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Pesticide Residue Reduction in Selected Vegetables Using Rice-Bran

Atsuko Adachi* **and Toshio Okano**

Department of Hygienic Sciences, Kobe Pharmaceutical University, Motoyamakitamachi 4-chome, Higashinada-ku, Kobe 658–8558, Japan

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The efficiencies of rice-bran for removing pesticides from vegetables was studied. Chlorothalonil was successfully removed from commercial eggplant and cucumber with an average removal efficiency of 95% after 5 min when vegetables were pickled in rice-bran paste. Moreover, tetradifon was removed from commercial eggplant, commercial cucumber, and cultivated cucumber with an average removal efficiency of 80% after 10 min. The mechanism of pesticides removal by rice bran was attributed to the uptake of pesticides into intracellular particles called spherosomes.

Key words —— chlorothalonil, tetradifon, rice-bran, eggplant, cucumber

INTRODUCTION

Pesticides are considered to be indispensable for the production of an adequate food supply for an increasing world population and for the control of insectborne diseases. Many pesticides are, however, toxic substances and persistent in character. Some of the pesticides are endocrine disrupting compounds.[1–3] Food is the main source of exposure of the general population to pesticides and accounts for more than 90% of total exposure.[4] Pesticide residues in food and crops are a direct result of application of pesticides to crops growing in the field, and to a lesser extent from pesticide residues remaining in the soil.[5] Photochemical behaviors of pesticides on the surface of solids such as agricultural leaves and fruits have been investigated.[6–8] Yoshida *et al.*[9] reported the removal rates of pesticide residues in vegetables and fruits by washing with water. Furthermore, there has been much interest in the use of organoclays as adsorbents to remove and remediate pesticide in contaminated agricultural soil.[10–12] Because of the hydrophilic, negative character of their surfaces, clay minerals, particularly phyllosilicates, have been shown to be very good adsorbents for cationic and highly polar pesticides, but their adsorption capacity for poorly soluble, nonionic organic compounds is usually low.[13–15] However, few detailed investigations have been made on the removal of pesticide residues in vegetables. We have previously reported that rice bran effectively removed pesticides from aqueous solution using the batch system in laboratory tests.[16] The objecticees of this study were to investigate the use of rice-bran for removing pesticide residues from vegetable skin.

MATERIALS AND METHODS

Materials —— Rice bran, cucumber, and eggplant were purchased at a local market. In addition, cucumber grown in our laboratory was also used. Plants of cucumber were grown in a plastic-house at Kobe Pharmaceutical University from February to May 2004. Three days prior to harvest, commercial tetradifon emulsion (8% tetradifon, Agro-Kanesho Co. Ltd., Tokyo, Japan) treatments were carried out with a sprayer. Pesticide standards for pesticide residue analysis were purchased from Wako Pure Chemical Industries Ltd. (Amagasaki, Japan). Sep-Pak silica and florisil cartridges were purchased from Waters Corporation (Milford, Massachusetts, U.S.A.).

Pickling Rice-Bran Paste and Samples —— Rice bran (200 g) and distilled water (200 ml) were mixed to form a paste. A pesticide standard solution (chlorothalonil or tetradifon 100 μg/ml acetone) was uniformly applied on the commercial vegetable (eggplant or cucumber, 20 g) skin using a micropipette (total volume 0.1 ml). After 30 min the vegetables were pickled in the rice-bran paste. Cultivated cucumbers were pickled in the rice-bran paste without application of standard solution.

Determination of Pesticides in Vegetables —— After pickling at various time intervals, vegetables were removed from the rice-bran paste and cleaned with paper (Wipers S-200, Kimberly-clark Corporation, Tokyo, Japan), cut into small pieces, and homogenized. Pesticides were extracted from the veg-

*To whom correspondence should be addressed: Department of Hygienic Sciences, Kobe Pharmaceutical University, Motoyamakitamachi 4-chome, Higashinada-ku, Kobe 658–8558, Japan. Tel.: +81-78-441-7525; Fax: +81-78-441-7565; E-mail: a-adachi@kobepharma-u.ac.jp

Table 1. Removal Efficiency of Rice Bran or Spherosomes Isolated from Rice Bran for Pesticides

Substance	Rice bran			Spherosomes		
	Concentration (μg/l)		Removal efficiency(%)	Concentration (μg/l)		Removal efficiency(%)
	Before treatment	After treatment		Before treatment	After treatment	
Chlorothalonil	50.0	6.5–8.7	85.2 ± 0.2[a)]	50.0	8.8–10.9	79.6 ± 1.0[a)]
Tetradifon	50.0	2.7–4.3	93.2 ± 1.1[a)]	50.0	6.5– 8.7	85.6 ± 1.0[a)]

a) Data represent the mean ± S.D. of four separate samples. Rice bran: 10 g/l, All spherosomes obtained from rice bran (1 g) were used for this experiment. Pesticide: 50 μg/l, Reaction time: 1.5 hr, pH: 7.0, Pesticide (10.0 mg) was dissolved in methanol (1 ml), and the solution was extended to 100 ml with distilled water. In addition, it was adequately diluted with distilled water, and 100.0 ml was used for the experiment. The rice bran (1.0 g) was added.

etables (20 g) with 100 ml acetone and the extract was concentrated to the volume of 50 ml under reduced pressure. After addition of 200 ml sodium chloride (5%) to the solution, pesticides were extracted with 100 ml *n*-hexane and the *n*-hexane layer was concentrated to 5 ml. The purification of the extract for chlorothalonil was carried out by transferring the 5 ml sample to a silica gel column (Waters, silica cartridges, 30 × 12 mm), eluting with 100 ml *n*-hexane-diethyl ether (95 : 5). The elute was concentrated to a final volume of 10 ml under reduced pressure before gas chromatography (GC) analysis. The purification of the extract for tetradifon was carried out using a florisil column (Waters, Florisil Cartridges, 30 × 12 mm), eluting with 100 ml *n*-hexane-diethyl ether (85 : 15). The elute was concentrated to a final volume of 10 ml under reduced pressure before GC analysis. The GC analysis was performed on a Shimadzu Model GC-14A (Shimadzu, Kyoto, Japan) gas chromatograph equipped with a flame ionization detector and a capillary column (ULBON HR-52, 30 m × 0.53 mm). The column and injection port were maintained at 250°C, with the detector maintained at 280°C.

Blank samples were used and no interference was found in the determination of pesticide. Residual pesticide (%) was calculated by the following equation:

$$\text{Residual pesticide (\%)} = (X/M) \times 100$$

where X is the total pesticide remaining in vegetable (μg) after pickling and M is the total pesticide remaining in vegetable (μg) before pickling. M values of tetradifon and chlorothalonil were 9.6 and 9.3 μg, respectively. The average recoveries of pesticides applied on the vegetable skin were 96.4% for tetradifon and 92.5% for chlorothalonil.

Isolation of Spherosome ——— Spherosomes were isolated according to a modification of the procedure of Moreau *et al.*[17)] Rice bran (1 g) was ground with a mortar and pestle in a 40 ml grinding medium (20 mM sodium succinate, pH 5.6, containing 10 mM $CaCl_2$). The paste was filtered through four layers of cheesecloth, and the filtrate was centrifuged at 30000 *g* for 20 min. The spherosome pad was removed from the top of the centrifuge tube with a spatula. It was washed by resuspending in 40 ml fresh medium, and the resuspension was recentrifuged at 30000 *g* for 20 min. This process was repeated two more times. The yield of spherosome extracted from 1g rice bran was 0.12 g.

Batch Method for Investigating Removal Mechanism ——— Experiments were conducted in a batch system. A 100 ml sample solution of pesticide (50.0 μg/l, The preparation is shown in Table 1[16)]) was placed in a 100 ml glass stoppered Erlenmyer flask, to which 1 g of rice bran, or all spherosomes from rice bran (1 g) was then added. The samples were mixed with a magnetic stirrer for 1.5 hr. During mixing, the flasks were stopperd to prevent evaporative loss. The reaction mixture was filtered through filter paper (quantitative ashless no. 5A Toyo Roshi, Ltd., Japan) to remove the rice bran or spherosome. The initial 10 ml of filtrate was discarded because of the adsorption of pesticides by the filter paper. In control samples lacking rice bran or spherosomes, the subsequent filtrate after the discarded portion contained the same amount of chemical compounds as those in the original solution. Fifty ml of this filtrate was placed in a separatory funnel and 5 ml *m*-xylene was added to the solution. The mixture was shaken for 1 min. The separated *m*-xylene layer was subjected to GC. Pesticides were analyzed using the same gas chromatographic conditions as for chlorothalonil and tetradifon in vegetables. Then the removal efficiency for pesticide was calculated by the following equation:

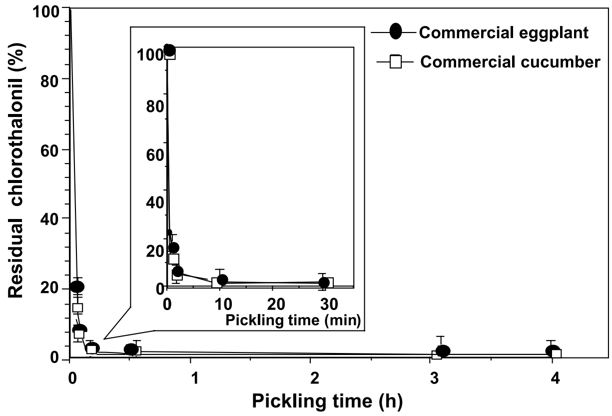

Fig. 1. Removal Efficiency of Chlorothalonil by Rice-Bran Paste
Data represent the mean ± S.D. of three separate samples.

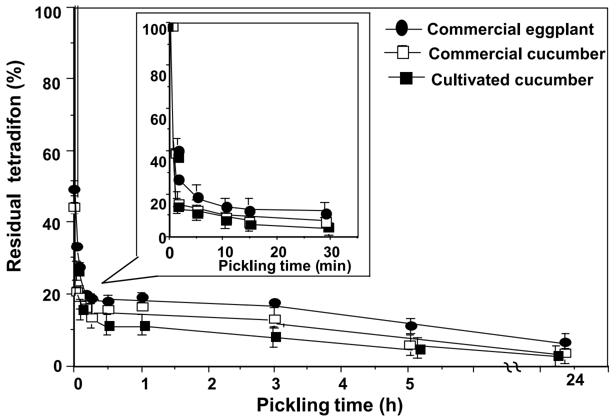

Fig. 2. Removal Efficiency of Tetradifon by Rice-Bran Paste
Data represent the mean ± S.D. of three separate samples.

$$\text{Removal efficiency (\%)} = [(Co - Ca)/Co] \times 100$$

where Co is the initial concentration of pesticide and Ca is the residual concentration of pesticide after reaction.

RESULTS AND DISCUSSION

Figure 1 shows removal efficiencies of rice-bran paste for chlorothalonil in commercial eggplant and cucumber. Chlorothalonil was successfully removed with an average removal efficiency of 95% after 5 min when vegetables were pickled in rice-bran paste.

Figure 2 shows removal efficiencies of rice-bran paste for tetradifon in commercial eggplant, commercial cucumber, and cultivated cucumber. The same pattern was also observed in cultivated cucumber. In all vegetables, tetradifon was removed with an average removal efficiency of 80% after 10 min. In cultivated cucumbers before pickling, tetradifon concentrations ranged from 0.35 to 0.72 μg/g (five separate cucumbers). Data represent the mean of three separate samples.

Next, we proposed the mechanism of removal of pesticides by rice-bran paste. We have previously reported that rice bran and defatted seed were effective in removal of organochlorine compounds such as chloroform, dichloromethane and benzene. Furthermore, it was confirmed that the spherosomes isolated from these materials were effective in removing these organic compounds.[18] Analytical and laser microscopic data have confirmed that the removal of organochlorine compounds and benzene is dependent on the uptake of these compounds into intracellular particles called spherosomes.[19] Spherosomes are widely distributed among plants and fungi.[20] Neither the function of spherosomes nor its analysis is well understood. Table 1[16] shows the removal efficiency of pesticides by both rice bran and spherosomes isolated from 1 g of rice bran. The removal by spherosomes was similar to that of rice bran. Based on these results, we concluded that removal of pesticides by rice-bran paste is dependent on the uptake into spherosomes as we already reported the removal of pesticides by batch method.[16]

Rice bran is a by-product of making polished rice from brown rice. Therefore, rice bran is very inexpensive, costing 1/50–1/40 that of activated carbon. Additionally, the use of rice bran is an example of effective utilization of waste matter. Taken together, this study suggest that the use of rice bran for removing pesticide residues from vegetables is efficient and cost-effective.

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