Evaluation of Residual Levels of Benomyl, Methyl Parathion, Diuron, and Vamidothion in Pineapple Pulp and Bagasse (Smooth Cayenne)

H. A. P. Cabrera,[†] H. C. Menezes,*,[†] J. V. Oliveira,[‡] and R. F. S. Batista[‡]

Department of Food Technology/FEA/UNICAMP, CP 6121, 13083-970, Campinas, SP, Brazil and Institute of Food Technology (ITAL), CP 139, 13073-001, Campinas, SP, Brazil

The objective of this research was to study the residual levels of benomyl, methyl parathion, diuron, and vamidothion in pineapple bagasse and pulp. Benomyl (benlate), methyl parathion (Folidol 600), diuron (Krovar), and Vamidothion (Kilval 300) were applied pre-harvest to pineapples (smooth cayenne). After harvesting, the fruits were washed (100 ppm sodium hypochlorite) and the pulp was separated from the sub-products (peel, core, tops, and tails). The pulp was not submitted to any heat treatment. The sub-products and the juice expressed from them, were submitted to a blanching process (95 °C for 1 min). After separating the juice, the bagasse and pulp were analyzed for residues of diuron and benomyl by high performance liquid chromatography, and for residues of vamidothion and methyl parathion by gas chromatography using a TSD detector. No residues of benomyl, diuron, vamidothion, or methyl parathion were detected in the pulp within the quantification limits of the methods (0.1 mg/kg, 0.1 mg/kg, 0.005 mg/kg, and 0.005 mg/kg, respectively). Only methyl parathion (0.052 mg/kg) and vamidothion (0.021 mg/kg) were detected in the bagasse. The presence of these residues in the bagasse was probably due to the action of the wax found in the peel, which prevented the methyl parathion and vamidothion from dissolving in the juice. According to these results, the pulp was fit for human consumption, as far as pesticide residues were concerned, and the bagasse was fit for animal feed and similar applications, because the residual levels found were below the limits established for these compounds.

Keywords: *Pineapple; by-products; pesticides; residues; processing*

INTRODUCTION

The pineapple is one of the most highly appreciated fruits in the world because of its delicious flavor, and also because it contains proteolytic enzymes which aid digestion (Bartolome et al., 1995; Tisseau, 1986). This fruit is cultivated in Brazil, which produces the most pineapples in the americas, in a total area of 44 318 hectares, resulting in an annual production of 314 229 tons. The State of Minas Gerais produces 311 079 tons of pineapple per year from 15 622 hectares, corresponding to 34 and 35.2%, respectively, of the total production area in Brazil and the total production in quantity (IBGE, 1996).

Pineapple juice is the third most preferred juice worldwide, with an estimated annual consumption of 200 000 tons. According to Savitici et al., 1995, only orange and apple juices are consumed in greater amounts than pineapple juice. The juice is normally obtained from the pulp, and the sub-products are discarded. In Australia, for example, 400 000 tons of these sub-products are produced per year (Tran & Mitchell, 1995). A total of 100 000 tons of pineapple is produced annually in the Ivory Coast, of which from 40 to 80% are sub-products (Ban-Koffi & Han, 1990). In Brazil, after processing pineapple for the production of fresh juice (13° Brix) and concentrated juice (60° Brix), 45.2% of

the fruit is discarded as sub-products. However, since 1989, Brazil has adopted the custom of processing the sub-products of pineapple to obtain juice, which is then exported (Savitci et al., 1995). These sub-products, obtained from the industrial processing, represent from 25 to 30% of the weight of the fruit; the principal component is the skin (Larauri et al., 1997). One of the undesirable aspects of discarding pineapple sub-products is the problem of environmental pollution from the high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) (almost 10 times that of an equivalent weight of domestic sewage) required to decompose the high sugar content (Askar & Treptow, 1997; Burbank & Kamagai, 1965; Noporatnaraporn et al., 1986). On the other hand, the use of pineapple sub-products results in a positive economic impact, as well as representing an important technological area, as it is a rich raw material which can be used to obtain viable economic products such as citric acid (Tran & Mitchell, 1995), alcohol (Ban-Koffi & Han, 1990), proteolytic enzymes (Ievleva et al., 1991), microorganisms (Noporatnaraporn et al., 1986), wine (Alian, 1976), vinegar (Satayavati, 1972), bromelin (Tisseau, 1986), biogas (Bordija, 1996), and animal feed (Tisseau, 1986; Larrauri et al., 1997).

The pineapple, when cultivated in the open, is subject to the attack of pests such as *Thecla basilides* and *Dysmicocus brevipes,* weeds such as *Imperata sp., Amaranthus hybridus* L., and *Cyperus rotundus,* and moulds such as *Fusarium monoliforme,* which is responsible for losses of pineapple of up to 30% in Brazil and which

* To whom correspondence should be addressed.
† Department of Food Technology/FEA/UNICAMP.
‡ Institute of Food Technology (ITAL).

Pesticide Residues in Pineapple Pulp and Bagasse

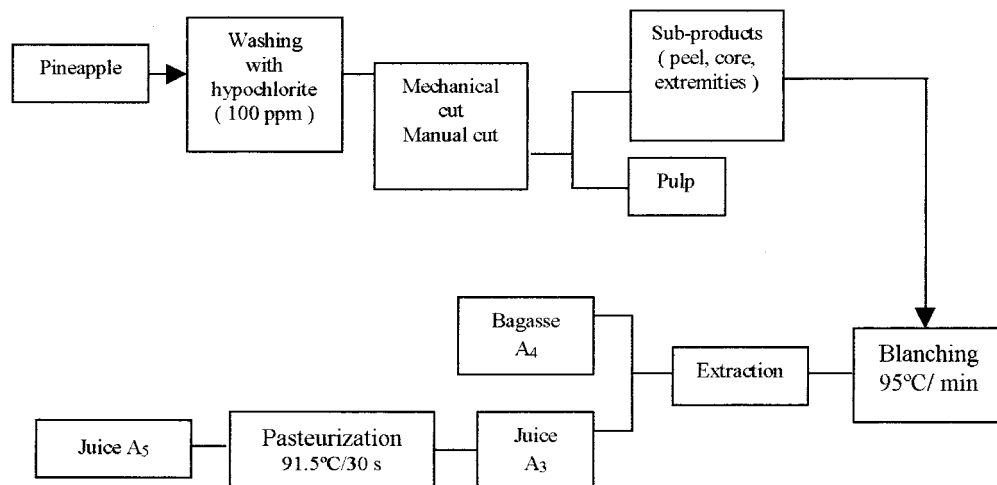*J. Agric. Food Chem.,* Vol. 48, No. 11, 2000 **5751**

Figure 1. Flow diagram of processing to obtain the bagasse.

only manifests itself during fruit development (Medina, 1987). To neutralize these adversaries and guarantee good quality fruit, the application of pesticides to pineapple palms is necessary, according to good agricultural practices. As a function of this cultural aspect, the sub-products are susceptible to the presence of residues of the pesticides used in pest control.

The pesticides used in pineapple plantations to combat the attack of insects, weeds, and moulds are methyl parathion, diuron, benomyl, and vamidothion.

Methyl parathion is a contact organophosphorated compound applied during fruit development. Diuron is a systemic selective herbicide, toxicological class III, derived from urea and applied to various cultures. In the case of pineapple, it is applied before flowering. Benomyl is a systemic fungicide, belonging to the benzimidazole group, applied to the soil to control a variety of fruit diseases. Benomyl rapidly degrades to carbendazim, its main degradation product, and this is also a systemic fungicide, used to combat a wide range of diseases. It is believed that the fungicidal activity of benomyl is due to the presence of carbendazim (Clemens & Sisler, 1969; Guan et al., 1994; Itak et al., 1993; Liu et al., 1990). Vamidothion is an insecticide discovered by the Rhône Poulenc laboratories in 1959. It is employed in various cultures to combat a variety of different types of insect. After application, vamidothion is slowly oxidized to vamidothion sulfoxide and vamidothion sulfone (Tsumura et al.., 1993; Dubosq & Dedde, 1972).

As a function of the aspects presented above, the objective of this research was to determine residual levels of methyl parathion, diuron, vamidothion, and benomyl in pineapple pulp and bagasse.

EXPERIMENTAL PROCEDURES

Field Trials. Hawaiian variety pineapples (*Smooth cayenne)* were planted in December 1996 and collected in March 1998 from the Nova Esperança farm in Canápolis, State of Minas Gerais, Brazil. The fruits were cultivated in an area of 15 hectares (31,250 pineapples/hectare), with a 1.0 m space between the double lines, 0.60 m between the individual lines of the double line, and 0.40 m between each fruit in the same line. Diuron (Krovar) was applied twice at a rate of 3 kg/ha. The first application was to the whole area with a tractor equipped with a nonadapted bar and the second was before floral induction with a fan-type-nozzle directed spray. Methyl parathion (Folidol 600) was applied three times (0.15%) after

planting to control *Dysmicoccus brevipes* and four times after flowering to control *Thecla basilides.* These sprayings were carried out at 15-day intervals with a tractorized bar sprayer with conical nozzles to allow for manual application to each fruit. Benomyl (Benlate) was applied 2 times (0.15%) to the soil during frutification, and was formulated with 0.05% of an adhesive spreader. After frutification these pulverizations were also carried out at 15-day intervals with a tractorized bar sprayer with conical nozzles to allow for manual application 2 times to each fruit. Vamidothion (Kilval 300) was applied 3 times (0.25%), using the tractorized bar sprayer with the conical nozzles to apply manually to each fruit, during the vegetative cycle and was aiming at controlling *Dysmicocus brevipes* and acarids. The first application was at least 60 days after planting. During the dry period (May to September) the pineapple plants were irrigated every 10 days with an 84-mm blade. The samples were collected 60 days after the last application of methyl parathion, vamidothion, and benomyl. Diuron was applied to the soil twice at intervals of 90 days and the pineapples were harvested 200 days after the last application.

Harvesting. Pineapple fruits were harvested at random. Fifty were collected from each of the three areas reserved for sampling. The fruits were immediately transported to the pilot plant for processing.

Fruit Processing Conditions. The processing of the pineapples to obtain the bagasse is shown in Figure 1. Each of the three samples (of 50 pineapples each) was processed separately, characterizing processing in triplicate. First the fruits were washed for 2 min in a 100-ppm solution of sodium hypochlorite, using brushes to remove the dirt. The pulp was then separated from the sub-products. The sub-products were submitted to steam blanching at 95 °C for 1 min. The bagasse was then separated from the juice and the bagasse stored in glass jars in the freezer (−20 °C) until analyzed.

Chemicals. Methyl parathion (98%) was acquired from Bayer. Vamidothion sulfone (99%) and Diuron (97%) were donated by Rhône Poulenc and Benomyl (98.7%) was donated by Du Pont. Stock solutions of methyl parathion and vamidothion sulfone at 100 ppm were maintained in acetone, and 100 ppm Diuron was maintained in methanol. The stock solution of benomyl in acetone at 100 ppm was allowed to stand at room-temperature overnight to ensure complete conversion to carbendazin. A conversion factor of 1.51 was used to convert carbendazin concentration into benomyl (Gilvydis & Walters, 1990).

Extraction procedures. Methyl parathion and vamidothion sulfone were extracted using a modification of Luke's method (Luke et al., 1975). Samples (10 g) were extracted with 150 mL of acetone in a 250-mL conical flask and shaken for 1 h on a horizontal shaker. The extraction mixture was vacuum filtered through Whatman *no.* 1, 125-mm filter paper in a

5752 *J. Agric. Food Chem.,* Vol. 48, No. 11, 2000

Cabrera, et al.

Büchner funnel connected to a kitasato flask, and the flask and funnel were rinsed with acetone. The filtrate was transferred to a 200-mL volumetric flask and completed to volume. A 20-mL aliquot was transferred to a 500-mL separating funnel, and 5 mL of 0.02% potassium permanganate (to degrade the vamidothion and vamidothion sulfoxide to vamidothion sulfone (Desmoras et al., 1973)) and 20 mL of phosphate buffer (0.1367 g $K_2HPO_4 \cdot 3H_2O$ plus 0.9886 g Na_2-$HPO_4 \cdot H_2O$ plus 100 mL of deionized water) were added. The mixture was extracted 3 times with 80 mL of dichloromethane and shaken vigorously, and the aqueous phase was discarded. The three lower dichloromethane phases were pooled and filtered through anhydrous sodium sulfate (previously dried in a muffler at 400 °C for 8 h and subsequently stored in a desiccator). The filtrate was collected in a 250-mL flask and evaporated in a rotary evaporator at 40 °C. The residue was dissolved in 10 mL of acetone in a concentration tube and evaporated to dryness using compressed air. This residue was completed to 1 mL with acetone and used for injection into the gas chromatograph. The results obtained for vamidothion sulfone were converted into vamidothion by multiplying by a conversion factor of 0.90.

Benomyl and Diuron were analyzed by a modification of the method of Bardalaye and Wheeler, 1985. Since benomyl degrades to carbendazin, the latter was quantified and the value converted into benomyl by multiplying by the conversion factor of 1.52 (White & Kilgore, 1972). A 5-g sample was weighed into a 250-mL conical flask with 100 mL of a mixture of methanol/buffer (60:40) and extracted for 30 min in a horizontal shaker. The extraction mixture was filtered through Whatman n*o*. 41 125-mm paper, and the funnel and flask were washed with the methanol/buffer (60:40) mixture. The filtrate was transferred to a 500-mL separating funnel and partitioned twice with 50 mL of chloroform. The aqueous phases were discarded and the chloroform phases were pooled and washed twice with water, again discarding the water phases. The chloroform phase was filtered through anhydrous sodium sulfate and collected in a 500-mL flat-bottomed flask. After evaporation to dryness on a rotary evaporator (40 °C), the residue was dissolved in 5 mL of methanol. The methanolic extract was filtered through a 0.25μ membrane and injected onto the HPLC.

Recovery Assay. Untreated samples were fortified with 0.1 mg/kg, 0.5 mg/kg, and 1.0 mg/kg of methyl parathion, vamidothion sulfone, diuron, and benomyl, and were processed according to the procedure described above. Recovery from three replicates gave values ranging from 80 to 120% with a maximum coefficient of variation (CV) of 16%.

Chromatography Apparatus. A Varian gas chromatograph, model 3.400, equipped with a thermoionic specific detector (TSD) was used for the analysis of methyl parathion and vamidothion. The following chromatographic conditions were used: mobile phase (N_2 = 10 psi), air (175 mL/min), H_2 (5 mL/min), bead probe (current 3.100), DB-608 megabore column, column temperature (245 °C), injector temperature (250 °C) and detector temperature (300 °C), injection volume (3.0 μL). The paper speed on the plotter was 30 cm/min, with a sensibility of 1 mV.

A Varian HPLC apparatus, model 9002 equipped with a Varian UV/Visible detector, model 9050, was used in the analysis of diuron and benomyl. Readings were taken at 254 nm for diuron and at 286 nm for benomyl. The mobile phase for diuron was a (60:40) mixture of methanol/phosphate buffer (0.1367 g $K_2HPO_4 \cdot 3H_2O$ plus 0.9886 g $Na_2HPO_4 \cdot H_2O$ plus 100 mL of deionized water), and for benomyl it was a (60:40) mixture of methanol/ammonium hydroxide (0.1%). A 15-cm (length) x 4-mm (i.d.) 5μm RP-18 column was used, with a 20 μL loop. RCDR/AUFS was 0.01 and the flow rate was 1.0 mL/min for both pesticides.

Calibration graphs were constructed for the pesticides using the external standard method, measuring peak area versus concentration. Good linearity was achieved in the range of 0.1–1.0 mg/kg for diuron, 0.1–1.0 mg/kg for carbendazim, 0.01–0.2 for methyl parathion, and 0.05–1.0 mg/kg for vamidothion

Table 1. Pesticide Residue Levels (mg/kg) in Pineapple Bagasse and Pulp

	sample type	
pesticide	pulp	bagasse
methyl parathion	nd[a,c]	0.052 ± 0.004[d]
diuron	nd[b]	nd[b]
vamidothion	nd[c]	0.021 ± 0.003[d]
carbendazim	nd[b]	nd[b]

[a] Not detected (nd), below the quantification limit of the method. [b]0.1 mg/kg. [c]0.005mg/kg. [d]Standard deviation (mean of three determinations).

sulfone, with correlation coefficients of 0.9915, 0.9930, 0.9996, and 0.9983, respectively.

RESULTS AND DISCUSSION

Table 1 shows the results of the analyses for pesticide residues in the pineapple pulp and bagasse. In Brazil the residue tolerance limits for diuron, methyl parathion, benomyl (as carbendazin), and vamidothion in whole pineapple fruit are 0.1 mg/kg, 0.2 mg/kg, 2.0 mg/kg, and 0.1 mg/kg, respectively. There is no register in CODEX for diuron in pineapple but for methyl parathion, benomyl (as carbendazin), and vamidothion the limits are 0.2 mg/kg, 20 mg/kg, and 1.0 mg/kg, respectively. None of the pesticides were found in the pineapple pulp in concentrations exceeding the detection limits of the methods used. The nondetection of pesticide residues in the pulp can be attributed to the pineapple peel, which acted as a protective shield, preventing the diffusion of benomyl, methyl parathion, diuron, and vamidothion. This result is in agreement with the results of Scalon et al., 1996, who detected no traces of the pesticide benomyl in mango pulp. It is important to emphasize that the degree of migration of residues from the peel to the pulp depends on the substrate and the chemical nature of the pesticide (Awasti, 1993).

Residues of diuron and benomyl were not detected in the samples of bagasse within the detection limits of the methods used (0.1 mg/kg). No data were found in the literature about residues of diuron or benomyl in pineapple. Because these pesticides were applied in the field, both probably suffered degradation to levels inferior to the detection limits due to climatic factors, irrigation, heat, or sunlight (Sheets, 1964).

On the other hand, Schnell et al., 1997, detected residues of benomyl at levels of 0.08 mg/kg and 0.05 mg/kg in sub-products of pears and apples, respectively, that had been cultivated in open fields. However, in those analyses, a fluorescence detector was used for the quantification of benomyl, which is more sensitive than the UV–Vis detector used in this analysis. Detection of the pesticides methyl parathion and vamidothion in the bagasse can be attributed to the presence of epicuticular wax in pineapple peel, which contains substances in which these pesticides are soluble (Melnikov, 1971). According to Riederer & Schreiber, 1995, after treatment with these pesticides, the molecules penetrate into the epicuticular wax of the peel by diffusion, impeding their transport into the pulp.

CONCLUSIONS

According to the results presented in this paper, it was concluded that both the pulp and the peel are fit

Pesticide Residues in Pineapple Pulp and Bagasse

J. Agric. Food Chem., Vol. 48, No. 11, 2000 **5753**

for human consumption and use in animal feed, according to the brazilian legislation and CODEX for pesticide residues.

LITERATURE CITED

Alian, A.; Museng, H. M. Utilization of pineapple waste for wine making. *J. Sci. Technol. (Peshawar, Pak.)* **1976**, *1* (1), 29–33.

Askar, A.; Treptow, H. Tropical Fruit Processing Waste Managment - Part II: Waste treatment and Disposal. *Fruit Process.* **1997**, *10*, 405–410.

Awasti, M. D. Decontamination of Inseticide residues in Mango by Washing and Peeling. *J. Food Sci. Technol.* **1993**, *30* (2), 132–133.

Ban-Koffi, L.; Han, Y. W. Alcohol production from pineapple waste. *World J. Microbiol. Biotechnol.* **1990**, *6*, 281–284.

Bardalaye, P. C.; Wheeler, W. B. Simplified method for the cleanup and reversed-phase high performance liquid chromatographic determination of benomyl in mangoes. *J. Chromatogr. Lib.* **1985**, *330*, 403–407.

Bartolome, A. P.; Ruperez, P.; Fúster, C. Pineapple fruit: morphological characteristics, chemical composition and sensory analysis of Red Spanish and Smooth Cayenne cultivars. *Food Chem.* **1995**, *53*, 75–79.

Bordija, N.; Samayaji, O.; Khanna, S. Biomethanation of banana peel and pineapple waste. *Bioresour. Technol.* **1996**, *58* (1), 73–76.

Brasil Ministério da Agricultura. *Legislação Brasileira de Resíduos de Pesticidas*, 1994.

Burbank, N. C.; Kamagai, J. S. Study of pineapple cannery waste. In *Proceedings of the 20th Industrial Waste Conference, Engineering Extension Series 118*; Purdue University: Lafayette, IN, 1966, Vol. 20, pp 365–397.

Clemens, G. P.; Sisler, H. D. Formation of a fungitoxic derivative from benlate. *Phytopathology,* 1969, *59* (5), 705–706.

Desmoras, J.; Laurent, M.; Buys, M. Vamidothion: Thin-layer and liquid chromatography and analyses of pesticides of international importance. In *Analytical Methods for Pesticides and Plant Growth Regulators*. Sherma, J. and Zweig, G., Eds.; Academic Press: New York, 1973, pp 479–494.

Dubosq, F.; Dedde, M. Dosage du vamidothion technique ou formulé par chromatographie en phase gazeuse. *J. Chromatogr.* **1972**, *71*, 557–561.

FAO/WHO. Codex Alimentarius Commission. Codex Committee on Pesticide Residues. Twenty-Sixth Session. FAO/WHO: The Hauge, 1994, pp 11–18.

Gilvydis, D. M.; Walters, S. M. Ion-pairing liquid chromatographic determination of benzimidazole fungicides in food. *J. Assoc. Off. Anal. Chem.* **1990**, *73* (5), 753–761.

Guan, X.; Davis, M. R.; Jin, L.; Baillie, T. A. Identification of S− (*n*-Butylcarbamoyl) glutathione, a reactive carbamoylating agent, as boliary metabolite of benomyl in the rat. *J. Agric. Food Chem.* **1994**, *42*, 2953–2957.

Instituto Brasiliero de Geografia e Estatistica (IBGE). *Anuário Estatístico do Brasil*, 1996, **p**p. 3–51.

Ievleva, E. V.; Zimacheva, A. V.; Huan, P. H.; Nhan, V. H.; Mosolov, V. V. Proteinases from proliferous tops of pineapple fruits. *Appl. Biochem. Microbiol.* **1994**, *30* (2), 172–179.

Itak. J. A.; Selisker, M. Y.; Jourdan, S. W.; Fleeker, J. R.; Herzog, D. P. Determination of benomyl (as carbedazim) in water, soil, and fruit juice by magnetic particle-based immunoassay. *J. Agric. Food Chem.* **1993**, *41* (12), 2329–2332.

Larrauri, J. A.; Ruperez, P.; Calixto, F. S. Pineapple Shell as a Source Dietary Fiber with Associated Polyphenols. *J. Agric. Food Chem.* **1997**, *45* (10), 4028–4031.

Liu, C. H.; Mattern, G. C.; Yu, X.; Rosen, J. D. Determination of benomyl by high performance liquid chromatography, mass spectrometry, selected ion monitoring. *J. Agric. Food Chem.* **1990**, *38* (1), 167–171.

Luke, M. A.; Froberg, J. E.; Masumoto, H. T. Extraction and clean up of orgachloorine, organophosphate, organonitrogen and hydrocarbon pesticides in produce for determination by gas–liquid chromatography. *J.—Assoc. Off. Anal. Chem.* **1975**, *58* (5), 1020–1026.

Medina, J. C. Abacaxi: Cultura, matéria prima e aspectos econômicos. In *Séries frutas tropicais 2* Ital 2nd ed; Imprensa Oficial do Estado S/A: Sao Paulo, Brazil, 1987; pp 57–70.

Melnikov, N. N.; Gunther, F. A.; Gunther, J. D. Chemistry of Pesticides. Vol. 36. Springer-Verlag: New York, 1971.

Noparatnaraporn, N.; Wonkkornchawalit, W.; Kantachote, D.; Nagai, S. C. P. M. Production of *Rhodopseudomonas sphaeroides* on pineapple wastes *J. Ferment. Technol.* **1986**, *64* (2), 137–143.

Riederer, M.; Schreiber, L. Waxes. The transport barriers of plant cuticles. In *Waxes: Chemistry, Molecular Biology and Functions;* Hamilton, R. J., Ed.; The Oil Press: Dundee, Scotland, 1995; Chapter 3, p 131.

Satyavati, V. K.; Bhat, A. V.; Varkey, A. G.; Mookerj, K. K. Preparation of vinegar from pineapple waste. *Indian Food Packer* **1972**, *26* (3), 50–53.

Savitci, L. A.; Gasparino Filho, J.; Moretti, V. A Perfil Industrial e de Mercado para Suco de Abacaxi *Coletânea do Instituto de Tecnologia de Alimentos.* **1995**, *25* (2), 153–168.

Scalon, S. P. Q.; Oliveira, J. J. V.; Lima, L. C. O. Resíduos de benomil em polpas de manga *haden* armazenadas em atmosfera modificada. Pesticidas: *R. Ecotoxicologia e Meio Ambiente;* Universidade Federal do Pararã; Curitiba, Brazil, 1996, vol. 6, pp 43–54.

Schnell, T. D.; Sofos, J. N.; Morgan, J. B.; Aaronson, M. J.; Tatum, J. D.; Smith, G. C. Pesticide residues in beef tissues from cattle fed fruits, vegetables and their byproducts. *J. Muscle Foods* **1997**, *8*, 173–183.

Sheets, Tj. Review of disappearance of Substituted Urea Herbicides from Soil. *J. Agric. Food Chem.* **1964**, *12* (1), 30–33.

Tisseau, R. Utilization of pineapple cannning waste. Potential extraction of bromelin. *Fruits* **1986**, *41* (2), 703–708.

Tran, Ch. T.; Mitchell, D. A. Pineapple Waste - A novel substrate for citric acid production by solid-state fermentation. *Biotechnol. Lett.* **1995**, *17* (10), 1107–1110.

Tsumura, Y.; Matsuki, H.; Tonogai, Y.; Nakamura, Y.; Kato, S.; Ito, Y. Simultaneous determination of vamidothion and its oxidation metabolites in potatoes and apples by gas chromatography. *J. Food Prot.* **1993**, *56* (5), 433–440.

White, E. R.; Kilgore, W. W. Determination of systemic MBC residues in food crops treated with benomyl fungicide. *J. Agric. Food Chem.* **1972**, *20* (6), 1230–1232.