ECLÉTICA Química
www.scielo.br/eq
Volume 31, número 2, 2006

Prochloraz residue levels in ginger submitted to Sportak 450 CE“ postharvest treatment

*L. Polese[1], E. F. G. Jardim[1], S. Navickiene[2], N. M. Brito[3], M. L. Ribeiro[1]**
[1]*Instituto de Química, UNESP, 14801-970. Araraquara, SP, Brazil*
[2]*Centro de Ciências Exatas e Tecnologia, UFS, 49100-000. Sergipe, SE, Brazil*
[3]*CEFET, 65025-001. São Luis, MA - Brazil*
**Corresponding author. E-mail: mlucia@iq.unesp.br (M. L. Ribeiro)*

Abstract: A preliminary study was conducted to determine the residue levels of prochloraz in ginger samples treated with Sportak 450 CE® (prochloraz as active ingredient) under laboratory conditions and cold-storage for 15 days at 10°C and 89% RH. Sampling was carried out at 10 and 15 days after Sportak 450 CE® dip treatment (450 and 900 μg mL^{-1}). Pesticide residues were determined by GC-ECD. During the study, residue levels in ginger ranged between 3.6 and 10.6 mg Kg^{-1} for prochloraz.

Keywords: ginger; prochloraz; fungicide; spices; dip treatment; GC-ECD.

Introduction

Ginger (*Zingiber officiale* Roscoe) is a plant used due to its medicinal properties as a stomachic, antiemetic, antidiarrheal and cardiotonic, for the treatment of several gastrointestinal and respiratory diseases. Ginger, propolis and honey are employed as active ingredients of syrups and sprays for treatment of throat diseases. Besides as a spice, ginger is used in beverages and cookery, specially in Asian cooking [1]. A substantial quantity of the Brazilian production of ginger is exported to different countries such as Canada, United Kingdom, United States and Netherlands. Once, fungal growth during cold storage is one of the main causes of deterioration in perishable food crops, which leads to very important economic losses [2]. So, the superficial and early infection may be eradicated with an effective fungicide treatment. In Brazil, studies dealing with the detection of pesticide residues in ginger have been lacking. Except for few works about residue analytical methods for determining organochloride pesticides in spices, including ginger, no method is described in the literature to analyze prochloraz in ginger matrix [3-5].

Prochloraz undergoes different transformations. In plants, the primary metabolic step is a breaking of the imidazole ring with the formation of *N*'-formyl-*N*-propyl-*N*-[2-(2,4,6-trichlorophenoxy)ethyl]urea (BTS 44596) and *N*-propyl-*N*-[(2-(2,4,6 trichlorophenoxy)ethyl)]urea (BTS 44595), which are then degraded to 2,4,6-trichlorophenol (2,4,6-TCP), present as free and conjugated metabolites, together with trace of 2-(2,4,6-trichlorophenoxy)-acetic acid [6, 7].

The objective of this work was to evaluate the residue levels of prochloraz in ginger samples submitted to Sportak 450 CE® postharvest treatment to verify the behaviour of prochloraz residues in ginger under cold storage.

Experimental

Chemicals and apparatus.

Dichloromethane, hexane, diethyl ether

and toluene (Mallinckrodt Baker Inc., Paris, KY, USA) were nanograde. Analytical grade pyridine hydrochloride was purchased from Sigma (St. Louis, MO, USA). Reference standards of prochloraz (98.4 % pure), 2,4,6-trichlorophenol (99.5 % pure), BTS 44596 (99.3 % pure) and BTS 44595 (98 % pure) were purchased from Dr. Ehrenstorfer (Augsburg, Germany). The individual stock solutions of the analytes were prepared by diluting 1.0 mg of the standards in 10.0 mL of toluene to obtain a concentration of 100 μg mL^{-1}. The working standard solutions were prepared by diluting the stock solutions as required.

The gas chromatographic analysis was carried out using a Varian 3300 gas chromatograph equipped with electron-capture detector (ECD), an on-column injector, and a connecting Varian 4290 reporting integrator. The megabore column was a DB-5 fused-silica column (30 m x 0.53 mm i.d., 0.83 μm; J&W Scientific, Folsom, CA, USA). The injector and detector were operated at 240 ºC and 300 ºC, respectively. The oven temperature was programmed as follows: 140 ºC for 1 min, increasing to 240 ºC (10 ºC min^{-1}) to 260 ºC (10 ºC min^{-1}) and holding for 10 min. Nitrogen (99.999 % pure) was the carrier (2 mL min^{-1}) and makeup (28 mL min^{-1}) gas.

Ginger postharvest treatment

The experiments were performed on mature ginger (*Zingiber officiale* Roscoe) under laboratory conditions. To carry out this study, ginger samples were placed in plastic boxes (20 gingers per box). The treatments were carried out in duplicate during 3 min, by dipping the samples in aqueous suspension. Each replicate was made up in two boxes. Prochloraz solutions were prepared as aqueous suspension, using the commercial formulation Sportak 450 CE® at the doses: 450 and 900 μg mL^{-1}. The gingers were left to dry at room temperature and stored for 15 days at 10 °C and 85-90 % relative humidity (RH). Samples were taken 10 and 15 days after Sportak 450 CE® application.

Preparation of laboratory sample and fortification.

300 g of each ginger sample was chopped with a stainless steel knife. The samples were triturated using a blender, homogeneized, and stored in closed glass flasks at temperature < –18ºC until analysis.

Fortified samples were prepared by adding 0.2, 1 or 2 mL of each standard solution to 10.0 g of untreated sample.

Analytical procedure.

An analytical sample of 10.0 g was weighed in a 80 mL screw-capped flask; 50 mL of dichloromethane was added and the flask was shaken in a mechanical shaker for 30 min (Thermolyne, Dubuque, Iowa, USA). The sample was allowed to settle and the supernatant was filtered in Whatman 40 filter. 5.0 mL of the organic layer was transferred to a round-bottom flask and concentrated to dryness using a rotary evaporator. A 1 g portion of dry pyridine hydrochloride was added and the flask was sealed with a stopper and heated to 220 °C for 90 min in the glycerin bath. The test tube was cooled and 10 mL of water was added. The aqueous solution was extracted two times with 5 mL of diethyl ether:n-hexane (1:4, v/v), and the organic phase was transferred into another test tube. 5 mL of 0.1 mol L^{-1} of KOH was added. The test tube was shaken for 1 min and the upper phase discarded; 5 mL of 1 M HCl were added to the aqueous phase and extracted two times with 5.0 mL of toluene. The final extract was reconstituted to an adequate volume (10.0 mL) with toluene.

Gas chromatographic analysis.

Suitable aliquots (1 μL) of ginger extracts and 2,4,6-trichlorophenol standard solutions were injected into a gas chromatograph. The percentages of recoveries were calculated by comparing the average chromatographic peak heights of the standard, fortified samples and unfortified samples. Quantification of prochloraz in ginger samples was performed using a five point linear calibration curve plotting peak heights versus concentrations. The amounts of 2,4,6-trichlorophenol obtained were corrected with the following factor to convert to the amounts of prochloraz [mol. wt. of prochloraz (376.7) / mol. wt. of 2,4,6-trichlorophenol (197.5) = 1.91].

Results and discussion

Method validation

The establishment of the experimental conditions was based on a method developed by de Paoli et al. [8] for the determination of prochloraz and its metabolites in vegetable, fruit and wheat samples that does not require steam distillation. The method involves extraction of the sample, hydrolysis with pyridine hydrochloride, extraction of the 2,4,6-TCP, derivatization with diazomethane and gas chromatographic analysis. In the present study, 2,4,6 TCP was analysed by GC-ECD without methylation with diazomethane.

The efficiency of the analytical procedure was evaluated by means of recovery analyses with ginger samples fortified at three different levels (0.2; 2 and 10 mg kg^{-1}). The mean recoveries of three replicates of fortified samples ranged from 75 % to 108 % with relative standard deviation (RSD) values of 1.6% to 12.7%, as shown in Table 1.

Table 1. Recovery of prochloraz, BTS 44595 and BTS 44596 from fortified ginger samples.

Compound	Spiked level (mg Kg^{-1})	Recovery (%)* Mean (%)	RSD (%)
prochloraz	0.2	96	1.6
	2.0	77	7.8
	10.0	108	1.8
BTS 44595	0.2	81	5.6
	2.0	86	7.1
	10.0	75	9.6
BTS 44596	0.2	104	8.5
	2.0	83	12.7
	10.0	101	11.3

*average of 3 replicates

Recoveries were calculated by the external standard method. Good linearity was achieved in the range from 0.4 to 5.0 μg mL^{-1}. The correlation coefficients obtained for the prochloraz, BTS 44595 and BTS 44596 were higher than 0.9991. The precision and accuracy were considered adequate for the validation of the method according to the validation criteria [9]. Standard solutions were injected after every ten samples to monitor changes in chromatographic conditions. The chromatograms of the ginger extracts were satisfactory, without any interference in the retention time of the fungicide. Under the chromatographic conditions described, the retention time of prochloraz as 2,4,6-trichlorophenol derivative was 4.8 min.

The criteria established by Thier and Zeumer [10] to find the detection limit (LOD) and the quantification limit (LOQ) were used in this study. The LOD and LOQ values for prochloraz and its metabolites were 0.1 and 0.2 mg Kg^{-1}, respectively.

Prochloraz degradation

The aim of this study was to verify the presence of prochloraz residues in ginger samples submitted to postharvest dip treatments with an aqueous suspension of Sportak 450 CE (doses used: 450 and 900 μg mL^{-1}). Samples were stored at 10 °C to simulate export conditions and analyzed in duplicate at two different times: 10 and 15 days. As shown in Figure 1, prochloraz residues were detected in all ginger samples submitted to the dip treatment.

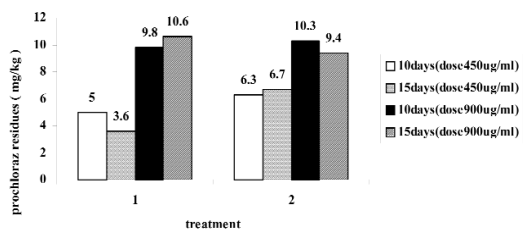

Figure 1. Ginger postharvest treatments with prochloraz.

In the first treatment, residues decreased from 5 to 3.6 mg Kg^{-1} (28 %) after 10 and 15 days, respectively at 450 μg mL^{-1} dose. No reduction in prochloraz level was obtained for the highest dose (900 μg mL^{-1}) between 10 and 15 days.

For the second treatment, the concentration values were c.a. 9% lower after 15 days (9.4 mg Kg^{-1}) than in 10 days (10.3 mg Kg^{-1}) at 900 μg mL^{-1} dose. Concentration values differences lower than 6 % were noted between 10 and 15 days at 450 μg mL^{-1} dose.

When both treatments were compared, no significant differences were obtained for the highest dose (900 μg mL^{-1}).

In this study, the period of 15 days was selected to simulate the time interval necessary to the shipment arrives in the import countries.

Conclusion

A decay study of prochloraz in ginger was carried out under laboratory conditions, in which it was observed that the remaining prochloraz residues quantified after 10 and 15 days ranged from 3.6 to 6.7 mg Kg^{-1} for 450 μg mL^{-1} dose with values between 9.4 and 10.6 mg Kg^{-1} for 900 μg mL^{-1} dose.

Acknowledgements

The authors acknowledge FAPESP and CAPES for the fellowships and Aventis Crop Science (Paulínia city, São Paulo State) for supplying Sportak 450 CE® formulation.

Recebido em: 30/03/2006
Aceito em: 08/06/2006

L. Polese, E. F. G. Jardim, S. Navickiene, N. M. Brito, M. L. Ribeiro. Resíduos de Prochloraz em gengibre submetido a tratamento pós-colheita com Sportak 450CE®.

Resumo: Um estudo preliminar foi realizado para determinar os níveis de concentração de procloraz em amostras de gengibre tratadas por imersão em solução aquosa com a formulação Sportak 450 CE (procloraz como ingrediente ativo) nas concentrações de 450 e 900 μg mL^{-1}, sob condições de laboratório e armazenadas por 15 dias em câmara fria a 10°C e com 89% de UR. Amostras de gengibre tratadas foram analisadas após 10 e 15 dias do armazenamento. Os resíduos de procloraz foram analisados por CG-DCE. Os níveis encontrados variaram entre 3,6 e 10,6 mg Kg^{-1} para procloraz.

Palavras-chave: gengibre; procloraz; fungicida; tratamento por imersão; CG-DCE.

References

[1] M. Thomson, K. K. Al-Qattan, S. M. Al-Sawan, M. A. Alnaqeeb, I. Khan, M. Prostaglandins, Leukotrienes Essent. Fatty Acids. 67 (2002) 475.
[2] F. Artés, M. C. Rodriguez, J. A. Martinez, J. G. Marin, Rev. Int. Froid. 18(1) (1995) 63.
[3] A. A. K. Abou-Arab, M. A. Abou-Donia, Food Chem. 72 (2001) 439.
[4] L. P. Srivastava, R. Budhwar, R. B. Raizada, Bull. Environ. Contam. Toxicol 67 (2001) 856.
[5] J. H. Sullivan, J. Agric. Food Chem. 28 (1980) 1031.
[6] T. R. Roberts, D. H. Hutson, Metabolic pathways of agrochemicals. Thomas Graham House, Cambridge, 1999.
[7] C. Tomlin, The pesticide manual. BCPC, Crop Protection Publications, Cambridge, 1995.
[8] M. De Paoli, M. T. Barbina, V. Damiano, D. Fabbro, R. Bruno, Journal of Chromatography A. 765 (1997) 127.
[9] INMETRO. Orientações sobre validação de métodos de ensaios químicos, DOQ-CGCRE-008, 2003.
[10] H. P. Thier, H. Zeumer, Manual of pesticide residue analysis. Deutsche Forschungsgemeinschagft, Pesticide Comm. Verlag Chemie, Weinheim, 1987.