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Multiresidue pesticide analysis to determine the influence of postharvest packinghouse handling on papaya residue levels

Abstract – The objective of this work was to adapt and validate a multiresidue method for pesticide analysis, to determine the influence of the current postharvest handling steps on the degradation of pesticide residues in papaya. 'THB' papaya of the Solo group were sampled at each postharvest handling step in the packinghouse. For the validation of the multiresidue analysis in papaya, the technique used for extraction was the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method, with modifications. Pesticide quantification was carried out using an ultra-high performance liquid chromatograph coupled to a triple quadrupole mass spectrometer with electrospray ionization (UHPLC-MS/MS). Fruit were analyzed before and after the handling procedures and were evaluated after ripening. The used methodology was successfully validated to detect the azoxystrobin, carbendazim, carbofuran, difenoconazole, imazalil, imidacloprid, flutriafol, prochloraz, pyraclostrobin, tebuconazole, thiabendazole, and thiacloprid pesticides in papaya. Fruit washing reduces azoxystrobin and difenoconazole residue levels in 78 to 90% and in 47 to 90% on fruit surface, respectively. Although the postharvest handling steps are ineffective in reducing the levels of thiabendazole, if this fungicide is applied at the appropriate rate, the residue levels will remain below the limits permitted by law.

Index terms: *Carica papaya*, food safety, QuEChERS, UHPLC-MS/MS.

Análise multirresíduos de pesticidas para determinar a influência do manuseio pós-colheita sobre os níveis de resíduos em mamão

Resumo – O objetivo deste trabalho foi adaptar e validar um método multirresíduo para análise de agrotóxicos, para determinar a influência das atuais etapas de manejo pós-colheita sobre a degradação de resíduos de agrotóxicos em mamão. Mamões 'THB' do grupo Solo foram amostrados em cada etapa de manejo pós-colheita, em casa de beneficiamento. Para a validação da análise multirresíduos em mamão, a técnica de extração utilizada foi o método *quick*, *easy*, *cheap*, *effective*, *rugged*, *and safe* (QuEChERS), com modificações. A quantificação foi realizada com cromatógrafo líquido de ultraeficiência, acoplado a espectrômetro de massa triplo, quadrupolo, com ionização por eletrospray (UHPLC-MS/MS). Os frutos foram analisados antes e depois dos procedimentos de manuseio e avaliados após o amadurecimento. A metodologia utilizada foi validada com sucesso para detectar os pesticidas azoxistrobina, carbendazim, carbofuran, difenoconazol, imazalil, imidacloprid, flutriafol, prochloraz, piraclostrobina, tebuconazol, tiabendazol e thiacloprid em mamão. A lavagem dos frutos reduz os níveis de resíduos de

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azoxistrobina e difenoconazol em 78 a 90% e em 47 a 90% na superfície dos frutos, respectivamente. Embora as etapas de manejo pós-colheita sejam ineficazes para a redução dos níveis do tiabendazol, se este fungicida for utilizado na dose adequada, os níveis de resíduos permanecerão abaixo dos limites permitidos por lei.

Termos para indexação: *Carica papaya*, segurança de alimentos, QuEChERS, UHPLC-MS/MS.

Introduction

Phytosanitary problems with papaya (*Carica papapa* L.) start in the field, as some postharvest diseases caused by fungi are usually due to field-established quiescent infections. Fungal diseases are the cause from 80 to 90% of the total losses (Demartelaere et al., 2017; Hernandez-Montiel et al., 2018; Liu et al., 2018).

The attempt to reduce the problem with these diseases leads to the indiscriminate use of pesticides, compromising the fruit quality. Additionally, the papaya production system still lacks registered phytosanitary products. In postharvest, the chemical method is the most traditional disease control measure, performed by immersing the fruit in fungicides (Hernandez-Montiel et al., 2018), which may leave residue levels above the maximum residue limits (MRLs) on the fruit. These residue, deposited as active ingredients, its metabolites, or breakdown products have the potential of detrimental effects on human health (Narenderan et al., 2020). Currently, only thiabendazole can be used in the postharvest of papaya fruit, and its MRL is 6 and 10 mg kg^{-1} for Brazil and Europe, respectively (Anvisa, 2023; European Commission, 2023).

Therefore, pesticide residue analysis studies are necessary for the establishment of efficient processes, to ensure the safety of food consumption, for which several methods are described in the literature. Among these, to date, the best known one is the quick, easy, cheap, effective, rugged, and safe (QuEChERS) method, which is used for multiclass and multiresidue pesticide analyses of food with high water content (Anastassiades et al., 2003). This method was later recognized as an official AOAC method for the analysis of various pesticides in fruits and vegetables (Mao et al., 2020). The simplified resources, practical benefits, and excellent results provided by the QuEChERS sample preparation approach, followed by gas chromatography tandem mass spectrometry (GC-MS/MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS) helped the QuEChERS concept to achieve significant popularity (Mao et al., 2020). However, there are few studies on this analysis methodology to determine pesticide residue in fresh papaya.

Several postharvest procedures and processing stages of domestic or industrial food can contribute to the removal/degradation of pesticide residue in food, such as washing with pure water or acidic or alkaline solutions, heating treatments, ozonation, peeling, grinding, cooking, freezing, drying, juicing, canning, and storing adequately (Oliva et al., 2017; Mir et al., 2022).

Studies with dissipation and migration of fungicides in papaya fruit are scarce, and more researches are necessary to better understand the risks of postharvest contamination, and to provide producers and consumers with guidelines on food management and safety, according to Freitas et al. (2023). However, there are still few studies showing the effect of each step of the papaya postharvest handling on the reduction of these residue in fruit.

The objective of this work was to adapt and validate a multiresidue method for pesticide analysis, to determine the influence of the current postharvest handling steps on the degradation of pesticide residues in papaya.

Materials and Methods

Fruit of 'THB' of the Solo papaya group were sampled on three dates, at each postharvest handling stage, in the packinghouse of a fruit exporting company located in the city of Mucuri, in the state of Bahia (BA), Brazil. The company has two postharvest processing lines: one for fruits intended for export to the United States of America (USA), and another one to Europe and the internal market (Brazil). In the USA processing line, postharvest handling steps involve fruit washing with chlorinated water, heat treatment with water (48±1°C for 20 min), packing, and cold storage. These fruit are produced in plots (areas), then separated and monitored for fruit flies, and they cannot receive postharvest products or wax. Thus, fruit samples were collected without any postharvest handling procedure (before washing), except for washing with chlorinated water and heat treatment. Part of the fruit collected before processing or heat treatment was also evaluated after full ripening, to assess the effect of handling and ripening on the pesticide degradation.

In the processing line of papaya fruit intended for the internal market and for export to Europe, the handling stages involved washing with chlorinated water, thiabendazole application (Tecto SC Syngenta, 400 mL L^{-1} or 194 g i.a. L^{-1}) (1st and 2nd samplings), carnauba wax application, drying, packing, and cold storage. In this case, fruit samples were collected without any postharvest processing, after washing with chlorinated water, followed by fungicide, and carnauba wax application and drying. Fruit were analyzed and after the handling procedures. Then, after ripening, fruit were also evaluated to assess the effect of handling and ripening on the pesticide degradation. The sampled fruit were packed and sent to the laboratory of residue and contaminants (Laboratório de Resíduos e Contaminantes) of Embrapa Meio Ambiente, in the municipality of Jaguariúna, SP, Brazil.

Twelve pesticides were analyzed on the fruit samples on the basis of products registered with the Agrofit system for papaya (Agrofit, 2023), considering also the most used products and pesticides detected in papaya samples collected from 2011 to 2015 – azoxystrobin, carbendazim, carbofuran, difenoconazole, flutriafol, imazalil, imidacloprid, pyraclostrobin, prochloraz, tebuconazole, thiabendazole and thiacloprid – by the Programa de Análise de Resíduos de Agrotóxicos em Alimentos (PARA) of the Agência Nacional de Vigilância Sanitária (Anvisa), Brazil.

The reagents, solvents, and other materials used in the present study are the following: sodium chloride, trisodium citrate dehydrate, and anhydrous magnesium sulphate J.T. Baker’s analytical grades (Avantor, PA, USA); sodium hydrogen citrate sesquihydrate, purity ≥ 99% (Sigma-Aldrich, St. Louis, MO, USA); methanol and acetonitrile HPLC grade (Tedia Company, Fairfield, OH, USA); ultra-purified water with Milli-Q Simplicity 185 water purification system (MilliporeSigma, Bedford, MA, USA); Bondesil-PSA (40 µm) was acquired from Varian (USA), syringe filter GV Millex 0.22 µm, 13 mm (MilliporeSigma, Burlington, MA).

The standards of the pesticides – azoxystrobin, carbendazim, carbofuran, difenoconazole, flutriafol, imidacloprid, imazalil, pyraclostrobin, tebuconazole, thiabendazole, thiacloprid, and prochloraz – were acquired from the companies Dr. Ehrenstorfer or Fluka, with purity grade ranging from 93 to 99.5%.

The papaya samples received in the laboratory were frozen and processed using a commercial food processor. The samples were stored at -20ºC until the pesticide residue were analyzed.

Sample removal from the fruit was performed manually with the aid of a knife, and the samples were separated into three groups: whole papaya (peel, pulp, and seed), pulp with peel (without seed), and pulp (without peel or seed). The extraction technique was the QuEChERS method (Anastassiades et al., 2003) with modifications, using citrate buffer, described as follows.

A total of 10 g of frozen sample was weighed in 50 mL Teflon tubes containing 10 mL acetonitrile. This mixture was shaken vigorously for 2 min at speed 10, in a Heidolph Multi Reax shaker. Subsequently, 4.0 g anhydrous magnesium sulfate, 1.0 g anhydrous sodium chloride, 1.0 g trisodium citrate dihydrate, and 0.5 g of disodium hydrogen citrate sesquihydrate were also added. The tube was then shaken for 2 min at 10 rpm in a Heidolph Multi Reax shaker and centrifuged for 5 min at 5,000 rpm and 20ºC. A supernatant aliquot of 5 mL was transferred to the centrifuge tube (10 mL) containing 125 mg of PSA and 750 mg of anhydrous magnesium sulfate. This mixture was shaken again for 2 min and centrifuged at 5,000 rpm and 20ºC. From this extract, 1 mL aliquot of the supernatant was evaporated to dryness with the aid of nitrogen. The extract was resuspended in 1 mL of mobile phase composed of 0.1% methanol: formic acid (50:50, v/v), sonicated for 30 s, and filtered through a 0.22 µm syringe filter directly into a vial that was taken to the injector for analysis. A UHPLC-MS/MS Waters system (Waters Corporation, Milford, MA, USA) was coupled with a Waters Quattro Premier XE triple quadrupole mass spectrometer (Waters Corporation) with electrospray ionization (ESI) source. Initially, direct infusions of the individual pesticide patterns into the mass spectrometer were performed to optimize the responses of the precursor ions. The responses of two ions were monitored in the multiple reaction monitoring (MRM) mode, with the most abundant ones being selected for quantification and the second abundant ion transition abundant for confirmation. The equipment was operated in the MRM mode with electrospray ionization in the positive ion mode.

Source temperature was 120ºC, and capillary voltage was optimized at 3.0 KV. Nitrogen was used as the desolvation gas at 400°C, with a flow rate of 500 L per hour, while argon was used as the collision gas with a flow rate of 0.2 mL min^{-1}.

The chromatographic separation was performed using 1.7 µm Kinetex C18 100Å column at 35ºC, with the following gradient elution: beginning at 40% (v/v) of phase B (methanol) and 60% of phase A (aqueous solution containing 0.1% formic acid), linearly increasing to 100% of phase B in 4.0 min, then returning to 40% of B in 4.2 min and remaining until 7.0 min, totaling seven minutes of analysis. The flow rate of the mobile phase was kept at 0.25 mL per minute, and the injection volume was 10 µL.

The validation of the analytical method was performed with pesticide-free papaya samples (blank samples). For that, blank samples were fortified with the standards at known concentrations, to obtain the validation parameters.

The adaptation and validation of the method were carried out for 12 pesticides, using UHPLC-MS/MS, and the compound detection was performed using the MRM mode by monitoring two pesticide transitions (Table 1), which shows the transitions for identification, confirmation, collision energy, and cone voltage, all obtained by compound infusion into the mass spectrometer. The validation procedure was conducted to determine the selectivity, linearity, limit of quantification (LOQ), accuracy (recovery), repeatability (RSD_r), intermediate precision (RSD_R), and the matrix effect was based on the parameters and criteria established by the Directorate-General for Health and Food Safety (SANTE), Document no. SANTE 11813/2017 (European Commission, 2017).

Linearity was evaluated based on the analytical curve, by external standardization in the solvent and matrix matched calibration curve, in the range 0.001, 0.0025, 0.005, 0.0075, 0.01, 0.0125, 0.0150, 0.0175, and 0.020 mg kg^{-1} for UHPLC-MS/MS. The limit of quantification (LOQ), was 0.005 mg kg^{-1}, and the limit of detection (LOD) was established as 0.0025 mg kg^{-1} with accuracy and precision. Thus, the lowest fortification level with accuracy and precision was established as the LOQ 0.005 mg kg^{-1} (Tables 2 and 3).

To verify the accuracy and precision (repeatability) of the method, six replicates of the control papaya samples, fortified with two concentration levels, were evaluated at 0.01 mg kg^{-1} and 0.005 mg kg^{-1} for the UHPLC-MS/MS analysis. The repeatability was expressed by the coefficient of variation (CV%) of three results of replicates analyzed on the same day, by the same analyst, using the same instrument.

Results and discussion

The method was adapted and validated for the pesticides azoxystrobin, carbendazim, carbofuran, difenoconazole, flutriafol, imazalil, imidacloprid, pyraclostrobin, prochloraz, tebuconazole,

Table 1. Optimized acquisition data of retention time (RT), two transitions (identification and confirmation), and collision energy and cone voltage for the adaptation and validation of pesticide determination method by UHPLC-MS/MS for the pesticide analysis in papaya (*Carica papaya*) fruit.

Pesticide	RT (min)	Transition 1 m/z	Collision energy (V) 1	Transition 2 m/z	Collision energy (V) 2	Cone voltage (V)
Carbendazim	1.33	192.1>160.0	28	192.1>132.0	18	20
Thiabendazole	1.52	202.1>175.1	32	202.1>131.1	26	47
Imidacloprid	1.71	256.1>209.2	16	256.1>175.1	16	30
Thiacloprid	2.70	253.1>126.0	38	253.1>90.1	22	40
Carbofuran	4.13	222.2>165.0	22	222.2>123.0	12	30
Imazalil	4.17	297.0>159.0	18	297.0>69.0	22	36
Flutriafol	4.49	302.1> 70.1	16	302.1>122.9	28	26
Azoxystrobin	4.71	404.1>372.1	30	404.1>329.0	15	26
Tebuconazole	5.07	308.1>70.1	22	308.1>125.0	36	36
Prochloraz	5.08	376.1>308.0	24	376.1>70.1	12	21
Pyraclostrobin	5.12	388.3>194.1	26	383.3>163.0	12	22
Difenoconazole	5.17	406.0>251.1	50	406.0>111.0	26	39

thiabendazole, and thiacloprid, using the UHPLC-MS/MS, and the compound detection was performed using the MRM mode. Under the optimized conditions, the recoveries varied from 76 to 111%, the CV% of intra-day precision varied from 0.88 to 18.27, and the CV% of the inter-day precision varied from 2.54 to 19.52 (Table 2), which is within the range (70 to 120%) and RSD ≤ 20% recommended by SANTE Guidelines (European Commission, 2017). Malonn et al., 2023 also obtained similar results using the QuEChERS with citrate in papayas, to validate this methodology for analyzing residue of azoxystrobin, carbendazim, carbofuran, difenoconazole, flutriafol, imazalil, imidacloprid, pyraclostrobin, thiabendazole and thiacloprid, among others.

The adapted and validated multiresidue method was applied in the analysis of 32 samples collected at each postharvest handling stage. In the papaya exporting company packinghouse, two postharvest processing lines were selected for the analysis: one for USA, and another one for Europe and the internal market, among which only the pesticides azoxystrobin, difenoconazole, and thiabendazole were detected (Table 4). These pesticides were detected as follows:

Table 2. Parameters for method validation of intra-day precision, inter-day precision, and recovery, at two fortification levels for whole papaya (*Carica papaya*) fruit.

Pesticide	Intra-day precision (CV %) (n=6)		Inter-day precision (CV %) (n=12)		Recovery (%) (n=6)	
	0.005 mg kg^{-1}	0.01 mg kg^{-1}	0.005 mg kg^{-1}	0.01 mg kg^{-1}	0.005 mg kg^{-1}	0.01 mg kg^{-1}
Azoxystrobin	18.27	9.40	13.98	11.44	89	86
Carbendazim	11.91	4.17	10.49	8.21	89	77
Carbofuran	16.13	4.66	15.62	6.85	105	96
Difenoconazole	3.63	10.68	19.12	15.41	83	76
Flutriafol	12.13	5.05	9.89	5.18	99	93
Imidacloprid	15.42	9.48	15.42	6.81	94	85
Imazalil	8.65	1.43	14.07	6.09	109	100
Pyraclostrobin	10.57	7.83	17.15	13.06	90	86
Prochloraz	14.05	6.18	19.52	12.69	102	102
Tebuconazole	13.04	6.37	18.47	12.60	111	106
Thiabendazole	8.70	0.88	8.49	4.64	90	77
Thiacloprid	10.39	2.50	10.37	2.54	102	95

Table 3. Limit of detection (LOD) and limit of quantification (LOQ) of the pesticides, and maximum residue limits (MRLs) established for papaya (*Carica papaya*) in Brazil, Europe, and the USA.

Pesticide	LOD (mg kg^{-1})	LOQ (mg kg^{-1})	MRLs (mg kg^{-1}) (Brazil – Anvisa)	MRLs (mg kg^{-1}) (Europe)	MRLs (mg kg^{-1}) (USA)
Azoxystrobin	0.0025	0.005	0.3	0.3	2.0
Carbendazim	0.0025	0.005	0.5	0.2	-
Carbofuran	0.0025	0.005	0.1	0.01	-
Difenoconazole	0.0025	0.005	0.3	0.2	0.6
Flutriafol	0.0025	0.005	0.5	0.01	-
Imidacloprid	0.0025	0.005	2.0	0.05	1.0
Imazalil	0.0025	0.005	1.0	0.05	-
Pyraclostrobin	0.0025	0.005	0.1	0.07	0.6
Prochloraz	0.0025	0.005	1.0	5.0	-
Tebuconazole	0.0025	0.005	1.0	2.0	-
Thiabendazole	0.0025	0.005	6.0	10.0	5.0
Thiacloprid	0.0025	0.005	0.3	0.5	-

Sources: Anvisa (2023), European Commission (2023), and USDA (2023).

azoxystrobin, in 23 samples; thiabendazole, in nine samples; and difenoconazole, in 29 samples. Only two samples had all three pesticides, corresponding to fruit intended for export to Europe: one of the samples was composed of ripe papayas after all procedures (after drying), while the other was composed of green papayas after treatment with carnauba wax. Difenoconazole was detected as below of the LOQ for ten samples, azoxystrobin in eight samples, and thiabendazole, in three samples (Table 4).

Thirteen samples of whole fruit whose residue values exceeded 0.03 mg kg^{-1}, for some of the three pesticides (azoxystrobin, difenoconazole, and thiabendazole), were selected for further analysis (Table 4). Thus,

Table 4. Residues of pesticides detected in samples of whole papaya (*Carica papaya*) fruit subjected to postharvest handling, for export to the USA and Europe, sampled on three dates.

Identification	Thiabendazole (mg kg^{-1})	Azoxystrobin (mg kg^{-1})	Difenoconazole (mg kg^{-1})
USA (First sampling)			
GP - Before postharvest handling	ND	ND	< LOQ
GP – After washing with chlorinated water	0.012	ND	ND
GP – After heat treatment	< LOQ	ND	ND
RP - After postharvest processing	< LOQ	ND	ND
Europe (First sampling)			
GP - Before postharvest handling	ND	0.009	< LOQ
GP – After washing with chlorinated water	ND	< LOQ	< LOQ
GP – After treatment with carnauba wax	0.833	< LOQ	< LOQ
RP – After postharvest handling	0.476	0.010	< LOQ
USA (Second sampling)			
GP - Before postharvest handling	ND	0.056	0.027
GP – After washing with chlorinated water	ND	0.006	0.007
GP – After heat treatment	ND	< LOQ	< LOQ
RP – After heat treatment	< LOQ	ND	< LOQ
RP – Without postharvest handling	ND	0.061	0.024
Europe (Second sampling)			
GP – Before postharvest handling	ND	0.031	0.020
GP - After washing with chlorinated water	ND	0.007	0.006
GP – After treatment with fungicide	ND	0.008	< LOQ
GP – After treatment with carnauba wax	1.316	0.011	0.006
GP – After drying	1.053	0.007	< LOQ
RP - After postharvest handling	1.322	0.009	0.005
RP – Without postharvest handling	ND	0.068	0.026
USA (Third sampling)			
GP – Before postharvest handling	ND	< LOQ	0.048
GP – After washing with chlorinated water	ND	< LOQ	0.005
GP – After heat treatment	ND	ND	0.007
RP – After postharvest handling	ND	ND	0.005
RP – Without postharvest handling	ND	0.009	0.013
Europe (Third sampling)			
GP – Before postharvest processing	ND	0.007	0.036
GP – After washing with chlorinated water	ND	< LOQ	0.019
GP – After treatment with fungicide	ND	ND	0.031
GP - After treatment with carnauba wax	ND	< LOQ	< LOQ
GP- After drying	ND	ND	0.026
RP - After postharvest handling	ND	< LOQ	0.017
RP - Without postharvest handling	ND	0.012	0.031

Samples: GP, green papaya; RP, ripe papaya. LOQ: limit of quantification. ND: not detected.

for these samples, fruit were analyzed as follows: the whole papaya, the pulp only, and the pulp with peel, separately (Table 5). In five of these samples, thiabendazole was detected at higher concentrations in the pulp with the peel, followed by whole papaya, then by pulp only. Azoxystrobin was detected in five whole papaya samples, pulp, and pulp with peel; in seven samples, the pesticide was detected in the whole papaya and in the pulp with peel. In only one of the green papaya samples – after fungicide application, intended for export to Europe – azoxystrobin was not detected in any of the parts. In turn, difenoconazole was detected in eight whole papaya samples, pulp, and pulp with peel. In the five remaining samples, difenoconazole was not detected in the pulp. In all samples with pesticide detection, the concentrations in the pulp were lower than in the whole fruit, constituting an indication that the fungicides remain on the fruit surface (Table 5) and were below the MRL permitted by law in Brazil, USA, and Europe (Table 3).

In the postharvest processing line of fruit exported to the USA, thiabendazole residue were only found in one sample collected after washing with chlorinated water. This may have occurred because thiabendazole is also used in the field to control anthracnosis and the black spot disease in papaya. However, after heat treatment, the values were well below the detection limit (Table 6). Azoxystrobin and difenoconazole were

Table 5. Residues of pesticides detected in samples of papaya (*Carica papaya*) subjected to postharvest handling, for export to the USA and Europe, sampled on three dates and separated into three parts: whole papaya (peel, pulp, and seed), pulp (without peel and seed), and pulp with peel (without seed).

Identification	Thiabendazole (mg kg^{-1})			Azoxystrobin (mg kg^{-1})			Difenoconazole (mg kg^{-1})		
	Whole fruit	Pulp	Pulp with peel	Whole fruit	Pulp	Pulp with peel	Whole fruit	Pulp	Pulp with peel
USA									
GP - Before postharvest handling (2nd sampling)	ND	ND	ND	0.056	0.005	0.188	0.027	0.002	0.045
RP - Without postharvest handling (2nd sampling)	ND	ND	ND	0.061	0.010	0.105	0.025	0.004	0.052
GP - Before postharvest handling (3rd sampling)	ND	ND	ND	0.003	ND	0.005	0.048	0.004	0.078
Europe									
GP – After treatment with wax (1st sampling)	0.833	0.351	1.800	0.004	ND	0.020	0.004	ND	0.014
RP – After postharvest handling (1st sampling)	0.476	0.132	1.066	0.010	ND	0.032	0.004	ND	0.017
GP - Before postharvest handling (2nd sampling)	ND	ND	ND	0.032	0.004	0.118	0.020	0.002	0.052
GP – After drying (2nd sampling)	1.053	0.421	2.925	0.007	ND	0.019	0.004	ND	0.014
GP - After treatment with wax (2nd sampling)	1.316	0.341	4.375	0.011	ND	0.060	0.006	0.001	0.024
RP – Without postharvest handling (2nd sampling)	ND	ND	ND	0.068	0.004	0.330	0.026	0.002	0.054
RP - After postharvest handling (2nd sampling)	1.322	0.306	2.250	0.009	ND	0.015	0.005	ND	0.008
GP - Before postharvest processing (3rd sampling)	ND	ND	ND	0.008	ND	0.017	0.036	ND	0.052
GP – After treatment with fungicide (3rd sampling)	ND	ND	ND	ND	ND	ND	0.031	0.002	0.073
RP - Without postharvest processing (3rd sampling)	ND	ND	ND	0.012	0.001	0.023	0.031	0.003	0.088

Samples: GP, green papaya; RP, ripe papaya. ND: not detected.

detected in the two last sampling dates. These fungicides are applied in in the pre-harvest, to control anthracnosis and the black spot disease in the field. However, after the postharvest processing, the fungicides were eliminated or reduced to below the MRL of the USA (Table 3).

Only the washing process in chlorinated water reduced the azoxystrobin residue levels approximately in 90%, and difenoconazole in 74% (2nd sampling) and 90% (3rd sampling) in the fruit. After heat treatment with hot water, the residue were virtually eliminated or remained within values well below those permitted by law in the USA (Table 3).

Washing can remove pesticide residue with reasonable efficiency, especially those with limited movement and penetration capacity. Washing effectiveness depends on the solubility of the pesticide in the water, or in different chemical solvents (Đjorđević & Đjurović-Pejčev, 2016). The washing with water or soaking in some chlorine-based solutions proved to be highly effective to reduce the pesticide content in fresh fruits and vegetables (Bhilwadikar et al., 2019). The reduction of azoxystrobin residue by 49.29% was observed in table grapes washed with chlorinated water (Wei et al., 2018).

Hot water increases the pesticide removal and can hydrolyze substantial fractions of nonpersistent compounds, favoring the volatilization and thermal degradation (Yigit & Velioglu, 2020). In the present study, the use of hot water for heat treatment of fruit exported to the USA favored the reduction of most detected pesticides.

For the postharvest processing line of papaya fruit exported to the European or commercialized in the internal markets, the fungicide thiabendazole was detected in the first two sampling dates (Table 6) at levels well below the MRL established by these countries (Table 3). This fungicide was not detected in the third sampling date, since the company was no longer using it. As expected, only the samples after the treatment with this fungicide showed residue; however, the processing steps after fungicide application, consisting of wax application and drying in a hot air tunnel, were ineffective in reducing the residue levels of thiabendazole.

Thiabendazole is a systemic fungicide of the benzimidazole chemical group. It is absorbed by the pulp even in postharvest applications (Table 6). Therefore, postharvest processing following the application of this fungicide (drying and packing) does not interfere with its degradation. Furthermore, no fruit washing procedure is performed after the fungicide application, which could remove residue in the peel.

Table 6. Samples analyzed at each stage of the postharvest processing line of whole papaya (*Carica papaya*) fruit exported to the USA, Europe, and commercialized in the internal market (Brazil).

Identification	Thiabendazole (mg kg^{-1})			Azoxystrobin (mg kg^{-1})			Difenoconazole (mg kg^{-1})		
	Sampling			Sampling			Sampling		
	1st	2nd	3rd	1st	2nd	3rd	1st	2nd	3rd
USA									
GP – Before postharvest handling	ND	ND	ND	ND	0.056	<LOQ	<LOQ	0.027	0.048
GP – After washing with chlorinated water	0.012	ND	ND	ND	0.006	<LOQ	ND	0.007	0.005
GP – After heat treatment	<LOQ	ND	ND	ND	<LOQ	ND	ND	<LOQ	0.007
RP - After postharvest handling	<LOQ	<LOQ	ND	ND	ND	ND	ND	< LOQ	0.005
RP - Without postharvest handling	-	ND	ND	-	0.061	0.009	-	0.024	0.013
Europe and Brazil									
GP – Before postharvest handling	ND	ND	ND	0.009	0.031	0.007	<LOQ	0.020	0.036
GP – After washing with chlorinated water	ND	ND	ND	<LOQ	0.007	<LOQ	<LOQ	0.006	0.019
GP – After treatment with wax + fungicide	0.833	1.316	ND	<LOQ	0.011	<LOQ	<LOQ	0.0056	<LOQ
GP – After drying	-	1.053	ND	-	0.007	ND	-	<LOQ	0.026
RP – After postharvest handling	0.476	1.322	ND	0.010	0.009	<LOQ	<LOQ	0.005	0.017
RP – Without postharvest handling	-	ND	ND	-	0.068	0.012	-	0.026	0.031

Samples: GP, green papaya; RP, ripe papaya. LOQ: limit of quantification. ND: not detected.

In papaya exported to Europe, azoxystrobin and difenoconazole were also detected in the three samplings (Table 6); however, they were at levels below the minimum limits permitted by law in European countries (Table 3). Similar to what occurred with papaya exported to the USA, fruit washing with chlorinated water reduced the azoxystrobin residue levels at about 78% (2nd sampling). From the first to the third sampling, the residue levels changed from 0.009 mg kg^{-1} and 0.007 mg kg^{-1} to levels below the limit of quantification (Table 6). As for difenoconazole residue, the values corresponded to reductions by 47% and 70%. Azoxystrobin is a fungicide of the strobilurin chemical group, while difenoconazole belongs to the triazole group. Although both are systemic fungicides, they are mostly deposited on the peels, as their largest amounts were detected in papaya samples of pulp with peel (Table 6).

Much of the azoxystrobin residue remained on the fruit peel, and, as a systemic fungicide, part of the residue was translocated to the pulp, thus hindering the total removal of this fungicide with washing. Krol et al. (2000) stated that some pesticides could be translocated to internal plant tissues, becoming inaccessible to washing with water. Oliva et al. (2017) associate it in some cases with the physicochemical properties of the pesticides and the vegetable surface characteristics.

The postharvest handling steps, especially washing with chlorinated water and heat treatment, showed to be effective in reducing the residue levels of azoxystrobin and difenoconazole found in the papaya samples. Ripe fruit analysis showed that fruit not subjected to postharvest processing had similar residue levels to those of green fruit sampled before postharvest handling. Such analysis also showed that ripe fruit subjected to postharvest handling had non-detectable or significantly reduced residue levels, in comparison with green fruit before postharvest handling (Tables 5 and 6).

Conclusions

1. The methodology using UHPLC-MS/MS and compound detection by the multiple reaction monitoring (MRM) mode is successfully adapted and validated to detect the pesticides azoxystrobin, carbendazim, carbofuran, difenoconazole, flutriafol, imidacloprid, imazalil, prochloraz, pyraclostrobin, tebuconazole, thiabendazole, and thiacloprid, in papaya (*Carica papaya*).
2. Washing step in the postharvest handling of papaya with chlorinated water reduces the azoxystrobin residue levels from 78% to 90%, and the difenoconazole residue levels from 47 to 90% on the fruit surface.
3. In papaya exported to the USA, the use of hot water for heat treatment significantly reduces the levels of azoxystrobin and difenoconazole.
4. Although the postharvest handling steps were ineffective in reducing the fungicide thiabendazole levels, if this fungicide is used at the appropriate dose, the residue levels will remain below the limits permitted by law in Brazil and in Europe.

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