	TEP 1	Ion Ratio	N/A	8552.6	NA	11.36	2.379e7	0.11	3.106e6	V	111
4185			Unknown	10.00		1	Pyrimethanil	TFP.1	101111010	N/A	9051.5	N/A	11.36	1.209e7	0.10	1.661e6	V	395
418			Unknown	10.00		Pyrimethanil.1	Pyrimethanil	TFP.1	_	N/A	8764.1	NA	11.30	3.796e6	0.10	5.311e5		
uo =						Pyrimethanil.2											V	102
434		1.1	Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	2917.8	N/A	11.36	3.138e7	0.13	3.625e6	7	293
434			Unknown	1.00		Pyrimethanil.2	Pyrimethanil	TFP.1		N/A	3170.7	N/A	11.36	1.106e7	0.10	1.512e6	V	104
4509			Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	2906.1	N/A	11.35	3.078e7	0.12	3.748e6	V	30
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Group 467	1 21929.3/31.05.2022 -SPALAT	E- DIL	Unknown	10.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	2852.9	N/A	11.36	4.381e6	0.10	6.288e5	V	30
4577	2 21929.3/31.05.2022 -SPALAT	'E- DIL	Unknown	10.00		Pyrimethanil.2	Pyrimethanil	TEP.1		N/A	2893.1	N/A	11.36	1.441e6	0.10	2.008e5	V	86
483	3 21929.3/31.05.2022 (Grapefri	uit)	Unknown	1.00		Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	1276.7	N/A	11.28	1.335e7	0.07	2.217e6	V	45
4834	4 21929.3/31.05.2022 (Grapefri	uit)	Unknown	1.00		Pyrimethanil.2	Pyrimethanil	TFP.1		N/A	1329.2	N/A	11.28	4.508e6	0.07	7.461e5	V	63
4995	15 21929.3/31.05.2022- DIL (Gra	apefruit)	Unknown	1.00	E	Pyrimethanil.1	Pyrimethanil	TFP.1		N/A	226.3	N/A	11.30	3.665e6	0.09	5.415e5	7	36
		anefruit)	Unknown	1.00		Pyrimethanil.2	Pyrimethanil	TEP 1	-		040.4	N/A	11.30	1.148e6	0.09	1.690e5	-	718
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^ Index	1	Sample Name	Sample ID	Sample Type	Dilution Factor	IS	Component Name	Component Group Name	IS Name	Outer Reasons	Actual Concentration	Calculated Concentration	Accuracy	Retention	Area	Width at 50%	Height	Used	l Si
3315	9 21929.3/31.05.2022 -	MIEZ-Rep 1		Unknown	1.00		Imazalil.1	Imazalil	TFP.1		N/A	74.1	NA	11.07	9.397e5	0.10	1.310e5		11
3320	0 21929.3/31.05.2022 -	MIEZ-Rep 1		Unknown	1.00	1	Imazalil 2	Imazalil	TEP.1		NA	71.7	NA	11.07	5.142e5	0.09	7.118e4	1	7
3481				Unknown	1.00		Imazalil.1	Imazalil	TFP.1		NA	73.2	NA	11.07	9.497e5	0.09	1.405e5	V	1
3480				Unknown	100		Imazalil 2	Imazalil	TEP 1		N/A	70.4	NA	11.07	5 158e5	0.10	7.061e4	V	7
3643			_	Unknown	10.00		Imazalil.1	Imazalil	TFP.1		NA	77.7	NA	11.07	1.053e5	0.10	1,474e4	V	1
364				Unknown	10.00		Imazalil.2	Imazalil	TFP.1		NA	76.9	NA	11.07	5.884e4	0.10	7.992e3	V	1
3805									TFP.1		NA	3510.3						_	1
,				Unknown	1.00		Imazalil.1	Imazalil					NA	11.05	2.433e7	0.12	3.131e6	V	
3806				Unknown	1.00		Imazalil.2	Imazalil	TFP.1		N/A	3703.9	NA	11.05	1.450e7	0.11	1.976e6	V	
3967				Unknown	1.00		Imazalil.1	Imazalil	TFP.1		N/A	3326.2	NA	11.06	2.395e7	0.12	3.034e6	V	
- 3968				Unknown	1.00		Imazalil.2	Imazalii	TFP.1		N/A	3480.1	NA	11.06	1.416e7	0.11	1.961e6	V	
4129	9 21929.3/31.05.2022 -	COAJA-DIL		Unknown	10.00		Imazalil.1	Imazalil	TFP.1		N/A	5324.9	NA	11.07	5.969e6	0.10	8.344e5	V	
4130	0 21929.3/31.05.2022 -	COAJA-DIL		Unknown	10.00		Imazalil.2	Imazalil	TFP.1		N/A	5168.9	NA	11.07	3.273e6	0.10	4.606e5	V	
4291	1 21929.3/31.05.2022 -	SPALATE-Rep 1		Unknown	1.00		Imazalil.1	Imazalil	TFP.1		N/A	1376.3	NA	11.06	1.243e7	0.10	1.808e6	V	
4292	2 21929.3/31.05.2022 -	SPALATE-Rep 1		Unknown	1.00		Imazalil.2	Imazalil	TFP.1		NA	1353.3	NA	11.06	6.903e6	0.10	1.039e6	V	
4453	3 21929.3/31.05.2022 -	PALATE- Rep 2		Unknown	1.00		Imazalil.1	Imazalil	TFP.1		NA	1468.0	NA	11.06	1.305e7	0.11	1.839e6		
4454				Unknown	1.00		Imazalil 2	Imazalil	TEP.1		N/A	1485.9	NA	11.06	7.463e6	0.10	1.101e6	V	+
4615				Unknown	10.00		Imazalil.1	Imazalil	TFP.1		NA	1674.5	NA	11.07	2 158e6	0.10	3.011e5	V	+
4616				Unknown	10.00		Imazalil.2	Imazalil	TFP.1		NA	1672.4	NA	11.07	1.218e6	0.10	1.671e5		
																		V	1
4777				Unknown	1.00		Imazalil.1	Imazalil	TFP.1		N/A	2145.8	NA	11.05	1.883e7	0.10	2.698e6	V	
4778				Unknown	1.00		Imazalil.2	Imazalil	TFP.1		N/A	2260.8	NA	11.05	1.121e7	0.10	1.738e6	V	
4939	9 21929.3/31.05.2022-1	JIL (Grapefruit)		Unknown	1.00		Imazalil.1	Imazalil	TFP.1		N/A	223.8	NA	11.07	3.043e6	0.09	4.457e5	V	
4940	0 21929.3/31.05.2022-1	JIL (Grapefruit)		Unknown	1.00		Imazalil.2	Imazalil	TFP.1		N/A	227.6	NA	11.07	1.749e6	0.09	2.578e5	V	
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Gaussian	Appi Smooth Width: 1.2	ly Manua points 21929.3/31.05.2022 Area: 9.397e5. Heig		2e5, Area: 9.4	97e5, Heig		Area: 5.158e5, Are		÷ ● 21929.3/31.05. Area: 5.884e4,			21929.3/31.05 1 Area: 1.450e7)	krea: 2.395e7, H	leig /	Area: 1.416e	15 21929.3(3) 7 Area: 5.96		+ • 219 Are	
	App n Smooth Width: 1.2 d RT:11.11	y Manu points 21929.3/31.05.2022 Area: 9.397e5. Heig min 1 stles	+ • 21929.3/3 Area: 5.14		97e5, Heig			sa: 1.053e5, Heig			7. Heig		krea: 2.395e7, H	leig /			9e6, Heig		ea:
Gaussian	App n Smooth Width: 1.2 d RT: 11.11	ly <u>(1000)</u> <u>(1000)</u> Marua points 21929.3/31.05.2022 Area: 9.397e5, Heig. min sec 1.2e5 11107	+ • 21929.3/3 Area: 5.14	2e5, Area: 9.4	97e5, Heig 7 11 <mark>.</mark> 07	1	Area: 5.158e5, Are			. Area: 2.433e 3.0e6 -	7. Heig 11.05 3	Area: 1.450e7,) 0e6 -	krea: 2.395e7, H	leig / 106 ^{3.1}	Area: 1.416e	7, Area: 5.96	9e6, Heig 11 <mark>1</mark> 07	Area	ва: 5 -
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Distribution and Qualitative and Quantitative Determination of Pesticide Residues from a Sample of Red Grapefruit, after Treatment with Fungicide Solutions

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.

REFERENCES

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