Residue Levels of Ethoxyquin, Imazalil, and Iprodione in Pears under Cold-Storage Conditions

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A gas chromatographic method is presented for the simultaneous determination of the antiscald ethoxyquin and the fungicides imazalil and iprodione in peel and pulp of Blanquilla pears. Fruits were cold-stored in commercial chambers in normal atmosphere and in controlled atmosphere with low oxygen content (oxygen and carbon dioxide were held at 2.5% and 1.5%, respectively). The method uses gas−liquid chromatography (GLC) with an alkaline flame ionization detector (detector of N−P, NPD) and allows the detection of the mentioned compounds to minimum levels of 0.08−0.12 mg/kg in fresh fruit. With this system the evolution of residues in fruit was monitored throughout the period of cold storage. In the surveys carried out the residue levels of these compounds were found to be below the limits allowed by the legislation of European Union. For the three studied products residues in pulp are lower and disappear more quickly than in peel.

Keywords: *Residue analysis; chromatographic methods; alkaline flame ionization detector; ethoxyquin; imazalil; iprodione; controlled atmosphere; pear; Blanquilla*

INTRODUCTION

The total production of pear in the Segriá region (Lérida province) is more than 200 000 metric tons per year. The Blanquilla variety is the most widely cultivated, representing almost half the total. The province of Lérida has a refrigerating capacity of about 2 million m^3, of which approximately half is used by Blanquilla pear in controlled atmosphere conditions, offering good possibilities for prolonging the conservation of pip fruit. This fruit is very suitable for cold storage and can be preserved without losing its organoleptic properties for 7−10 months, so it is possible to provide the product practically during the whole year.

During the cold-storage period pears can be attacked by a variety of infectious diseases caused by fungi and micomicetes (*Penicillium, Botrytis, Rhizopus, Geosporium*, etc.), and besides different physiological disorders (core flush, scald, ...) can also appear. These are the most important causes of losses during the storage period.

For the control of these illnesses several fungicides are recommended, two of which, iprodione and imazalil, are mainly used by the producers. Likewise, ethoxyquin is recommended to avoid surface scald of the fruit in the postharvest period.

The antioxidant ethoxyquin [1,2-dihydro-2,2,4-trimethylquinolin-6-yl ethyl ether] is widely used to protect animal feedstuffs, apples, and pears in storage. In postharvest treatments it is applied on the surface of the fruit for the prevention of surface scald (Warman and Austin, 1988), and although it remains mostly in the peel, it has also been found to have a certain penetrating power (Muller and Hansen, 1980). The European Community Scientific Committee for Food has expressed concern about the limited chronic toxicity data available for this product (Johnson et al., 1980). More recently studies on ethoxyquin toxicity have been published by Hard and Neal (1992). At present in Spain its presence in fruits is authorized at a maximum residue level of 3 mg/kg in whole fresh fruit (Royal Decree-Law 280/1994).

The fungicide imazalil [(±)-allyl 1-(2,4-dichlorophenyl)-2-imidazol-1-yl ethyl ether] is an imidazole used as a curative and preservative fungicide. It has a certain systemic power, is very effective against a great variety of fungi affecting fruit (pears, apples, bananas, citrus, ...), vegetables, and ornamental plants, and is also recommended for the protection of seeds. It has high activity against *Fusarium, Helminthosporium*, and *Septoria* spp. and has also been presented as highly effective against benzimidazole-resistant strains of *Penicillium* spp. The current legislation allows levels of up to 5 mg/kg for its use in fresh fruit (*Official Journal of the European Communities*, 1993).

Iprodione, 3-(3,5-dichlorophenyl)-*N*-isopropyl-2,4-dioxo-1-imidazolidine carboxamide, is a particularly effective fungicide against putrefaction fungi (*Botrytis, Monilia,* and *Sclerotinia* spp.) and also works against *Alternaria, Helminthosporium, Rhizoctonia,* and *Septoria* spp. It is normally used as a seed treatment for cereal and oil seeds and for the conservation of ripe fruit, vegetables, and grapes, as a postharvest dip. The current legislation allows levels of up to 10 mg/kg in the fresh fruits (*Official Journal of the European Communities*, 1993).

The application of these products in fruit destined for cold storage is carried out mostly by immersion of the fruit in an emulsion of the crop protection products at the moment of the entry in cold store. As a consequence of these treatments small amounts of the fungicides and antiscald product remain in the fruit. These amounts

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need to be controlled to (1) provide appropriate control of pathogens and prevent the scald and (2) minimize the exposure to consumer in edible portions of fruit. Analytical methods are needed to determine residue levels in the fruit and verify they do not exceed legislative regulations.

This paper describes a simple method for simultaneous determination of ethoxyquin, imazalil, and iprodione residues in pears and studies their evolution in postharvest fruit during cold storage.

MATERIALS AND METHODS

All the solvents used in the analyses were of high purity and suitable for use in residue analysis. They were purchased from Merck and used without further purification. Samples of fungicides imazalil (>99.7% active ingredient (ai)) and iprodione (>99.6% ai) were kindly submitted by Pennwalt-Jansen (Madrid) and Rhône Poulenc-Condor (Lyon), respectively. These were used to prepare standard pesticide solutions. Ethoxyquin of 95% purity was obtained in our laboratory from commercial product (70% ai, Pennwalt-Jansen, Madrid), using a purification process in column chromatography (SiO_2/hexane−ether).

Analytical Method. For routine chromatography analysis a Hewlett-Packard Model HP 5890 series II gas chromatograph, equipped with a split/splitless injector and an alkaline flame ionization detector (NPD), was used. The same model gas chromatograph, interfaced to a Hewlett-Packard HP 5971 quadrupole mass spectrometer, equipped with a HP 7673 autoinjector and a HP Vectra 486/33N data Station, was employed for confirmatory analysis.

Chromatographic separation for routine analysis was performed on a packed GLC-column (1 m × 6 mm (i.d.)) containing 10% SE−30 on silanized WAW-DMCS 80/100 mesh. The gas chromatograph parameters were as follows: injector temperature, 225 °C; detector temperature, 250 °C; oven temperature, 170 °C for 5 min, then increased to 240 °C at 3 °C/min, and finally kept at this temperature for 15 min. The carrier gas was nitrogen at 50 mL/min, and the flows of hydrogen and air were 40 and 200 mL/min, respectively.

In GC-MS analyses chromatographic separation was carried out on a capillary column (HP-1, 12 m × 0.2 mm (i.d.), 0.33 μm film). For these analyses the same GLC oven temperature program was used. The carrier gas was helium at 1 mL/min. Injection of the sample was carried out using on column mode.

The mass spectrometer was operated in the electron impact mode, with a filament voltage and current of 70 eV and 80 μA, respectively. Electron multiplier gain was 10^5, and scan range was from 35 to 550 amu at 1 s/scan. Transfer line and manifold temperatures were 250 and 220 °C, respectively. Single Ion Monitoring (SIM) technique was used for MS identification of compounds, and the selected ions for each chemical were as follows: *m*/*z* 217, 202, 174 (for ethoxyquin); *m*/*z* 249, 215, 173 (for imazalil); and *m*/*z* 316, 314, 187 (for iprodione).

Range of Linearity and Detection Limits. Solutions of the three studied products in toluene at concentrations levels of 1, 2, 3, 4, 6, 8, 10, 14, 25, 30, 40, and 60 μg/mL were prepared and injected in quadruplicate in the gas chromatograph. The NPD-responses were recorded for linearity.

From a concentration of 1 μg/mL using successive dilutions, the limits of detection for every compound were calculated through the respective chromatographic responses, when 1 μL of each solution was injected. These limits are the concentrations corresponding to a height of the chromatographic peak equal to three times the background noise of the chromatogram (S/N = 3).

Treatment of the Pears. The postharvest treatments were performed on fruit from several commercial orchards at the moment they were introduced in the cold-storage chamber by applying the corresponding products through a shower of an emulsion. The emulsions of the fungicides imazalil and iprodione were prepared from commercial products (Decoprocil, 7.5% of imazalil and 10% of iprodione) and were applied in two doses: 40 and 80 g/100 L for imazalil and 50 and 100 g/100 L for iprodione. Two doses of ethoxyquin, 250 and 500 g/100 L, obtained from a commercial product (Deccoscald, 72% ai), were also used. The highest dose corresponded in all the cases to the one recommended and used ordinarily in the cold-storage fruit industries.

Table 1. Characteristics of the Treatments in Pears from Cold Storage

dose (g/100 L) imazalil/ iprodione/ ethoxyquin	technology of conservation	period of conservation (months)	capacity of the chambers (m^3)
80/100/500	NCS[a]	0	1000
80/100/500	NCS[a]	2	
80/100/500	NCS[a]	4	
80/100/500	NCS[a]	6	
80/100/500	NCS[a]	8	
40/50/250	NCS[a]	0	1000
40/50/250	NCS[a]	2	
40/50/250	NCS[a]	4	
40/50/250	NCS[a]	6	
40/50/250	NCS[a]	8	
40/50/250	CA-LO[b]	0	1050
40/50/250	CA-LO[b]	8	

[a] Chambers of normal cold storage (gas content 21% O_2, 0.03% CO_2). [b] Chambers of controlled atmosphere with low oxygen contents (gas content 2.5% O_2, 1.5% CO_2). The relative humidity was 92%, and the temperature was −0.5 °C.

Residue evolution was studied in peel and pulp of pears stored under two refrigeration systems: normal cold storage (NCS) and controlled atmosphere with low oxygen content (CA-LO).

Controls were performed in the five different conservation periods specified in Table 1. The sampling was made following the guidelines of the European Community for pesticide control in fruits and vegetables (Royal Decree-Law 280/1994). Thus, a sample of 30 pears chosen at random was selected for each dose of treatment, storage period, and cold-storage technique. The pears were peeled, and all the peel and 20 g of pulp were used to obtain the analytic laboratory sample. The sample was then lyophilized and stored in the freezer at −18 °C up to the moment in which it was analyzed. Before the analysis the sample was crushed to fine dust and homogenized, and five replicates of 1 g each were collected for processing.

Extraction Process. Each of the five retorts (of 1 g) of peel and pulp was submitted to an extraction procedure with 20 mL of methanol in an ultrasonic device for 25 min. The methanolic extract was separated, and the operation was repeated another three times to increase the extraction yield. Further extractions did not increase the extraction yield. The three methanolic extracts were brought together and evaporated to dryness at low pressure (20 Torr, 30 °C). The residue was dissolved in 20 mL of distilled water, and this solution was later extracted with diethyl ether (4 × 10 mL) in a decantation funnel. The combined organic extracts were washed with saline solution and dried on anhydrous magnesium sulfate. After filtering and evaporating the solvents a residue was recovered, dissolved in 1 mL of toluene, and concentrated at 0.2 mL using a nitrogen stream. Thus, the sample, corresponding to an amount of 5 g of substrate per milliliter of injected extract, was ready to be injected into the gas chromatograph.

For confirmatory purpose the same procedure was also carried out on three retorts of 1 g of fresh material (peel and pulp).

Identification and Quantification of the Samples. The identification of the different products was carried out in GLC-NPD analysis by comparison of the retention times of the peaks of the chromatogram with the ones corresponding to authentic samples. Confirmatory MS-GLC analysis was done for comparison of the corresponding mass spectra. The quantification of the products in the samples was carried out by

Table 2. Chromatographic Response and Detection Limits of the Fungicides Imazalil and Iprodione and the Antiscald Ethoxyquin in CGL-NPD

product	retention time (min)	chromatographic response in the range (1−60 μg/mL)	estimated limit of detection: toluene solution (μg/mL)	estimated limit of detection: lyophilized peel extract (mg/kg)	estimated limit of detection: lyophilized pulp extract (mg/kg)
ethoxyquin	11.41	$y = 0.047 \times +0.0095$ $R^2 = 0.9913$	1.5	1.3	0.8
imazalil	25.16	$y = 0.0233 \times +0.0003$ $R^2 = 0.9940$	3.0	1.6	0.6
iprodione	29.54	$y = 0.0184 \times +0.0358$ $R^2 = 0.9949$	2.5	1.6	0.6

Table 3. Characteristics of the Determination of Ethoxyquin, Imazalil, and Iprodione in CGL-EM Coupling, Using the SIM Methodology

product	retention time	selected *m/z*	EM response range (1−60 μg/mL)	limit of detection (μg/mL)[a]
ethoxyquin	5.80	217, 202, 174	$y = 4.34$ (E5) $\times -$ 8.88 (E4) $R^2 = 0.997$	0.2
imazalil	8.68	240, 215, 173	$y = 2.97$ (E4) $\times -$ 8.37 (E4) $R^2 = 0.971$	3.0
iprodione	10.18	314, 316, 187	$y = 9.22$ (E4) $\times -$ 1.41 (E5) $R^2 = 0.985$	1.5

[a] Estimated from injection of standard toluene solutions of the studied chemicals.

comparison of the respective areas of the peaks with those of external standards.

Recoveries of the Extraction Method. To test the recovery and the reproducibility of the method, different samples of untreated vegetal material (pulp and peel of freeze-dried and homozenized pear) were fortified with different concentrations of the studied crop protection products in the range of 0.5−15 mg/kg. Three replicates of each concentration were prepared, and then the samples were extracted and quantified according to the above procedure.

RESULTS AND DISCUSSION

Linearity of the Chromatographic Response and Limits of Detection. A linear relationship was obtained between the chromatographic response and the injected concentration of each of the three chemicals in the whole range of concentrations studied (1−60 μg/mL). These results are presented in Table 2. In all the cases linear regression coefficients higher than 0.99 were obtained.

The limits of detection of the three products in toluene solution and in extracts of peel or pulp samples are also presented in Table 2. These studies of sensitivity proved that ethoxyquin can be detected in concentrations of 1.5 μg/mL in toluene solutions and in concentrations of 6.5 and 4.0 μg/mL in toluene extracts of peel and pulp samples, respectively. For imazalil we found minimum detectable level values of 3.0, 8.1, and 3.0 μg/mL for their detection in standard toluene solution, in peel extract solution, and in pulp extract solution, respectively. For iprodione the three detection limits found were 2.5, 7.8, and 2.8 μg/mL. These limits can be expressed as mg/kg of lyophilized pears resulting values between 0.5 and 1.6 for the three chemicals. According to the recoveries calculated for these compounds the following minimum levels of the crop protection product residues can be estimated for detection in fruit: for ethoxyquin 0.4 and 0.15 mg/kg in fresh peel and fresh pulp, respectively, and for imazalil and iprodione 0.45 and 0.15 mg/kg in fresh peel and fresh pulp, respectively.

Although the three products can be analyzed by high performance liquid chromatography with good sensitivity, the suggested method of GLC-NPD is faster and cheaper, allowing the detection and simultaneous quantification of the three chemicals without noticeable interferences and with sufficient sensitivity according to the residue levels found in the pears from cold-storage chambers.

For the ethoxyquin the limits found are of the same range as the ones described by Corti et al. (1992) in apples and pears using the HPTLC and HPLC-UV detector or by Warman and Austin (1988) in Bramey apples by GLC with a thermoionic bead nitrogen-selective detector. For imazalil Yamazaki and Nimomiya (1996) give quite similar minimum limits of detection in lemons, using GLC with mass-selective detection. The limits obtained for residues of iprodione in pulp are similar to those Adaskavez and Ogawa (1994) obtained in cherries using a similar procedure and slightly higher than the ones described by Lentza-Rizos (1995) in preserved peaches.

In a coupled GLC/MS system using the single ion monitoring (SIM) technique, minimum detectable levels of 0.2, 3.0, and 1.5 μg/mL were obtained for ethoxyquin, imazalil, and iprodione, respectively, evaluated from injected toluene solutions of the studied products (see results of Table 3). This procedure allows the detection of the three products with similar sensitivity and with a good improvement of the selectivity. Comparing the sensitivity of the detection systems (NPD and MS (SIM)) in toluene solutions, according to results in Tables 2 and 3, we can conclude that they show a similar sensitivity for imazalil and iprodione, whereas ethoxyquin is detected with higher sensitivity (a factor of 7) by the MS (SIM) detector.

In Figure 1 chromatograms, obtained from injection of toluene standard solutions of the three compounds using the two proposed detection systems NPD and MS-SIM, are presented.

Identification of the Residues of Ethoxyquin, Imazalil, and Iprodione in Pulp and Peel. Previously, extracts from nontreated samples of freeze-dried pulp and peel had been analyzed to verify the absence of interfering peaks at the times of retention of the products in the corresponding chromatogram. The chromatogram obtained was quite clean, and only the one

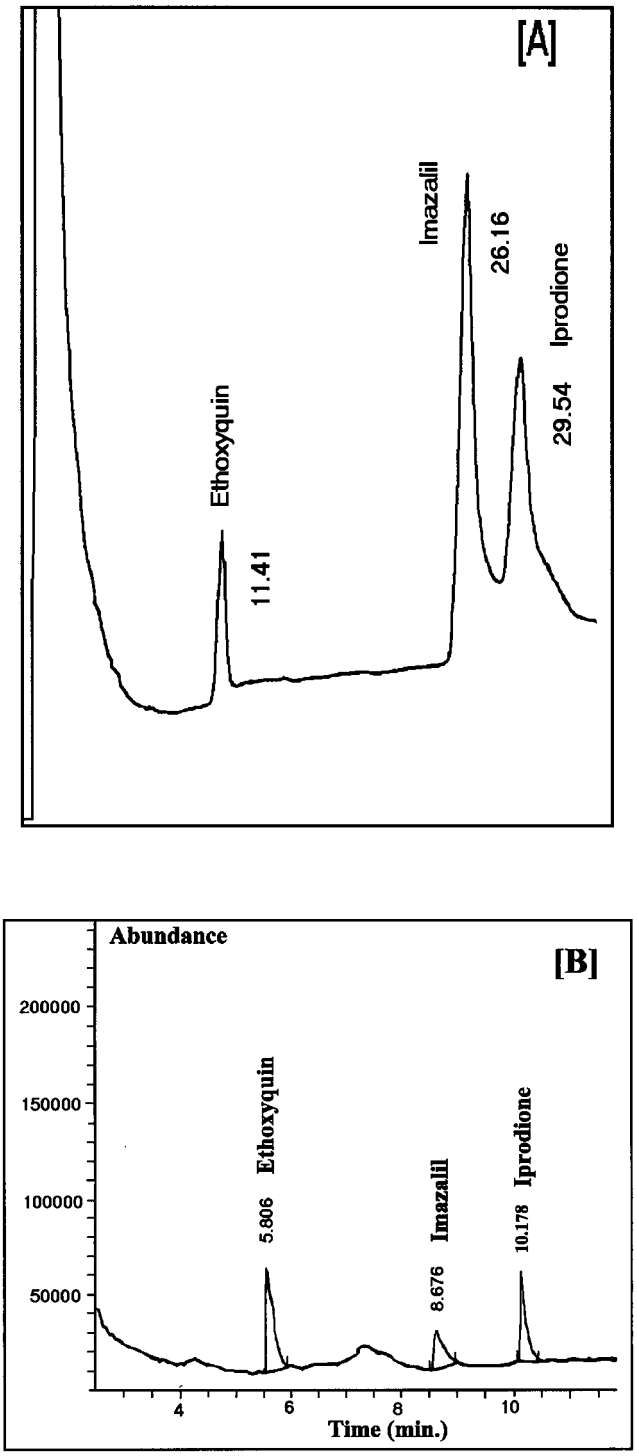

Figure 1. Chromatograms obtained by injection of 1 μL of standard toluene solutions of 60 μg/mL of ethoxyquin, imazalil, and iprodione using (A) CGL-NPD and (B) CGL-EM (SIM).

corresponding to the peel extracts showed a certain number of peaks of low intensity corresponding to several extracted components of the fruit cuticle. That fact supposed higher values (a factor of 3) of the limit of detection than those obtained in analysis of pulp extracts but did not jeopardize the detection of the compounds above these limits.

In Figures 2 and 3 we presented the chromatograms obtained by injection of extracts from freeze-dried peel and pulp spiked with the three mentioned crop protection products. These chromatograms can be compared

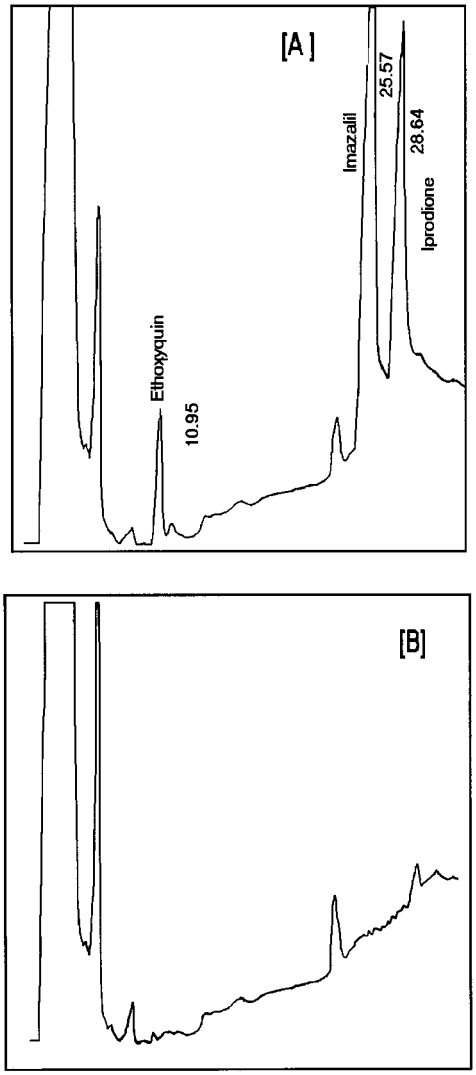

Figure 2. Chromatograms obtained by injection of 2 μL of extract from freeze-dried peel (A) spiked with 40 mg/kg of ethoxyquin, 60 mg/kg of imazalil, and 60 mg/kg of iprodione and (B) nonspiked.

with those obtained when we injected extracts from nontreated samples.

In the chromatographic analysis the fungicide iprodione undergoes a certain thermal decomposition; in this process traces of acid products are formed which remain in the "liner" of the injection block or at the head of the chromatographic column and in turn autocatalyze the process. This inconvenience was minimized by periodically eliminating these deposited residues from the chromatographic system.

Studies of the Percentages of Recovery. The percentages of recovery of the antiscald product ethoxyquin and of the fungicides imazalil and iprodione obtained from the corresponding extractions of spiked freeze-dried samples are shown in Table 4.

In the range of concentrations of studied residues of ethoxyquin (16.5−2.5 mg/kg) we obtained good recovery percentages in peel (77−97%) and pulp (77−82%). These percentages are quite similar to those obtained by other authors in freeze-dried of samples of "Bramley" apples (Warman and Austin, 1988) as well as in samples of pears using HPLC (Geiger, 1986; Corti et al., 1982). The percentages of recovery for the fungicide imazalil in peel samples are quite high and variable (96−114%). For the

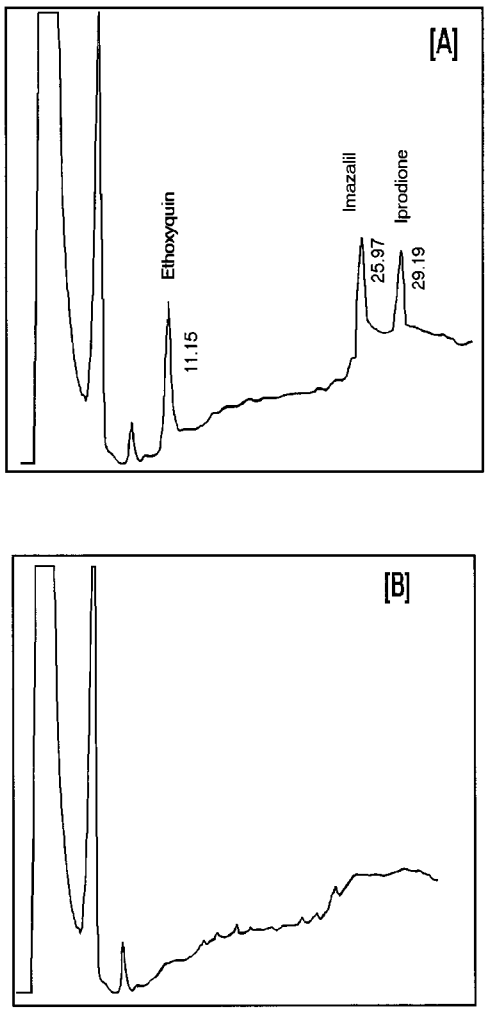

Figure 3. Chromatograms obtained by injection of 2 μL of extract from freeze-dried pulp (A) spiked with 40 mg/kg of ethoxyquin, 10 mg/kg of imazalil, and 10 mg/kg of iprodione and (B) nonspiked.

Table 4. Percentages of Recovery of Ethoxyquin, Imazalil, and Iprodione from Samples of Nontreated Freeze-Dried Peel and Pulp Spiked with Different Concentrations[a]

	freeze-dried peel		freeze-dried pulp	
product	spiked amount (mg/kg)	recovery (%)	spiked amount (mg/kg)	recovery (%)
ethoxyquin	16.5	76.8 ± 2.7	15.0	76.7 ± 6.5
	11.5	83.5 ± 0.1	11.5	70.1 ± 0.8
	5.0	85.4 ± 4.6	5.0	82.3 ± 7.7
	2.5	96.9 ± 4.6	3.0	80.6 ± 12.3
			1.0	80.4 ± 3.0
imazalil	28.0	96.8 ± 12.5	26.0	82.2 ± 3.0
	17.5	105.7 ± 9.5	17.5	86.2 ± 7.3
	10.5	114.5 ± 13.5	5.2	86.5 ± 2.3
	5.5	103.9 ± 10.5	1.5	84.6 ± 2.8
			0.5	92.0 ± 4.5
iprodione	26.5	83.3 ± 0.1	24.0	74.4 ± 8.0
	18.5	99.5 ± 8.0	18.5	85.3 ± 4.0
	10.5	96.4 ± 1.0	5.0	93.9 ± 1.0
	5.0	97.9 ± 15.0	1.2	96.7 ± 9.1
			0.4	100.0 ± 5.5

[a] The values of the percentages are mean of three replicates.

studies carried out in pulp we found values with lower dispersion (82−92%), similar to those obtained for other researchers in apples (Tuinstra et al., 1991; Matsumoto et al., 1973) and in pears (Garrido et al. 1997). Recoveries of 83−98% were obtained for iprodione from peel samples and 74−100% from pulp samples. Using different extraction methods Tonogai et al. (1995) obtained recovery rates of 71% in pears. Adaskavez and Ogawa (1995) in cherries and Lentzen-Rizos (1995) in apples obtained recovery rates higher than 95%.

Table 5. Percentages of Recovery of Ethoxyquin, Imazalil, and Iprodione from Samples of Peel and Pulp Spiked with 10 mg/kg[a]

	from freeze-dried material		from fresh material	
product	peel	pulp	peel	pulp
ethoxyquin	67.0 ± 0.8	69.0 ± 3.4	75.3 ± 0.1	80.1 ± 0.8
imazalil	76.2 ± 13.2	77.0 ± 5.8	82.5 ± 6.0	80.0 ± 7.1
iprodione	79.4 ± 1.0	69.0 ± 5.4	70.1 ± 1.0	65.0 ± 4.0

[a] Recoveries were carried out on fresh material and on previously freeze-dried material. The values of the percentages are mean of three replicates.

We could not carry out all the analyses of the samples as soon as they arrived in the laboratory, so we needed to preserve them until the moment they could be processed. The lyophilization process is not at all an aggressive technique and allows the water elimination of the samples with minimum changes in the chemical composition of their components. To confirm that the losses of the chemicals during lyophilization process are not very important, we conducted the following experience: Two samples of fresh peel and pulp of 1 g each were spiked with a dose of 10 mg/kg of the three studied products. One of the samples was directly extracted and processed according to the described method, and the other was submitted to a freeze-dried procedure before processing. The results are shown in Table 5, and from them we can confirm that quite a good correlation can be observed between the levels of imazalil and iprodione residues in fresh and in freeze-dried samples. Losses of less than 10% were detected for ethoxyquin in the lyophilization process. According to these results it is assumed that the lyophilization process does not affect basically the contents of chemicals studied. It also allows more uniform samples to be obtained, and with the removal of the water the process of extraction and sample preparation for chromatography is facilitated.

Analysis of Fungicide Residues in Pears under Cold Storage. At the doses applied in the two storage systems fruits affected by the scald or by fungi attack were not detected in any of the treatments. Accordingly we may deduce that the crop protection treatments were effective in all the cases, even at the lowest dose.

The present work monitors the evolution of the residues in Blanquilla pear subjected to the two doses of treatments and stored under habitual cold conditions (NCS). We did not arrange enough samples to monitor the evolution of the residues in fruits submitted to conservation in controlled atmosphere with low oxygen content (AC-LO), for which we only have data of residues after 8 months of storage.

Evolution of Residues in Pears during Cold Storage. During cold storage the residue concentration of the studied chemicals decreases, as is confirmed by the results shown in Tables 6 and 7 and Figures 4−6). In these tables we present the results obtained in the analyses of pears from normal cold storage, which had been treated with formulations of these products at normal dose (500 g/100 L for ethoxyquin, 80 g/100 L for imazalil, and 100 g/100 L for iprodione) or at half-dose, respectively. The concentration values of each product

Table 6. Mean Concentrations in mg/kg of Residues of Ethoxyquin, Imazalil, and Iprodione Found in Blanquilla Pears after Cold Storage[a]

period (months)	product	freeze-dried peel[b]	freeze-dried pulp[b]	fresh peel[c]	fresh pulp[c]	fresh fruit[c]
0	ethoxyquin	14.4 ± 3.5a	6.7 ± 1.4a	4.1	1.1	1.4
2		14.2 ± 1.5a	6.0 ± 0.3a	4.1	1.0	1.3
4		13.8 ± 1.4a	5.8 ± 0.3a	4.0	0.9	1.2
6		12.0 ± 1.1a	4.9 ± 1.0a	3.7	0.7	1.0
8	imazalil	8.0 ± 1.8a	2.0 ± 0.2a	2.6	0.3	0.5
0		23.9 ± 1.8a	1.5 ± 0.3a	7.0	0.3	1.0
2		23.6 ± 2.4a	1.3 ± 0.1a	6.7	0.2	0.8
4		23.3 ± 2.3a	1.0 ± 0.3a	6.7	0.2	0.8
6		21.9 ± 1.9a	0.6 ± 0.1b	6.8	0.1	0.8
8	iprodione	19.1 ± 2.3a	0.7 ± 0.3ab	6.1	0.1	0.7
0		41.0 ± 3.8a	2.1 ± 0.8a	11.7	0.3	1.4
2		35.0 ± 4.0a	1.5 ± 0.2a	10.0	0.2	1.2
4		31.0 ± 3.9a	1.4 ± 0.1a	8.9	0.2	1.1
6		23.8 ± 1.9b	0.9 ± 0.1b	7.6	0.1	0.8
8		17.3 ± 1.3c	0.5 ± 0.1c	5.5	0.1	0.6

[a] The pears had been submitted to normal dose treatments of the fungicides. [b] Mean concentration values followed by the same letter do not differ significantly according LSD test ($p > 0.05$). Results are mean of five replicates. [c] Calculated from analysis of freeze-dried fruit.

Table 7. Mean Concentrations in mg/kg of Residues of Ethoxyquin, Imazalil, and Iprodione Found in Blanquilla Pears after Cold Storage[a]

period (months)	product	freeze-dried peel[b]	freeze-dried pulp[b]	fresh peel[d]	fresh pulp[d]	fresh fruit[d]
0	ethoxyquin	8.1 ± 0.7a	6.0 ± 2.2a	2.5	0.9	1.1
2		7.9 ± 3.1a	5.3 ± 2.1a	2.4	0.8	0.1
4		7.5 ± 1.4a	4.1 ± 0.7a	2.3	0.6	0.8
6		6.8 ± 0.8a	1.5 ± 0.3b	2.1	0.2	0.4
8		3.9 ± 0.5a	1.5 ± 0.1b	1.2	0.2	0.3
0	imazalil	18.9 ± 3.5a	1.7 ± 0.8a	6.0	0.3	0.9
2		18.8 ± 3.6a	1.3 ± 0.8a	5.7	0.2	0.7
4		17.2 ± 1.3a	1.0 ± 0.2a	5.2	0.2	0.7
6		12.7 ± 0.9b	0.3[c] ± 0.1b	4.0	0.1	0.4
8		11.4 ± 0.9b	0.4[c] ± 0.1b	3.6	0.1	0.4
0	iprodione	15.6 ± 3.1a	1.5 ± 0.2a	5.0	0.2	0.7
2		12.2 ± 4.1a	1.3 ± 0.4a	3.7	0.2	0.5
4		11.5 ± 1.8a	0.9 ± 0.1a	3.5	0.1	0.4
6		10.2 ± 0.8a	0.5[c] ± 0.2a	3.2	0.1	0.4
8		9.9± 1.3a	0.5[c] ± 0.2a	3.1	0.1	0.4

[a] The pears samples had been submitted to half-dose treatments. [b] Mean concentration values followed by the same letter do not differ significantly according to Turkey's LSD test ($p > 0.5$). The results are mean of five replicates. [c] Results are near the limit of detection. [d] Calculated from analysis of freeze-dried fruit.

in freeze-dried peel and pulp, in fresh peel and pulp, and in the whole fresh fruit are shown.

The values of the concentrations in lyophilized fruit were calculated directly from the chromatographic analysis. The corresponding values for the fresh product have been determined by extrapolation, after calculating the reduction factors due to the losses of water in the lyophilization process. The values referred to whole fruit were calculated considering the respective percentages of pulp and peel in the fruit. According to results in Table 4 one would expect a good correlation between the levels of residues in the corresponding fresh and freeze-dried samples.

The residue values found in samples of fresh fruit submitted to conventional or half-dose treatments after the different periods of conservation are always below the statutory ceilings established by the Spanish legislation and guidelines of the European Community, which are set at 3 mg/kg for ethoxyquin, 5 mg/kg for imazalil, and 10 mg/kg for iprodione. According to these results we believe that the current recommendations to guarantee acceptable pollution levels of these crop protection products, which consider the period between application and consumption of the fruit, submitted to this conservation method to be 1−3 months, are suitable.

We did not find proportionality between the applied dose of the chemicals and the amount deposited on the fruit, and, according to our results, the application of half-dose is sufficient to control diseases in Blanquilla pears during the whole conservation period.

1. Evolution of Ethoxyquin in NCS. Of the three products studied, this is the one that has the greatest penetration capacity into the fruit. If we define a penetration index as the ratio of the amount of the chemical found in the peel and to the amount of the chemical found in the pulp, this index rises to 25−36% for ethoxyquin compared with 2−5% for imazalil and iprodione. The higher the dose of the chemical applied, the higher the penetration index.

The statistical analyses carried out on the results of the freeze-dried material reveal that in the evolution of the residues no significant differences were found in the first surveys (at 2, 4, and 6 months), and only the level of residues in the product of the last sampling was lower.

In samples of fresh peel from the high-dose treatment (Table 6), ethoxyquin exceeds the statutory ceilings up to 6 months in conservation; in pulp samples these limits are never reached. For the whole fruit values about the levels of tolerance are never reached neither. The percentage of degradation of the product in fresh

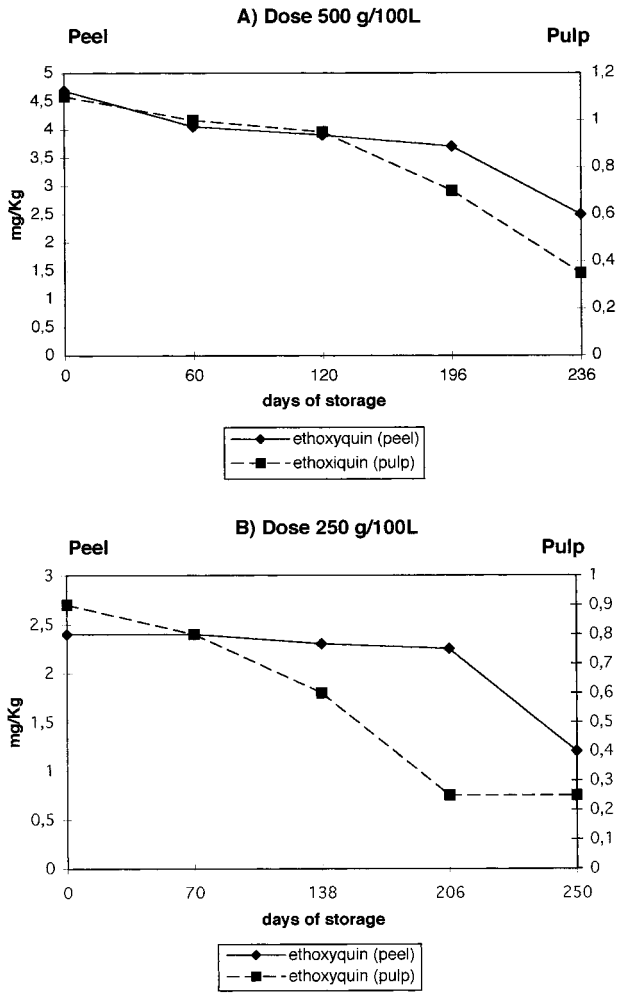

Figure 4. Evolution of the residues of the antiscald ethoxyquin in peel and pulp of pears under normal cold-storage conditions: (A) pears treated with a dose of 500 g/100 L and (B) pears treated with a dose of 250 g/100 L.

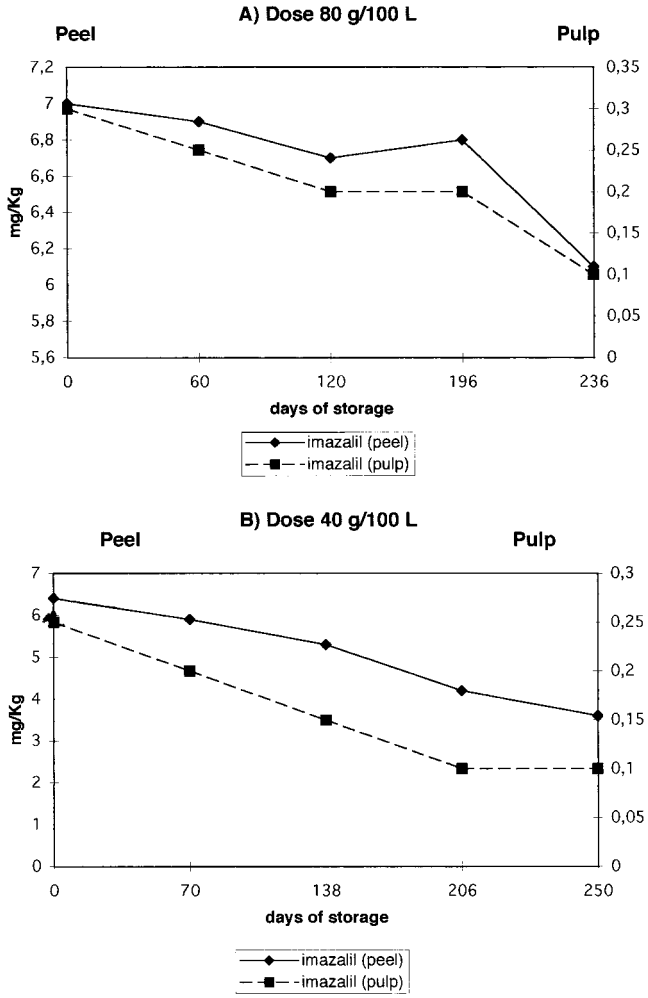

Figure 5. Evolution of the residues of fungicide imazalil in peel and pulp of pears under normal cold-storage conditions: (A) pears treated with a dose of 80 g/100 L and (B) pears treated with a dose of 40 g/100 L.

peel in the whole period of cold storage is around 37%, whereas in pulp the degradation is higher, reaching values of around 73%.

When half the normally used dose is applied (Table 7), we find a similar behavior, with overall degradations of the antiscald product of 52% and 78%, in peel and in pulp, respectively.

2. Evolution of Imazalil in NCS. The imazalil is more stable than ethoxyquin (Figures 4 and 5). This degradation is more uniform and occurs at a lower rate throughout all the period of study, that means a less pronounced gradient of the corresponding curves for the two doses. The good persistence of the product, particularly in peel, guarantees a good efficiency in the protection of the fruit during the whole period of conservation.

The results of the statistical treatment carried out on the residue values found in freeze-dried peel and pulp do not show significant differences from the levels of all the controls carried out after 6 months of cold storage, especially for half-dose treatments.

In the high-dose treatment (see Table 6) the degradation in fresh peel is very low (around 13%), and again in pulp we find higher values (around 67%). Nevertheless, as has been stated, the capacity of penetration of the fungicide into the fruit is lower than in ethoxyquin (penetration index values of around 2−4%). At the end of the process of conservation the surface residues represent around 87% of the ones found on entry to cold storage. In the samples treated at half-dose (Table 7) we find again a certain parallelism, though in this case a higher rate of degradation in peel during the whole process of conservation is confirmed and the residual levels detected at the end of the storage period are of the order of 60% of the initial levels.

The low capacity of penetration of this pesticide is also reported by other researchers working on several kinds of fruits. For example, Cuñat and Room (1989) do not give significant values of fungicide in pulp of treated citrus, and Lafuente and Tadeo (1985) state that the product also shows a good superficial persistence in citrus.

The treatments at the highest dose (see Table 6) show residue values in peel that are slightly higher than the statutory ceilings in the controls carried out after 8 months. However in pulp as well as in the whole fresh fruit dangerous values are not reached.

3. Evolution of Iprodione in NCS. Iprodione presents a very low penetrating power, comparable to imazalil, as we can see in Tables 6 and 7. The concentration of residues in fruit during cold storage decreases quite uniformly and more sharply than with the other fungicide (Figure 6). On the other hand, the statistical treatment of the data obtained from freeze-dried peel and pulp samples shows that the effect of cold-storage

Table 8. Mean Concentrations in mg/kg of Residues of Ethoxyquin, Imazalil, and Iprodione in Blanquilla Pears after Normal Cold Storage and Atmosphere Controlled Cold Storage with Low Content of Oxygen[a]

period (months)	product	chamber cond	freeze-dried peel[b]	freeze-dried pulp[b]	fresh peel[d]	fresh pulp[d]	fresh fruit[d]
0	ethoxyquin	NCS	8.1 ± 0.7a	6.0 ± 2.2a	2.5	0.9	1.1
8			3.9 ± 0.5b	1.5 ± 0.1b	1.2	0.2	0.3
0		AC-LO	8.7 ± 0.8a	5.7 ± 0.8a	2.6	1.2	1.3
8			8.1 ± 0.6a	1.2 ± 0.1b	2.6	0.2	0.4
0	imazalil	NCS	18.9 ± 3.5a	1.7 ± 0.8a	6.0	0.3	0.9
8			11.4 ± 0.9b	0.4[c] ± 0.1b	3.6	0.1	0.4
0		AC-LO	18.7 ± 3.5a	1.7 ± 0.8a	5.6	0.3	0.8
8			16.0 ± 1.4a	0.4[c] ± 0.1b	5.1	0.1	0.6
0	iprodione	NCS	15.6 ± 3.1a	1.5 ± 0.2a	5.0	0.2	0.7
8			9.9 ±1.3b	0.5 ± 0.1b	3.1	0.1	0.4
0		AC-LO	15.7 ± 2.8a	1.7 ± 0.8a	4.7	0.3	0.7
8			11.5 ± 1.2b	0.4[c] ± 0.1b	3.7	0.1	0.4

[a] Half-dose pear treatments were applied. [b] Mean concentrations values followed by the same letter do not differ significantly (test LSD, signification level of 95%). The results are mean of five replicates. [c] Results near the limit of detection. [d] Calculated from analysis of freeze-dried fruit.

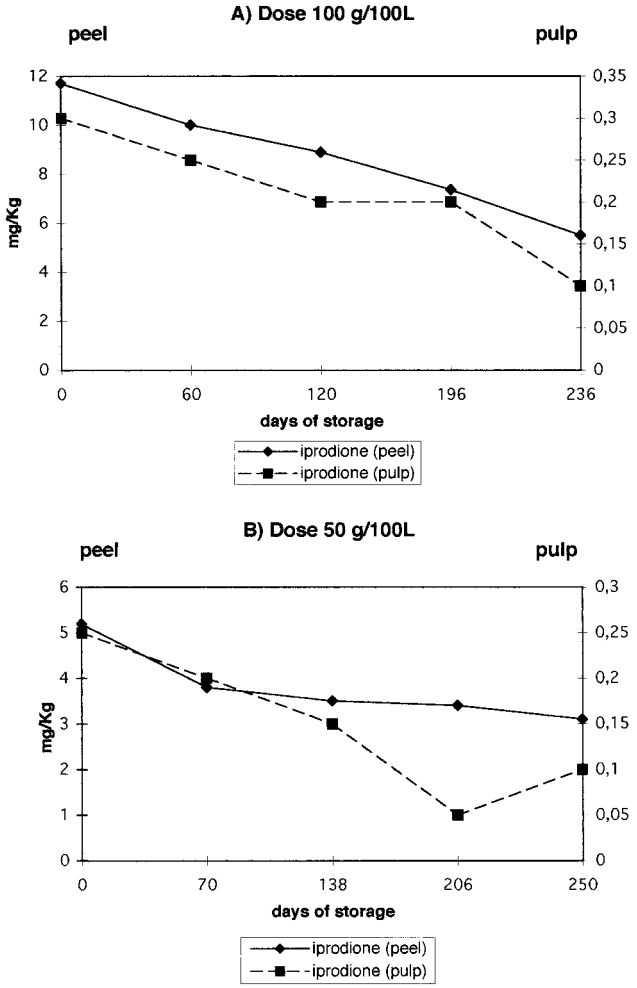

Figure 6. Evolution of the residues of fungicide iprodione in peel and in pulp of pears under normal cold-storage conditions: (A) pears treated with a dose of 100 g/100 L and (B) pears treated with a dose of 50 g/100 L.

period is significant with regard to its concentration in the samples treated at the normal dose.

In the samples treated at the normal dose it undergoes greater degradation in peel and pulp than in those treated at half-dose. Thus, for example, when normal dose is used, the product degrades by about 53% in peel and 67% in pulp, whereas in the samples treated at half-dose we found degradations values of around 38% in peel and 50% in pulp. We must note that in this case the controls carried out in the final periods of the experiment gave concentrations of fungicide near to the limit of detection of the analytical method that was used.

In all the periods of control during cold storage the residue levels are below the limit allowed, even in the treatments at high dose.

Bertolini et al. (1985) give half-life values of 120 days in apples and 180 days in pears. When analyzing residues of iprodione in peach, Lentza-Rizos (1995) does not detect noticeable degradation after the first 20 days of cold storage.

Influence of the Cold-Storage Technology. Table 8 compares the residual values of the chemicals found in pears conserved in normal cold storage (NCS) and in cold storage with controlled atmosphere with low oxygen contents (CA-LO), corresponding to half-dose treatments.

The statistical analysis shows that the ethoxyquin and imazalil residues in peel are higher in pears preserved 8 months in CA-LO than in the pears stored in normal conditions. For iprodione the same trend of the corresponding averages seems be observed, though the differences are not significant. The presence of a greater oxygen content in the atmosphere thus seems to influence the disappearance of these chemicals from the outer surface of the fruit.

Nardin and Trevisani (1986) also found higher concentrations of ethoxyquin in the peel of apples preserved in CA-LO than in those kept in normal cold storage.

In the pulp we did not find significant differences in the levels of residues of any of the three studied products according to the conservation system. Inside the fruit the degradation rate for the three compounds is similar in the two cold-storage systems.

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