Correlation between Organochlorine Compounds or Benzene Adsorption Efficiency and Number of Spherosomes from Different Plant Sources

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Rice bran, wheat bran, rapeseed, linseed, okara, and sakekasu were evaluated for correlations between their effectiveness in adsorbing organochlorine compounds such as chloroform, dichloromethane or benzene and the number of spherosomes. Wheat bran contained the highest concentration of spherosomes, with a mean level of 4.37 × 10^{10} spherosomes/g. The lowest was sakekasu, with a mean level of 2.28 × 10^{10} spherosomes/g. There was a high correlation between removal efficiency and the number of spherosomes.

Key words —— rice bran, wheat bran, okara, sakekasu, spherosome

INTRODUCTION

We have previously reported that rice bran and defatted rice seed were effective in adsorbing pesticides and organochlorine compounds such as chloroform, dichloromethane and benzene.[1,2] Furthermore, it was confirmed that the spherosomes isolated from these adsorbents were effective in removing these organic compounds.[1,2] 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.[1,2] Spherosomes are widely distributed among plants and fungi but have not been observed in animal cells.[3] However, spherosomes occur prominently in seeds.[4] The function of spherosomes is not well understood. Spherosomes are intracellular oil-containing particles about 1 μm in diameter, with an osmiophilic matrix, and they are bound by unusual single-line membranes.[5] We hypothesize that these special membranes may be influential in the uptake of chemical compounds into the spherosomes. We assumed that lees materials such as wheat bran, okara, and sakekasu would contain spherosomes, as would rice bran. Lees materials, which are residues from the manufacturing processing of food plants, are considered a waste product. However, lees materials could be efficiently used in industry for separation, purification, and recovery processes, then a considerable reduction in costs would be possible. The measurement of spherosome levels is important for using lees materials as adsorbent materials for the removal of organochlorine compounds and benzene. Since no previous studies have been conducted to evaluate the activity of spherosomes, we tried to count the number of spherosomes using a hemacytometer under a light microscope. In addition, the relationship between removal efficiency and number of spherosomes was investigated.

MATERIALS AND METHODS

Procedure for Removal —— Rice bran, okara, and sakekasu were purchased at a local market. Wheat bran and defatted seed (rapeseed, linseed), provided by Fujiwara, Inc. (Osaka, Japan) and Nissin Oil Mills, Inc. (Yokohama, Japan), respectively, were used. 100 ml of sample solution, including chemical compounds, was taken in a 100 ml glass stoppered Erlenmyer flask, to which 0.1–1 g of lees materials was added, and the solution was mixed with a stirrer. The reaction mixture was filtered through a filter paper to remove the lees materials. The initial 10 ml of filtrate was discarded because of the adsorption of chemical compounds by the filter paper. In control samples lacking lees materials, the subsequent filtrate after the discarding 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 of m-xylene was added to the solution. The mixture was shaken for 1 min. The separated m-xylene layer was subjected to gas chromatography (GC) to assess the concentrations of these compounds. To quantify the evaporation loss of the chemical compounds, control experiments were performed following the same

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procedure, except for the absence of lees materials. Maximum loss was about 5 (4.7 ± 0.22) %, although almost no loss was detected in most cases. The removal efficiency of lees materials was calculated by eliminating the contribution due to evaporation loss. The assay of chemical compounds was performed on a Shimadzu Model GC-14B gas chromatograph equipped with an electron capture detector and a capillary column (ULBON HR-52, 30 m × 0.53 mm) or Shimadzu Model GC-6A gas chromatograph equipped with a flame ionization detector and glass column (3 m × 3 mm) packed with 20% silicon DC 550 on 60–80 mesh Chromosorb W. Both the column and injection port were maintained at 90°C, and the detector at 120°C.

Sample Solution ——A chemical compound (1.0 g) was dissolved in distilled water, and the volume was made up to 1000 ml with distilled water. In addition, it was diluted 10 fold, and 100.0 ml was used for the experiment.

Determination of Number of Spherosomes —— 0.5 g of sample was ground in 40 ml of a grinding medium of 0.15 M Tricine buffer (pH 7.5), containing 0.6 M Sucrose, 1 mM EDTA, 10 mM KCl, 1 mM $MgCl_2$ and 2 mM dithioerythritol with a mortar and pestle.[6] The suspension was diluted to 1000 ml with distilled water, and the number of spherosomes was determined using a hemacytometer under a light microscope.

Statistical Analysis —— Values are shown as means ± S.D. Data were analyzed using one-way analysis of variance (ANOVA) and, when appropriate, by a Student-Newman-Keul test. Results were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The removal of chloroform, dichloromethane or benzene was examined using 6 different lees materials (8 kinds of rice bran, 2 kinds of rapeseed, 3 kinds of linseed, 4 kinds of wheat bran, 9 kinds of okara, 9 kinds of sakekasu). Table 1 shows the removal efficiencies of these materials for chloroform, dichloromethane or benzene at a reaction time of 90 min. Data represents the mean of four separate samples. The removal efficiencies for chloroform, dichloromethane and benzene varied from 48.6% to 93.3%, 37.3% to 87.3% and 45.7% to 94.4%, respectively. When the average removal efficiencies for these three chemical compounds by rice bran, rapeseed, linseed, wheat bran, okara, or sakekasu were compared, wheat bran was highest and the next was linseed (Fig. 1). We previously reported that the removal of pesticides and organochlorine compounds by rice bran and defatted seed can be attributed to the uptake by intracellular particles called spherosomes.[1,2] The historical background of the spherosome, which is considered by some authors to be the direct precursor of the oil body, has been reviewed by Frey-Wyssling *et al.*[7] Sorokin[8] concludes that spherosomes and oil bodies represent separate entities, and that spherosomes are a common feature of most vegetative cells in higher plants, even in cells which do not produce oil, whereas oil bodies are restricted to cells producing oil. She further indicated the differences between the two structures by stating that spherosomes have a limiting membrane, whereas oil bodies do not. Spherosomes have been observed in situ by several investigators,[9–11] but have not been isolated from ungerminated seeds. Clifford and Novellie[12] reported the isolation of spherosomes from ungerminated seeds and described their composition.

The measurement of spherosome levels is important for using lees materials as adsorbents for the removal of organochlorine compounds and benzene. We noticed that spherosomes are particles, and tried to count the number of spherosomes using a hemacytometer under the light microscope. Spherosomes from all six of the sources (rice bran, rapeseed, linseed, wheat bran, okara, and sakekasu) we examined were very similar under the light microscope. The particles have well defined edges, possibly surrounded by a membrane. The abundance of particles with diameters of 1 to 10 μm was evident. The number of spherosomes in all the samples was independently counted. The number varied from 1.82×10^{10} for sakekasu to 4.95×10^{10} particle/g for wheat bran (Table 1). As shown in Table 1, wheat bran contained the highest concentration of spherosomes, with a mean level of 4.37×10^{10} spherosomes/g. The lowest was sakekasu, with a mean level of 2.28×10^{10} spherosomes/g.

The relationship between removal efficiency and the number of spherosomes was investigated (Fig. 2). The number of spherosomes in lees materials increased with increasing removal efficiency of organochlorine compounds and benzene by the lees materials. The correlation coefficients between the number of spherosomes and removal efficiency for chloroform, dichloromethane and benzene were 0.794,

Table 1. Removal Efficiency of Rice Bran, Rapeseed, Linseed, okara, Sakekasu or Wheat Bran Hydrocarbon and Number of Spherosomes

Substance	Number of spherosomes (particle/g)	Removal efficiency (%)		
		Chloroform	Dichloromethane	Benzene
Rice bran				
1	3.33×10^{10}	64.9	67.6	72.5
2	3.32×10^{10}	61.9	66.7	72.2
3	3.18×10^{10}	69.7	71.5	94.4
4	3.21×10^{10}	70.8	63.7	78.2
5	3.44×10^{10}	76.2	65.4	71.8
6	4.43×10^{10}	85.6	78.9	74.8
7	3.64×10^{10}	83.1	77.4	72.4
8	4.04×10^{10}	82.3	75.4	71.4
Mean ± S.D.	$(3.57 \pm 0.44) \times 10^{10}$	74.3 ± 8.9	70.8 ± 5.8	76.0 ± 7.8
Rapeseed				
1	4.43×10^{10}	72.4	69.5	47.8
2	3.59×10^{10}	71.4	68.4	45.7
Mean	4.01×10^{10}	71.9	69.0	46.8
Linseed				
1	3.63×10^{10}	87.6	86.8	78.3
2	3.93×10^{10}	88.3	87.3	80.7
3	3.60×10^{10}	86.5	86.0	72.7
Mean ± S.D.	$(3.72 \pm 0.18) \times 10^{10}$	87.5 ± 0.7	86.7 ± 0.5	77.2 ± 3.4
Wheat bran				
1	4.95×10^{10}	93.3	86.7	90.8
2	4.45×10^{10}	87.6	86.7	89.0
3	4.37×10^{10}	85.3	85.0	85.7
4	3.72×10^{10}	84.9	84.8	84.5
Mean ± S.D.	$(4.37 \pm 0.51) \times 10^{10}$	87.8 ± 3.4	85.8 ± 0.9	87.5 ± 2.5
Okara				
1	4.06×10^{10}	77.0	63.4	74.1
2	4.41×10^{10}	78.3	72.4	79.0
3	4.41×10^{10}	78.6	71.3	77.3
4	3.43×10^{10}	86.2	84.9	82.7
5	3.72×10^{10}	88.5	86.2	74.6
6	3.95×10^{10}	88.7	87.2	81.2
7	4.17×10^{10}	82.1	84.8	86.2
8	3.51×10^{10}	79.0	80.2	82.2
9	3.40×10^{10}	77.5	78.0	80.9
Mean ± S.D.	$(3.90 \pm 0.40) \times 10^{10}$	81.8 ± 4.5	78.7 ± 7.7	79.8 ± 3.7
Sakekasu				
1	1.82×10^{10}	57.5	56.6	51.5
2	2.04×10^{10}	55.8	49.7	58.8
3	2.42×10^{10}	57.4	38.5	63.0
4	2.40×10^{10}	54.4	48.2	57.0
5	2.58×10^{10}	59.8	37.3	58.1
6	2.36×10^{10}	50.2	50.9	52.7
7	2.34×10^{10}	50.0	50.0	50.5
8	2.32×10^{10}	49.5	48.5	49.5
9	2.28×10^{10}	48.6	47.8	47.3
Mean ± S.D.	$(2.28 \pm 0.24) \times 10^{10}$	53.7 ± 3.9	47.5 ± 5.7	54.3 ± 4.9

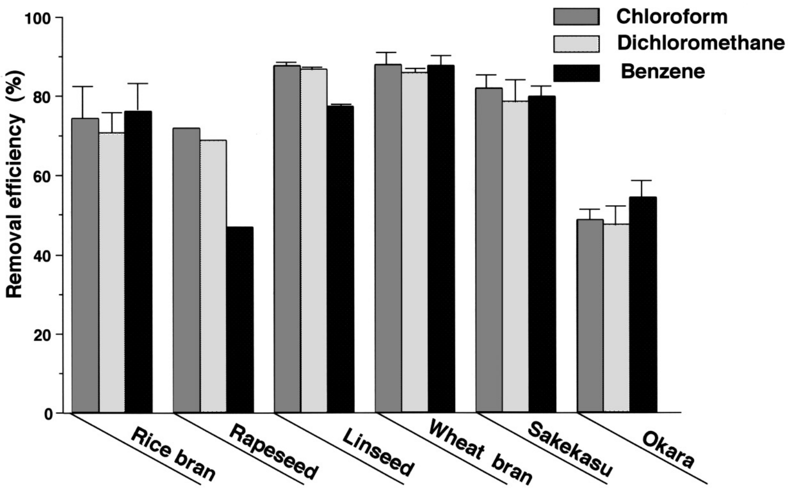

Fig. 1. Removal Efficiency of Rice Bran, Rapeseed, Linseed, Wheat Bran, Sakekasu or Okara for Hydrocarbon

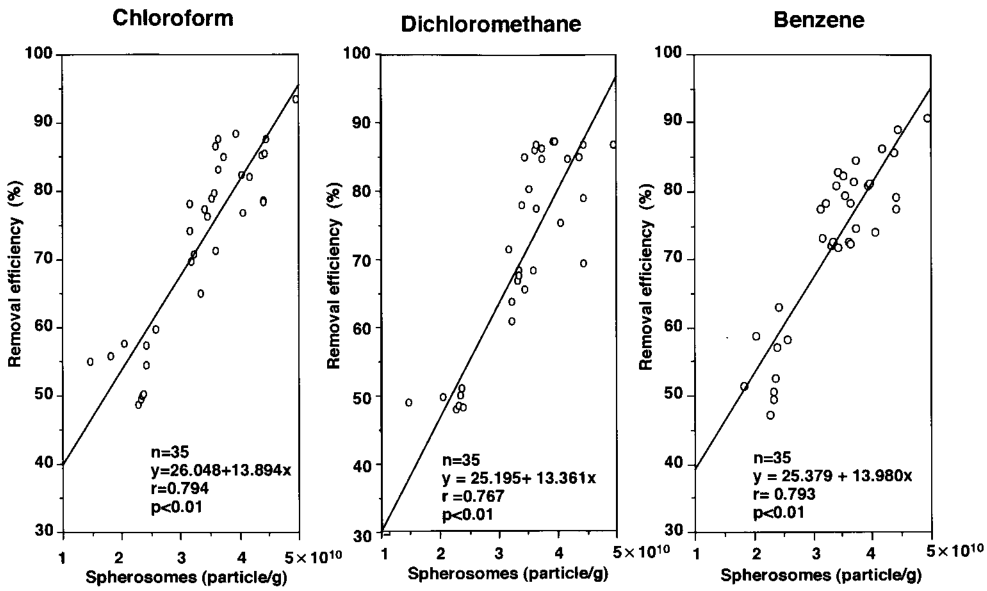

Fig. 2. Correlation between Removal Efficiency and Number of Spherosomes

0.767 and 0.793, respectively ($p < 0.01$). Significant correlations between the respective removal efficiency and number of spherosomes were observed. These results suggest that counting spherosomes using a hemacytometer is useful in the evaluation of removal efficiency of organochlorine compounds and benzene by spherosomes. These lees materials which we examined are residues from the manufacturing process of food plant, and are therefore a waste product. This process also offers a significant use for the lees materials in terms of recycling. From this perspective, the use of these lees materials as adsorbents *et al.* are prospective.

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