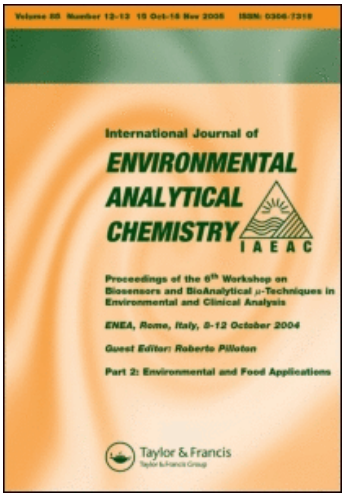

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STUDY OF IMAZALIL RESIDUES IN GLASSHOUSE MINICUCUMBER PICKLES

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Brined minicucumbers, brine, pickles, and pickling juice were analysed for imazalil (IMA) residues and T_{824} by HPLC after glasshouse minicucumbers IMA-treated were submitted to pickling. The distribution of IMA on minicucumber prior to pickling, seven days after treatment, increased somewhat from peel to flesh and core. Most of it was detected in the peel (65 %), being 18 % transferred to the flesh and 7 % detected in the minicucumbers core when they were IMA treated at 5 mg kg^{-1}. The pickling process behaved like an extended extraction. The whole process reduced IMA residue on the minicucumber through the two stages of pickling: part of it was removed by the (a) brining and (b) pickling stages, remaining ca. 7 % of IMA on the pickled minicucumbers. The process had quite a beneficial effect on IMA residue, producing pickles with lower amount of IMA than the MRL and no T_{824} formation was observed at any stage of pickling, even after seven days under the acidic medium contact. As far as the pKa of IMA is concerned, the acetic acid medium surrounding the IMA-treated minicucumbers improved IMA solubility leading to a better removal.

Keywords: Imazalil; pickling; minicucumber; gherkins; processing; fungicide

INTRODUCTION

The cucurbits, apart from being eaten raw, as salads, and cooked, either boiled, steamed, grilled, broiled or stir-fried, are commonly preserved by pickling. Minicucumbers and gherkins are the most important type of vegetable used for this process and are used also in brined or vinegar products. In North America, the name pickles is synonymous with cucumber pickles and their consumption exceeds that of other pickled vegetables by a very considerable margin[1,2]. The crop grown for pickling in some of the principal growing states in the USA

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exceeds 10,000,000 tons per annum. They are also very popular in Europe, where numerous kinds are to be found. The Swedish production of cucumber pickles is estimated to be approximately 5,000 tons each year. Gherkins and minicucumbers used for pickling have similar problems with fungi as cucumbers and the compounds applied in the field to protect them are similar. Minicucumbers, are vegetables mostly eaten with their peel on and due to their high water content are very perishable, therefore it is necessary to protect them against diseases and pests by using fungicides. Imazalil (Figure 1), a low toxicity fungicide, effective against the benzimidazole-resistant strains of *Penicillium*, typical storage fungi, is now used quite widely in some vegetables.

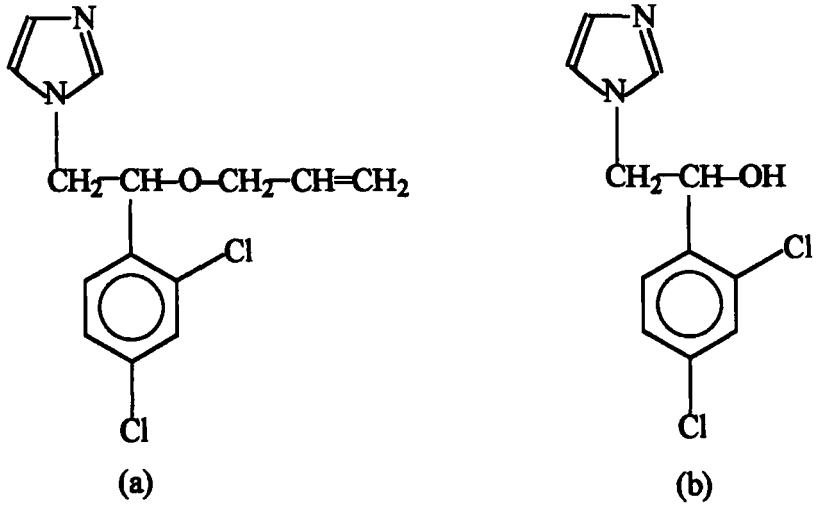

FIGURE 1 Chemical structures: [a] imazalil (1-[2-(2,4-dichlorophenyl)-2-(2-propenyloxy)ethyl]-1 *H*-imidazole) and [b] T_{824} (1-(2,4-dichlorophenyl)-1 *H*-imidazole-1-ethanol)

Although at present the fungicide is not allowed to be used postharvest on vegetables, several researchers are suggesting the possibility of using imazalil due to its effectiveness to those resistant strains. Surveys have reported imazalil residue on raw cucumbers and other fruits[3–9], however, there are no data on the effect of processing, on its fate, especially when it is applied postharvest. Therefore, studies on imazalil removal, distribution and/or degradation during pickling are of considerable interest and a survey to find out the effect of pickling on imazalil residue from treated glasshouse minicucumber was carried out.

EXPERIMENTAL

Materials

Samples

261 minicucumbers, variety Danimas F_1, Class 1, freshly harvested, no imazalil applied, from Slough Nurseries, Slough, UK.

Standards

Imazalil sulfate (1 g, 94.8% pure), Makhteshim Chemical Works Ltd, Beer-Sheva, Israel and T_{824} (50 g, 98 % pure), Janssen Chimica.

System for solvent filtration

Filtration funnel with fritted glass (porosity 0.7 μl), and filter membrane discs for solvent filtration, non sterile, pore size 0.45 μ with 47 mm diameter.

SPE system

Cartridges (C_{18}, 500 mg, 3 ml bed volume), column reservoir (10 ml), luer stop-cocks, and vacuum manifold.

Pickling materials

Cooking salt and distilled malt vinegar.

Other materials

2.0 L jars with lids, sample concentrator, ultrasonic bath, and heating block. For the minicucumber extracts that presented particulates to be injected in the HPLC column.

Syringe Filter holder and filter membrane discs

Nylon membrane, pore size 0.2 μ, 13 mm diameter.

Solvents and gas

Acetone, acetonitrile, methanol, and water (HPLC grade), helium (Research grade), and nitrogen.

HPLC System

Injector Rheodyne. Pump: Spectra-physics SP 8810, isocratic. Integrator: Hewlet Packard 3369[A]. Pre-column filter: Upchurch, Scientific. *Detector*: Kratos Spectroflow 757. Guard column and column: both, Zorbax SBCN (Scussel, 1995a, 1998), 5 μm particle size, and 10 and 250 mm long, respectively.

HPLC Conditions

Mobile phase: acetonitrile:water (6:4 vlv), flow rate: 1 ml/min, chart speed: 0.5 cm/min, injection volume: 20 µl. *Wavelengh*: 202 nm.

Methods

The methodology for studying the effect of pickling on imazalil removal from minicucumbers was divided into the following steps: (1) imazalil treatment, (2) pickling procedure and (3) imazalil residue analysis.

Minicucumber preparation

Minicucumbers presenting mechanical damage, abnormal shape, discoloration, and/or other deterioration were discarded. After weighing, they were allocated to 6 groups according to the time of storage prior to pickling: freshly, one and seven days, with two levels of imazalil treatment, 0.5 (MRL) and 5.0 mg kg^{-1}. The same number of minicucumbers (for each time of storage) was prepared for the pickling process, but taken only up to the brining step, to assess any imazalil removal at that stage. A control group without imazalil treatment was also prepared. Therefore, Group 1: raw minicucumbers, not imazalil treated, Group 2: raw freshly treated, Group 3: raw imazalil-treated, one and seven day stored, Group 4: imazalil-treated one and seven days stored and brined, Group 5: imazalil-treated, one and seven day stored, brined and pickled and Group 6: pickled, no imazalil treatment. For three trials: 261 samples were used.

Imazalil dipping treatment

The imazalil dipping solution was prepared in distilled water after a study of liquid retention on minicucumber in order to obtain on each minicucumber *ca.* 0.5 and 5.0 or kg^{-1} of imazalil. Dipping Procedure: Each minicucumber, held by its extremity (stem) with tweezers, was immersed twice in the dipping solution contained in a 600 ml beaker (simulating dipping tanks), for 3 s each time, allowed to drip until no more liquid was observed to drain and then stored in clean lockers, free of odour, at room temperature (*ca* 20°C) for seven days.

Pickling procedure

The pickling procedure had two steps: brining and pickling. Brining: the samples (control and stored) were prepared for pickling as follows. Groups of 5 minicucumbers each were soaked overnight in sufficient brine (60 g of salt dissolved in 600 ml hot water and boiled) to cover the whole sample contained in a 2.0 L jar. They were left 24 hours in the dark, in lockers, at room temperature. Pickling

(vinegar addition): after the 24 hours soaking, the brine was drained and kept for analysis, its volume being measured. The salted minicucumbers were transferred to another jar, which was filled with hot vinegar (after boiling) to the top and closed with lid for seven days. Another group of cucumbers was brined, but not treated with acetic acid and kept for analysis after brining to check on imazalil removal at that stage (Figure 2).

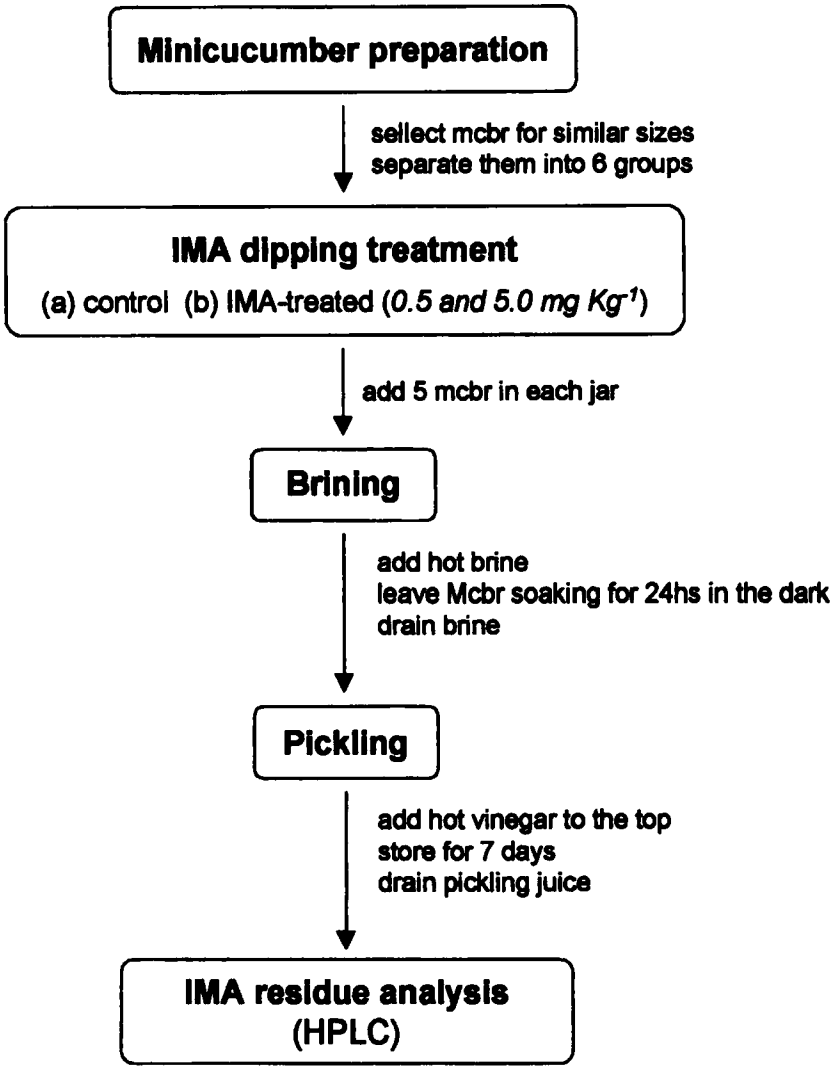

FIGURE 2 Chartflow of the pickling study using IMA-treated minicucumbers (*Mcbr.* minicucumber, *IMA*: imazalil)

Imazalil residues analysis

Samples of raw, brined, and pickled minicucumbers (IMA-treated and control), at the two levels of application, fleshly and after one and seven days of storage, as well as, brine and pickling juices were used for analysis. The methodology was that developed by Scussel (1995b) with extraction/clean-up by reversed-phase SPE, separation by HPLC (SBCN column) and detection by UV at 202 nm (limit of determination (LDM) / limit of detection (LDC) = 0.1 mg kg^{-1}). See HPLC conditions in Materials. Figure 2 shows the chartflow of the pickling study using IMA-treated minicucumbers.

RESULTS AND DISCUSSION

Raw and pickled ratio of minicucumbers

Minicucumbers lost *ca.* 6.5% in weight at the brining stage, and 26.5 % after the whole pickling process (one week). For example: a group of raw minicucumbers weighing 743.0 g submitted to brining would reach approximately 688.9 g, and 558.8 g after seven days of pickling. Pickles not treated with imazalil and the treated ones were indistinguishable by texture, colour, and aroma. All had a firm consistency to pressure with light green/brownish colour, characteristic of cucumber pickles. After 1 week of pickling the pH reached 3.8.

Imazalil residue in raw minicucumber – distribution

When minicucumbers were analysed fresh, most of the imazalil applied was detected on the peel, both at 0.5 and 5.0 mg kg^{-1}. However, when they were analysed 24 hours after imazalil treatment, the residues on the peel decreased somewhat and traces and 10 % were detected in the flesh for the two concentration (Figure 3), respectively. Traces of imazalil at the most were detected in the core of 5.0 mg kg^{-1} at Day 1. After seven days of application, 63 and 65 % were detected on peel and 12 and 18 % transferred to the flesh for original concentrations of 0.5 and 5.0 mg kg^{-1}, respectively and 7 % of imazalil were detected in the core at 5 mg kg^{-1}, but none was detected at the lower level of application. A relevant factor is that the peel of minicucumber is rather thinner than that of cucumber and this may speed the transfer of imazalil to the flesh than that on the ordinary cucumbers. As the core is bigger, imazalil can also reach it more quickly than in ordinary salad cucumbers.

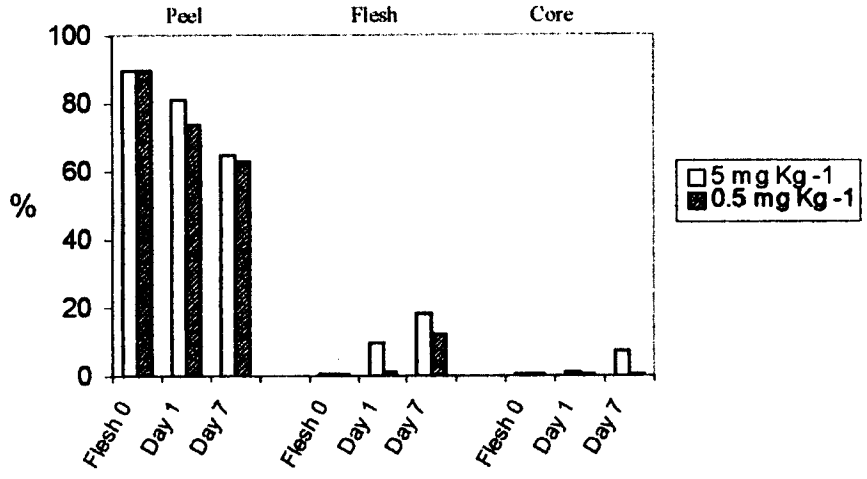

FIGURE 3 Imazalil distribution in peel, flesh and core of raw minicucumbers trated with 5 and 5.0 mg Kg^{-1} and stored for seven days at 20° C. (n=3)

Brined and pickled minicucumbers – distribution, removal, and degradation

The effects of brining and pickling on imazalil residues in the brined and pickled minicucumbers are summarised in Figure 4. Several factors might contribute to the imazalil removal and/or distribution in minicucumbers during the overall process: (a) in the brining: (a.1) brine soaking and (a.2) osmosis, and (b) in the pickling: (b.1) acetic acid soaking, (b.2) osmosis, (b.3) skin tenderising, (b.4) temperature that the acetic acid was added, and (b.5) time of pickling. For *both* stages also the (c) length of time prior pickling, that the minicucumbers IMA-treated were stored, and (d) imazalil concentration applied, could interfere on imazalil extractions. It is not considering here different imazalil treatments, only dipping.

(a) Brined

When minicucumbers were used for brining, 24 h after the treatment with 0.5 mg kg^{-1} of imazalil, just over half of it was detected in the sample and a mean of 34 % of imazalil was detected in the brine (30, 32, and 40% for Trials 1, 2, and 3, respectively) (Figure 4A).

Soaking effect: Imazalil is water-soluble and so part of it can readily be removed by soaking the sample in the brine, especially when they were prepared for pickling after only a short time after its application. When samples with 5.0 mg kg^{-1} of imazalil were brined after 24 h, a similar effect took place and

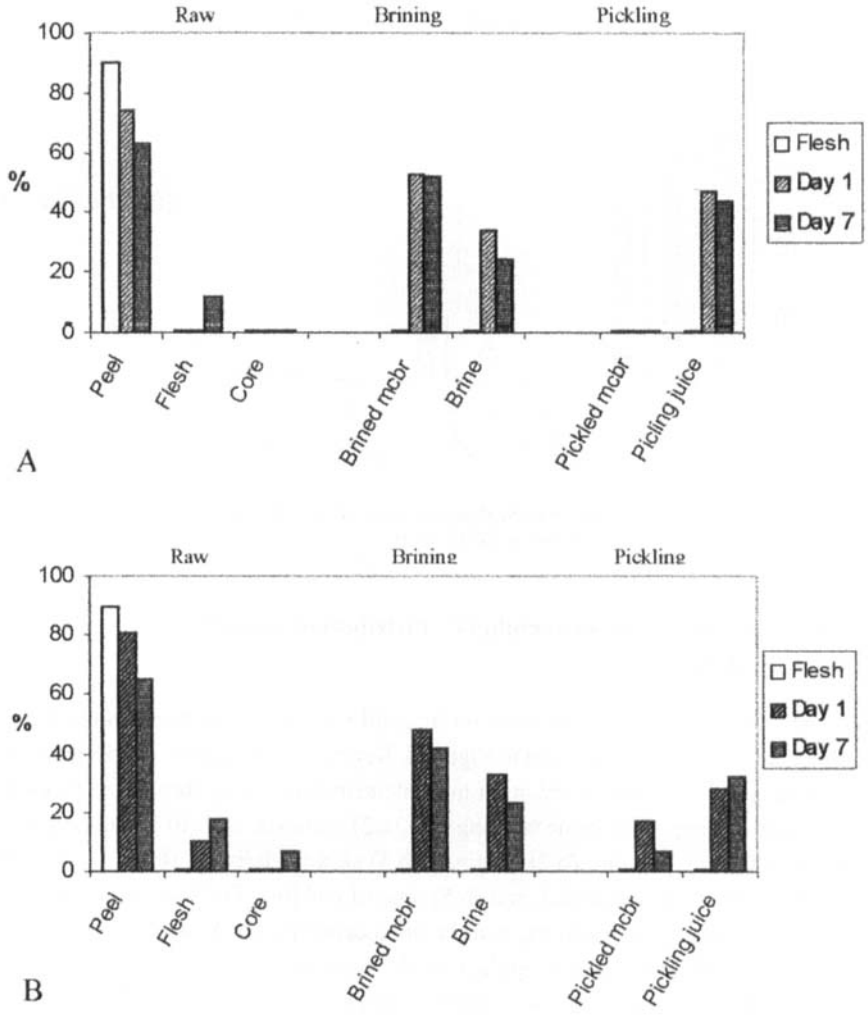

FIGURE 4 Imazalil distribution in raw minicucumber (mcbr) trated at [A] 0.5 and [B] 5.0 mg Kg^{-1} levels and its removal by brining and pickling after seven days of storage at 20° C. (n=3) (See Color Plate at the back of this issue)

similar percentage remained on the brined samples (48 %) 49, 47, and 48 % for the three trials, respectively, and an average of 33 % in the brine. After seven days, the situation changed, as relatively less imazalil was removed, more of it being kept in the brined minicucumbers.

Osmosis: The water transfer from the samples to the brine (due to difference on salt concentration) could play an important role on imazalil extraction from

the surface of the samples, as it can provoke its removal together with the liquid released from the inner of the minicucumber. However, from the results obtained, it was shown that the first situation was more likely to take place, in combination with the physical effect of soaking. The combined effect gradually would release imazalil from skin.

(b) Pickled

Acetic acid soaking: When samples IMA-treated (24 hs after application) were in contact /soaked in acetic acid solution for seven days, most of the remaining imazalil, from the previous step, was removed, leaving only a mean residue of 17 % of the 5 mg kg^{-1} applied (Figure 4B). With 0.5 mg kg^{-1} applied, imazalil was not detected down to the limit of detection of the method used. The pickling juices presented means of 47 and 28 % of imazalil for the two dipping levels, 0.5 and 5 mg kg^{-1}, respectively. On the other hand, when minicucumbers were used for pickling 7 days after imazalil application, the situation was rather different. At that stage, the raw minicucumbers taken for pickling presented already some penetration of imazalil into the inner layers. As seen in Figure 3, the penetration ratio peel/flesh/core was at freshly, 24 h, and at 7 days of storage: 90/ND/ND, 74/traces/ND, and 63/12/ND % for 0.5 mg kg^{-1} IMA-treated minicucumbers, and 90/ND/ND, 81/10/traces, and 65/18/7 % for 5.0 mg kg^{-1}, respectively. The effect of brining and pickling on imazalil removal would therefore expected to be less after 7 days than at 24 hs after application, either due to the length of time or due to its transfer to inner layers (Figures 4A and B). A mean removal of 24 and 23 % of imazalil from the minicucumbers into the brine was observed (after 7 days imazalil treatment) and of 24 and 32 % into the pickling juice for the two concentrations, leaving therefore only low amounts of imazalil in the final pickled product, i.e., < MRL at 5.0 mg kg^{-1} (7 % = 0.35 mg kg^{-1}) and none (not detected) at 0.5 mg kg^{-1}. As far as the pKa of imazalil and vinegar addition are concerned, the low pH medium produced by the addition of the acetic acid solution around the imazalil-treated minicucumbers, improved its solubility, contributing also to a better removal.

Temperature that acetic acid was added: Another effect that could also contribute to imazalil removal, was the temperature at which the vinegar is added onto the minicucumbers at the pickling stage. It was added boiling and that could improve the release of imazalil from the skin.

Skin tenderising: the acetic acid softens the minicucumber skin during pickling by hydrolysing pectin, thus reducing skin integrity, with consequent leakage of liquids from within the minicucumbers – another factor to release imazalil to the acidic medium.

Time: (b.4.1) The time that the minicucumbers were immersed in the acetic acid solution was quite long, and thus plays an important role on imazalil removal, compared to that of other cucurbits (courgette and marrow) which are submitted only to a relatively short immersion in boiling water as part of the cooking process; however, in the latter case, there was the added effect of temperature. (b.4.2) Another phenomenon, that could take place, is the re-adsorption of the imazalil removed. That means: after a certain period of time, instead of the imazalil removed staying in the juice, it could be re-adsorbed into the pickles and that could occur during long storage of the ready pickles i.e., during its shelf life.

In relation to degradation of imazalil, it should be noted that no T_{824} (Figure 1b) was detected at any stage of pickling, i.e., for the IMA-treated minicucumbers stored for 7 days and pickled for two weeks. It is reported in the literature that imazalil is totally transformed to T_{824} after 24 days of application on the leaves of a living cucumber plant, in accord with its systemic action[13]. However, that occurs before harvest while the metabolism of the plant is fully active. Once non-climacteric fruits are separated from the plant, metabolism slows down. The acetic acid and temperature did not have any effect on its degradation. As far as the bound residues are concerned, some reduction of the total residue was observed, but other factors could contribute to losses apart from binding to cell constituents.

CONCLUSIONS

The results obtained showed that the pickling process had a substantial effect on imazalil removal by both stages of processing: (a) brining and (b) pickling solutions. The pickling process behaved like an extended extraction. The contact with acetic acid solution after the brine dip (24 hours) lead to a maximum transfer of the remaining imazalil to the pickling juice at the MRL level. The percentage of removal from minicucumbers to the brine/pickling juice of 34/47 and 33/28 %, for 0.5 and 5 mg kg^{-1} after 24 h, respectively, remaining some residue in the 5.0 mg kg^{-1} level. As far as the pKa of imazalil is concerned, the acetic acid medium surrounding the IMA-treated minicucumbers improved imazalil solubility leading to a better removal.

Different domestic processings lead to different fungicide removal from vegetables extensive to cucurbits[3]. Also the imazalil treatment used on traw samples and time of storage prior to processing might interfere on removal (Scussel, 1995b). Although the ordinary pickling process carried out in a household kitchen could lead to only low imazalil residues for the concentrations under

study, the same may not be true for higher concentrations than those used here (>5.0 mg kg^{-1}). Considering that, minicucumbers should reach consumers with imazalil residues at/or below the MRL, from the data obtained, one can conclude that the likelihood of imazalil being detected at the end of an ordinary pickling process is remote (for the amounts studied and 5 times its value).

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