Pesticide Residues in Raisin Processing

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The fate of the residues of benalaxyl, dimethoate, iprodione, metalaxyl, phosalone, procymidone, and vinclozolin in sunlight and oven raisin processing was studied. The drying process caused a fruit concentration factor of 4, while the decreases in residue with the two drying processes were different for the different pesticides. In sunlight-drying the residue level in the raisins was identical to that in the fresh fruits for benalaxyl, metalaxyl, and phosalone, whereas it was higher for iprodione (1.6) and lower for vinclozolin and dimethoate (one-third and one-fifth, respectively). The oven-drying process was preceded by washing, which caused residue decreases for iprodione and procymidone of 57 and 41%, respectively, whereas no decrease was observed in all of the other pesticides. During oven-drying pesticide residues in raisins with respect to fresh fruits showed an increase of 2.7 for phosalone, the same values for benalaxyl, metalaxyl, and procymidone, and lower values for vinclozolin and dimethoate. Sunlight-drying was more effective for phosalone and vinclozolin, whereas oven-drying was more effective for iprodione and procymidone, which was due to the washing effect rather than to dehydration. The experiments carried out with a model system showed that the decrease in dimethoate is attributable to heat, whereas in benalaxyl, procymidone, and phosalone it is due to codistillation and in iprodione and metalaxyl to the combined action of heat and codistillation.

Keywords: *Raisin; drying processes; pesticides; residues*

Grapes are one of the most important fruit plants in the Mediterranean area. Many cultivars give grapes with different basic characteristics. Some are suitable to produce wine, whereas others are eaten directly. Among these are some cultivars that produce apyretic grapes that are used in the production of raisins. Two methods are used to produce raisins: the natural process (exposure to sunlight) and the industrial process (oven-drying) (Striegler et al., 1996). The drying process produces a concentration factor of 4 in the fruits. Therefore, theoretically the pesticide residues in the grapes at harvest time could increase by the same factor. Some recent works have pointed out that the drying process could cause an important decrease in pesticide residues in fresh fruits (Cabras et al., 1997, 1998). This result was obtained in apricots and plums, whereas no data on grapes have been found in the international literature. Pesticides on fruits are submitted to different actions during the drying process. In sunlight-drying they are subjected to both heat and light. The effect of the latter is very important in pesticide decrease. During oven-drying the effect of light is totally absent, whereas the temperature is even higher than with sunlight-drying. The aim of this paper is to give a contribution to the knowledge of the drying process by comparing the effect of the two drying processes on pesticide residues in the production of raisins.

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EXPERIMENTAL PROCEDURES

Field Trials. The trial was carried out in a grape vineyard owned by CRAS, located at Uta, (Ca, Italy). The vineyard was planted in 1992 with a tree spacing of 3 × 2 m; the cultivar was Imperatrice. A random-block scheme was used with four replications for each test, and each block contained 12 plants. Treatments were carried out with an AM-150 portable motor sprayer (Oleo-Mac, Reggio Emilia, Italy). Three experiments were carried out: the commercial formulations were Galben R 4.33 [active ingredient (AI) 4% of benalaxyl], Rovral (AI 50% of iprodione), and Tetrafos 200 (AI 19% of parathion), at doses of 120, 450, and 288 g/ha AI, respectively, in the first experiment; Sumisclex (50% procymidone), Ridomil R (3.5% metalaxyl), and Aragol L40 (38% dimethoate) at doses of 450, 84, and 342 g/ha AI, respectively, in the second experiment; and Ronilan (50% vinclozolin), Topas 10 EC (10.2% penconazole), and Zolone L34 (33.6% phosalone) at doses of 600, 18.4, and 403 g/ha AI, respectively, in the third experiment. Two treatments were carried out on August 12 and September 2, 1997, with 600 L of suspension/ha by spraying the plants until completely wet. Samplings (on dry plants) started about 1 h after treatment; random 5-kg samples of grapes were collected from each plot and immediately analyzed for fungicide residues. The samplings and analyses were repeated weekly. The environmental conditions [maximum and minimum temperature (°C), relative humidity, and rainfall] were continuously monitored with an AD-2 automatic weather station (Silimet, Modena, Italy). During the experiments the maximum and minimum average temperatures were, respectively, 32.8° and 18.5 °C; no rain was observed during the experiments.

Sample Preparation. Each sample was made up of 5 kg of grapes in bunches. The samples were collected at harvest time on September 24. To determine the average weight, 200 grapes were weighed. The sample was divided into three groups: one was immediately analyzed for pesticide residues,

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while the other two were dried prior to residue analysis. Each sample was chopped and homogenized before extraction.

Fruit Drying Conditions. After the molded parts were removed, the samples were subjected to two drying processes: the sunlight process and the oven process. Except for periodic rotation, the samples exposed to sunlight did not receive any pretreatment. During the sunlight experiments the maximum and minimum average temperatures were, respectively, 28.5° and 19.1 °C. Oven-drying consisted of three steps: immersion in water at 99 °C for 5−10 s, immediate curbing with cold water, and drying in a ventilated oven at 70 °C for 24 h (Striegler et al., 1996). In both procedures the dried samples were weighed before and after drying to determine the concentration factor.

Model System. To extract epicuticular waxes, untreated grapes were extracted with chloroform according to the procedure of McDonald et al. (1993). The total amount of waxes was calculated by evaporation of 10 mL of chloroformic extract (165 $\mu g/cm^2$). The chloroformic solution was fortified with pesticides in a concentration similar to that found on the grapes at harvest time. Part of the solution was placed on a regenerated cellulose membrane (2.5 cm in diameter with 0.45 μm pores) (Sartorius, Göttingen, Germany). After evaporation of the solvent, the membrane showed the same amount of waxes and pesticides found in the fruits.

Test A. A filter was put in a vial with a screw cap with a center hole. The vial contained a sugar solution (20%) at pH 3. The water during evaporation passed through the filter. This experiment allows evaluation of the codistillation effect.

Test B. A filter was placed in a vial with a screw-closed cap. During the drying process any pesticide that might have evaporated should deposit on the vial surface after freezing at −20 °C. This test allows evaluation of the evaporation effect; the loss of pesticide on the filter would point out the degradation effect.

The two vials were placed in the oven at 70 °C for 24 h, while the control filter was kept in the dark at room temperature. At the end of the experiment the screw-closed vials were placed in a freezer at −20 °C to condense the evaporated pesticide on the glass surface. Each experiment was done in quadruplicate.

Chemicals. Benalaxyl**,** dimethoate, iprodione, metalaxyl, parathion, penconazole, phosalone, procymidone, and vinclozolin were analytical standards obtained from Dr. Ehrenstorfer (Augsburg, Germany); triphenyl phosphate (99%) was used as the internal standard (i.s.) and was of analytical grade (Janssen, Geel, Belgium). Standard stock solutions (∼500 mg/L) were prepared in methanol. Working standard solutions containing the i.s. at 0.1 mg/kg were obtained by dilution with hexane. Hexane, chloroform, dichloromethane, and methanol were of HPLC grade (Carlo Erba, Milan, Italy).

Extraction Procedure. A 10 g aliquot of sample was weighed in a 30 mL screw-capped tube; 10 mL of hexane with the i.s. was added and the tube agitated for 30 min in a rotary shaker. The phases were allowed to separate, and the organic layer was injected for GC analysis. For experiment B a 10 g aliquot of sample was extracted with CH_2Cl_2; 1 mL of the extract was then evaporated under a gentle nitrogen stream and redissolved with 1 mL of hexane with the i.s. at 0.1 mg/kg.

Apparatus and Chromatography. An HRGC Mega 2 (Carlo Erba, Milano, Italy) gas chromatograph equipped with a split−splitless injector and an AS 800 autosampler (Carlo Erba), connected to an HP 3396-A reporting integrator (Hewlett-Packard, Avondale, PA), was used. The capillary column was a CP Sil 8 CB (5% phenyl methylsilicone, 25 m × 0.25 mm, film 0.12 μm, Chrompack, Middelburg, The Netherlands). The injector and detector were operated at 240 and 300 °C, respectively. The sample (2 μL) was injected in the splitless mode (30 s), and the oven temperature was programmed as follows: 110 °C for 1 min, raised to 250 °C (10 °C/min), raised to 280 °C (20 °C/min). Helium was the carrier gas at 120 kPa. The detector was a nitrogen−phosphorus detector (NPD-80), and the gases were H_2 at 60 kPa, N_2 at 80 kPa, and Ar at 130 kPa; the current was 2.75 A and the voltage 3.5 V. Calibration graphs for the AI were constructed with the i.s. method by measuring peak heights versus concentrations. A good linearity was achieved in the 0.01−2.00 mg/kg range, with correlation coefficients between 0.9987 and 0.9993.

Recovery Assay. Untreated samples were fortified with 0.05, 0.5, and 2.0 mg/kg of pesticides and processed according to the above procedure. The average recovery from four replicates showed values ranging from 91 to 108% with a maximum coefficient of variation (CV) of 9%.

RESULTS AND DISCUSSION

The average weight of the fresh grapes was 1.93 ± 0.15 g, whereas the average weights of sun- and oven-dried fruits were 0.48 ± 0.04 and 0.46 ± 0.05 g, respectively. During the drying process the fruits were concentrated 4 times. Therefore, the amount of pesticide residues in grapes at harvest time should increase by a similar rate not considering the dehydration effect.

Treatments were carried out with nine AI, but at harvest time the residue levels of parathion-methyl and penconazole could not be determined (<0.01 mg/kg).

Benalaxyl. The presence of benalaxyl in grapes after sun-drying was not different from that in the fresh fruit. Therefore, because in the drying process the fruit is subjected to a concentration factor of 4, this means that the residue is decreased by the same factor. A similar behavior can be noticed in the oven-drying process; preliminary washing did not affect the residue levels.

Dimethoate. The residue of this insecticide decreased from 1.02 mg/kg in fresh fruit to 0.19 mg/kg in grapes dried by sunlight. Therefore, considering the concentration factor, the drying process caused a residue loss of a factor of 20. During the oven-drying process, preliminary washing did not cause any losses in the pesticide level, whereas subsequent dehydration at 70 °C caused a decrease similar to that obtained by sunlight.

Iprodione. The residue level in sun-dried grapes (2.79 mg/ kg) showed a value 1.6 times higher than that in the fresh fruit (1.74 mg/kg), but it was smaller than the concentration effect. This means that during the drying process the residue decreased by 2.5 times. Washing before the oven-drying process caused a residue decrease of 2.3 times. In the subsequent drying process the residue level was unchanged, suggesting a decrease identical to the concentration factor (4 times).

Metalaxyl. This AI belongs to the same chemical family as benalaxyl, the acylalanine, and presents the same behavior.

Phosalone. During oven-drying washing did not affect the residue level, whereas subsequent dehydration caused a residue increase of 1.7 times. Considering the concentration effect, the drying process caused a residue decrease of 2.5 times. A higher decrease was obtained with sun-drying. The amount of pesticide residue found in raisins was similar to that studied in the fresh fruits, pointing out a residue decrease of 4 times.

Procymidone. As with benalaxyl, metalaxyl, and phosalone, fresh grapes and raisins have the same residue level. The drying process caused a residue decrease of 4 times. During oven-drying washing caused a decrease of 60%, and after completion of the drying process the residue was identical. This indicates that in the two different processes the residue decrease follows the same rate.

Vinclozolin. Raisins obtained by sun-drying presented a residue level corresponding to one-third the

Table 1. Pesticide Residues in Fruits during the Drying Process

pesticide	fresh fruit, mg/kg ± SD	sun-dried fruit, mg/kg ± SD	oven-dried fruit	
			washing, mg/kg ± SD	drying, mg/kg ± SD
benalaxyl	0.05 ± 0.02	0.04 ± 0.02	0.04 ± 0.02	0.07 ± 0.03
dimethoate	1.02 ± 0.09	0.19 ± 0.06	1.06 ± 0.24	0.28 ± 0.08
iprodione	1.74 ± 0.30	2.79 ± 0.67	0.75 ± 0.21	0.81 ± 0.24
metalaxyl	0.13 ± 0.02	0.10 ± 0.03	0.12 ± 0.03	0.09 ± 0.02
phosalone	0.97 ± 0.25	0.69 ± 0.24	1.08 ± 0.26	2.73 ± 0.72
procymidone	2.63 ± 0.53	2.42 ± 0.55	1.55 ± 0.20	1.58 ± 0.30
vinclozolin	0.30 ± 0.06	0.08 ± 0.03	0.30 ± 0.07	0.19 ± 0.03

Table 2. Pesticide Residues (Milligrams per Kilogram ± Standard Deviation) during the Drying Process in a Model System

pesticide	control (filter)	hole cap vials (filter)	screw-capped vial	
			filter	vial
benalaxyl	0.13	<0.01	0.11 ± 0.03	<0.01
dimethoate	1.05	0.19 ± 0.04	0.13 ± 0.02	<0.01
iprodione	1.30	0.69 ± 0.09	0.99 ± 0.17	<0.01
metalaxyl	0.15	<0.01	0.05 ± 0.01	<0.01
phosalone	0.81	0.26 ± 0.05	0.82 ± 0.05	<0.01
procymidone	1.47	<0.01	1.30 ± 0.23	<0.01
vinclozolin	0.34	<0.01	0.05 ± 0.01	<0.01

level observed in the fresh fruits; therefore, the concentration factor is 12. Considering that washing did not affect the residue levels, during oven-drying the decrease was about half compared to sun-drying.

Mechanism of Residue Decrease in a Model System. The pesticide deposited on the fruit after treatment rapidly propagates inside the epicuticular waxes and cuticle (Riederer and Schreiber, 1995). During the drying process, when the water contained in the juice passes through these two layers, it could entrain pesticide molecules (codistillation), while heat could cause evaporation and degradation. The residue decrease is mainly due to these three factors (evaporation, degradation, and codistillation). A model system in which a cellulose membrane serves as cuticle was used to study these three effects.

The data relating to the residues of the two experiments are reported in Table 2.

In experiment B no residue was found on the vial walls. This means that none of the studied pesticides were subjected to evaporation in the experimental conditions. The filters from these vials had the same residues as the control with benalaxyl, phosalone, and procymidone, whereas with dimethoate, iprodione, metalaxyl, and vinclozolin the residues were 12, 76, 33, and 15% of control, respectively. This decrease was attributed to degradation.

In experiment A, the filter crossed by the water vapor did not show residues of benalaxyl, metalaxyl, procymidone, or vinclozolin. Since in the previous experiment benalaxyl and procymidone were stable to heat, their disappearance was completely due to codistillation. The decrease in metalaxyl and vinclozolin was due to the combined effect of heat and codistillation.

Dimethoate had the same residue levels in the filter of both experiments; this fact pointed out that there was no codistillation effect and the decrease in dimethoate was due only to heat. Phosalone and iprodione had smaller residues in the filter crossed by water vapor than in that of experiment B. The decrease in phosalone could be attributed to codistillation; in fact, this pesticide was not affected by heat. The decrease in iprodione is due both to codistillation and to degradation.

Conclusion. The drying process caused a concentration factor of 4. Therefore, the residue level should increase by this factor if no losses occur due to dehydration.

In sunlight drying the residue level in raisins was identical to that in fresh fruits for benalaxyl, metalaxyl, and phosalone, higher (1.6) for iprodione, and lower (one-thrid and one-fifth, respectively) for vinclozolin and dimethoate. The oven-drying process was preceded by washing, which caused residue decreases for iprodione and procymidone (both dicarboxymides) of 57 and 41%, respectively. No decrease due to washing was observed for the other pesticides. Compared to the fresh fruits, during oven-drying, pesticide residues in raisins increased by 2.7 in phosalone, gave the same values in benalaxyl, metalaxyl, and procymidone, and were lower in vinclozolin and dimethoate. This residue variation was only apparent because the concentration factor has to be taken into account. The two drying processes caused a residue decrease that was, however, different for the different pesticides. The same behaviors were observed only with benalaxyl and metalaxyl. Sunlight-drying was more effective in phosalone and vinclozolin, whereas oven-drying was more effective in iprodione and procymidone, due to the washing effect rather than to dehydration

The experiments carried out with the model showed that the decrease in dimethoate was attributable to heat, that in benalaxyl, procymidone, and phosalone to codistillation, and that in iprodione and metalaxyl to the combined action of heat and codistillation.

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