Determination of Spherosomes from Lees Materials

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To evaluate the effectiveness in adsorbing organochlorine compounds such as chloroform, dichloromethane, or benzene by lees materials, the determination of spherosomes from different lees materials was established by using a hemacytometer under an optical microscope. Rice bran, wheat bran, rapeseed, linseed, okara, and sakekasu were used for this investigation, and activated carbon was also used as a standard adsorbent material. The number of spherosomes varied from 1.82×10^{10} particles/g for sakekasu to 4.95×10^{10} particles/g for wheat bran. There was a high correlation between the removal efficiency in adsorbing organochlorine compounds such as chloroform, dichloromethane, or benzene by lees materials and the number of spherosomes from different lees materials.

KEYWORDS: Spherosome; rice bran; wheat bran; rapeseed; chloroform

INTRODUCTION

Organochlorine compounds such as chloroform, dichloromethane, or benzene have been used in large quantity in various manufacturing fields for their excellent chemical properties. A U.S. National Cancer Institute (NCl) report shows that chloroform caused cancer in rats and mice under laboratory test conditions (*1*). Furthermore, both dichloromethane and benzene are now recognized to be two of the stable carcinogens in our environment. The problem with regard to the treatments of these compounds has not yet been solved, although several methods for the decomposition of organochlorine compounds such as ozonation (*2*) or ultraviolet irradiation (*3*−*5*) have been proposed. We have previously reported that the removal of pesticides and organochlorine compounds by rice bran and defatted seed can be attributed to uptake by intracellular particles called spherosomes (*6, 7*). Spherosomes are widely distributed among plants and fungi (*8*). Neither the function of spherosomes nor its analysis is well understood. The measurement of spherosome levels is important for evaluating lees materials as adsorbents for the removal of organochlorine compounds and benzene. This study was conducted to determine the number of spherosome in lees materials using a hemacytometer.

MATERIALS AND METHODS

Apparatus. The assay of chloroform, dichloromethane, or benzene 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 a Shimadzu model GC-6A gas chromatograph equipped with a flame ionization detector and a glass column (3 m × 3 mm) packed with 20% silicon DC 550 on 60−80 mesh Chromosorb W. An improved Neubauer counting chamber

Table 1. Number of Spherosomes from Rice Bran, Rapeseed, Linseed, Okara, Sakekasu, or Wheat Bran

substance	no. of spherosomes (particles/g)	substance	no. of spherosomes (particles/g)
rice bran		okara	
1	3.33×10^{10}	1	4.06×10^{10}
2	3.32×10^{10}	2	4.41×10^{10}
3	3.18×10^{10}	3	4.41×10^{10}
4	3.21×10^{10}	4	3.43×10^{10}
5	3.44×10^{10}	5	3.72×10^{10}
6	4.43×10^{10}	6	3.95×10^{10}
7	3.64×10^{10}	7	4.17×10^{10}
8	4.04×10^{10}	8	3.51×10^{10}
mean ± SD	$(3.57 \pm 0.44) \times 10^{10}$	9	3.40×10^{10}
		mean ± SD	$(3.90 \pm 0.40) \times 10^{10}$
rapeseed			
1	4.43×10^{10}	sakekasu	
2	3.59×10^{10}	1	1.82×10^{10}
mean	4.01×10^{10}	2	2.04×10^{10}
		3	2.42×10^{10}
linseed		4	2.40×10^{10}
1	3.63×10^{10}	5	2.58×10^{10}
2	3.93×10^{10}	6	2.36×10^{10}
3	3.60×10^{10}	7	2.34×10^{10}
mean ± SD	$(3.72 \pm 0.18) \times 10^{10}$	8	2.32×10^{10}
		9	2.28×10^{10}
wheat bran		mean ± SD	$(2.28 \pm 0.24) \times 10^{10}$
1	4.95×10^{10}		
2	4.45×10^{10}		
3	4.37×10^{10}		
4	3.72×10^{10}		
mean ± SD	$(4.37 \pm 0.51) \times 10^{10}$		

(Kayagaki Works, Tokyo, Japan) and an optical microscope (Leica Microsystems, Tokyo, Japan) were used for counting the number of spherosomes.

Materials. Rice bran, okara, and sakekasu were purchased at a local market. Wheat bran and defatted seed (rapeseed, linseed) were provided by Fujiwara, Inc. (Osaka, Japan) and Nissin Oil Mills, Inc. (Yokohama,

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Table 2. Removal Efficiency of Rice Bran, Rapeseed, Linseed, Okara, Sakekasu, Wheat Bran, or Activated Carbon for Hydrocarbons

substance	removal efficiency (%)			substance	removal efficiency (%)		
	chloroform	dichloromethane	benzene		chloroform	dichloromethane	benzene
rice bran				okara			
1	84.9	77.6	72.5	1	77.0	63.4	74.1
2	81.9	76.7	72.2	2	78.3	72.4	79.0
3	79.7	71.5	94.4	3	78.6	71.3	77.3
4	80.8	73.7	78.2	4	86.2	84.9	82.7
5	86.2	75.4	71.8	5	88.5	86.2	74.6
6	85.6	78.9	74.8	6	88.7	87.2	81.2
7	83.1	77.4	72.4	7	82.1	84.8	86.2
8	82.3	75.4	71.4	8	79.0	80.2	82.2
				9	77.5	78.0	80.9
mean ± SD	83.1 ± 2.3	75.8 ± 2.4	76.0 ± 7.8				
				mean ± SD	81.8 ± 4.5	78.7 ± 7.7	79.8 ± 3.7
rapeseed				sakekasu			
1	72.4	69.5	47.8	1	57.5	56.6	51.5
2	71.4	68.4	45.7	2	55.8	49.7	58.8
mean	71.9	69.0	46.8	3	57.4	38.5	63.0
linseed				4	54.4	48.2	57.0
1	87.6	86.8	78.3	5	59.8	37.3	58.1
2	88.3	87.3	80.7	6	50.2	50.9	52.7
3	86.5	86.0	72.7	7	50.0	50.0	50.5
				8	49.5	48.5	49.5
mean ± SD	87.5 ± 0.7	86.7 ± 0.5	77.2 ± 3.4	9	48.6	47.8	47.3
wheat bran				mean ± SD	53.7 ± 3.9	47.5 ± 5.7	54.3 ± 4.9
1	93.3	86.7	90.8				
2	87.6	86.7	89.0	activated carbon			
3	85.3	85.0	85.7	1	92.5	85.7	89.6
4	84.9	84.8	84.5	2	90.5	87.5	90.0
				3	90.2	84.3	94.4
mean ± SD	87.8 ± 3.4	85.8 ± 0.9	87.5 ± 2.5	4	88.5	87.5	90.3
				mean ± SD	90.4 ± 1.4	86.0 ± 1.2	91.2 ± 2.1

Japan), respectively. Activated carbon (powder, coal base carbon) of practial grade was purchased from Wako Pure Chemical Industries Ltd. (Amagasaki, Japan).

Determination of Spherosomes. Sample (0.5 g) 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 (*9*). The suspension was diluted to 1000 mL with distilled water, and 100 μL of the solution was subjected to the improved Neubauer counting chamber. The chamber is placed on the microscope, and the light is adjusted. Improved Neubauer ruling consists of a square measuring 3 by 3 mm subdivided into nine secondary squares, each 1 by 1 mm. The total number of the smallest 0.05 by 0.05 mm squares in the central square is 400. As a rule, 5 of the tertiary squares, amounting to 80 of the smallest squares, were used. The number of spherosomes was calculated according to the formula

$$\text{no. of spherosomes per } \mu\text{L} = E \times 400/80 \times 10$$

where *E* is the total number of spherosomes counted in 80 squares and 10 is a factor transforming the surface of the square millimeter to the volume in cubic millimeters.

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 and then diluted 10-fold; 100.0 mL was used for the experiment.

Procedure for Removal. One hundred milliliters 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 or activated carbon was added, and the solution was mixed with a stirrer for 90 min at room temperature (22 ± 2 °C). The reaction mixture was filtered through a filter paper to remove the lees materials or activated carbon. The initial 10 mL of filtrate was discarded because of the adsorption of chemical compounds by the filter paper. In control samples without lees materials, the subsequent filtrate after the discarded portion contained the same amount of chemical compounds as those in the
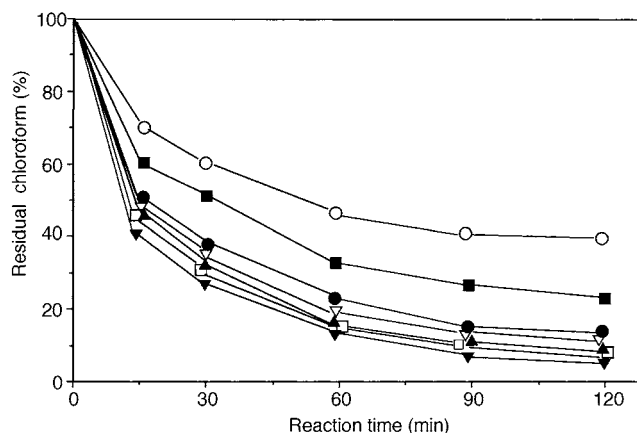

Figure 1. Removal efficieny of rice bran, rapeseed, linseed, okara, sakekasu, wheat bran, or activated carbon for chloroform: (▼) activated carbon; (□) wheat bran; (▲) linseed; (▽) rice bran; (●) okara; (■) rapeseed; (○) sakekasu. Experimental conditions: adsorbent, 10 g/L; chloroform concentration, 0.1 g/L; pH, 7.

original solution. The filtrate (50 mL) 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 procedure as the sample treatment, except for the absence of lees materials or activated carbon. 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 gas chromatograph. Both the column and injection port were maintained at 90 °C, and the detector was maintained at 120 °C.
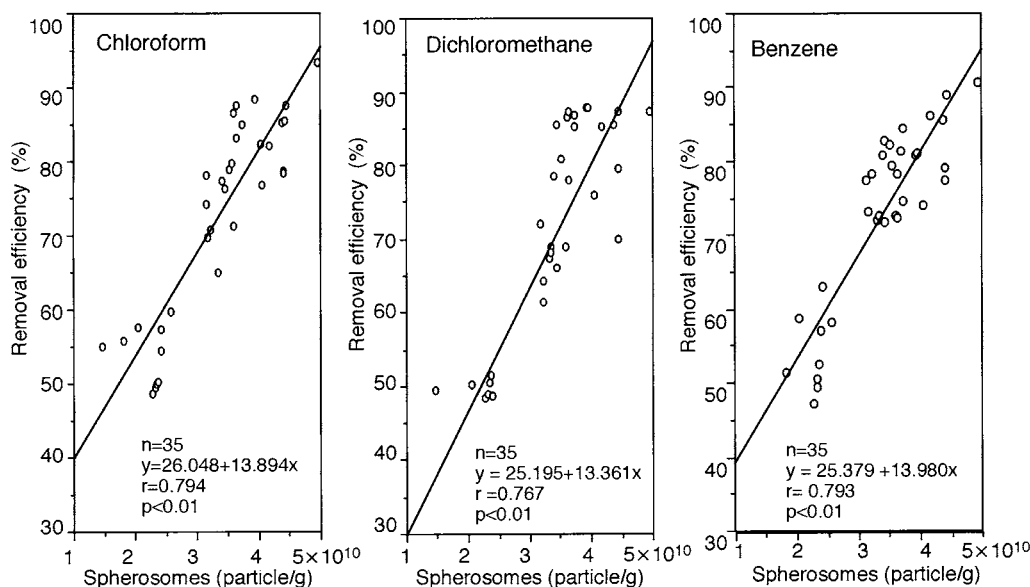
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Figure 2. Correlation between removal efficiency and number of spherosomes.

Statistical Analysis. Values are shown as means ± standard deviation (SD). Data were analyzed using one-way ANOVA and, when appropriate, by a Student−Newman−Keul test. Results were considered to be significant at $p < 0.05$.

RESULTS AND DISCUSSION

The hemacytometer method was applied to the determination of spherosomes in the six different lees materials (eight rice bran, two rapeseed, three linseed, four wheat bran, nine okara, nine sakekasu). Spherosomes were very similar under the optical microscope. The particles have well-defined edges, possibly surrounded by a membrane. The abundance of particles with diameters of $1-10$ μm was evident. The number of spherosomes in all of the samples was independently counted. As shown in **Table 1**, the number varied from 1.82×10^{10} particles/g for sakekasu to 4.95×10^{10} particles/g for wheat bran. Wheat bran contained the highest concentration of spherosomes, with a mean level of 4.37×10^{10} particles/g. The lowest was sakekasu, with a mean level of 2.28×10^{10} particles/g.

When the removal of chloroform, dichloromethane, or benzene was examined using six different lees materials and the activated carbon as a standard adsorbent material, the removal efficiencies for chloroform, dichloromethane, and benzene varied from 48.6 to 93.3%, from 37.3 to 87.3%, and from 45.7 to 94.4%, respectively (**Table 2**). Also, 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. The removal rates by wheat bran, linseed, rice bran, and okara were similar to that by activated carbon as a standard adsorbent material. The removal rate is initially fast, but after a short time the rate is reduced and the removal appears to plateau (**Figure 1**).

The number of spherosomes in lees materials increased with increasing removal efficiency of organochlorine compounds and benzene by the lees materials (**Figure 2**). The correlation coefficients between the number of spherosomes and the removal efficiency for chloroform, dichloromethane, and benzene were 0.794, 0.767, and 0.793, respectively ($p < 0.01$). Significant correlations between the respective removal efficiency and the number of spherosomes were observed. These

results suggest that employing a hemacytometer for the determination of spherosomes from different lees materials is useful in the evaluation of the removal efficiency of organochlorine compounds and benzene by spherosomes.

In conclusion, the determination of spherosomes from different lees materials was established by using a hemacytometer under a light microscope. The proposed method could be used for evaluating the effectiveness in adsorbing organochlorine compounds such as chloroform, dichloromethane, or benzene by lees materials.

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