

# Selective Fractionations of Rice Bran Oil Ester and $\gamma$ -oryzanol with Supercritical Carbon Dioxide

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## 「超臨界二酸化炭素による米ヌカ油エステル及び $\gamma$ -オリザノールの選択的抽出分離」

米ヌカ油関連物質の選択的抽出分離を、超臨界二酸化炭素を抽剤として超臨界抽出-カラム装置を使用し、検討した。

米ヌカ油エステルの抽出分離の選択性は、カラム温度の上昇と共に向上し、カラム温度343Kで、抽出初期にパルミテートが純度86%で得られた。硝酸銀シリカゲルカラムを用いると高選択的に抽出分離され、抽出圧力11.7-16.7 MPa で、パルミテート（純度91%）及びオレート（純度81%）がそれぞれ収率94%、91%で得られ、リノレートも純度84%で38%が回収された。また $\gamma$ -オリザノール、トリオレイン、オレイン酸の等量混合物から、抽出圧力12.7-21.6 MPa で、純度100%の $\gamma$ -オリザノールが86%の収率で抽出残留物として得られた。

### Introduction

World paddy production is about 400 million tons per year, 6% of which is bran; rice bran contains about 15% rice bran oil<sup>1)</sup>. Rice bran is a valuable oil resource that is potentially available in large quantities in Japan and other east Asian countries, however, rice bran oil is used less than other edible oils because of its high content of free fatty acids. The pharmacologically important fatty acids of rice bran oil are mainly composed of palmitic acid, oleic acid and linoleic acid. Rice bran oil also contains 1.5-3% of  $\gamma$ -oryzanol, a mixture of esters of furulic acid and triterpenalcohols<sup>2)</sup>.  $\gamma$ -Oryzanol is also pharmaceutially important because of its biological activities, such as promotion of growth, anti-oxidation capability, and

platelet aggregation<sup>3)</sup>. From these points of view, several attempts have been reported to utilize rice bran oil by esterification of free fatty acids with lipase<sup>4)</sup> or by refining with miscella<sup>5)</sup>. Supercritical carbon dioxide (SC-CO<sub>2</sub>) extraction, which utilizes the specific properties of SC-CO<sub>2</sub><sup>6, 7)</sup>, is a recognized technique for extracting of oils from lipids bearing materials<sup>8, 9)</sup> and for selectively separating of fatty acid methyl esters<sup>10)</sup>. Zhao<sup>11)</sup> and Taniguchi<sup>12)</sup> reported that the rice bran oil extracted with SC-CO<sub>2</sub> was lighter in color and contained less phosphorus than that extracted with hexane, and hence the extraction method might be effective in simplifying the process of edible rice bran oil. On the basis of these informations described above, we noticed that selective fractionation of useful components in rice bran oil with SC-CO<sub>2</sub> was of interest and an useful technique to utilize rice bran oil, because the components in rice bran oil enable to make effective use of their particular properties.

Fatty acids in rice bran oil differ not only

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in number of carbon atoms, but in the degree of unsaturation; thus, selective fractionation of these compounds is very difficult using only the SC-CO<sub>2</sub> extraction method because of the chemical and physical similarities of these acids. To enhance the degree of fractionation, a distillation column was used in conjunction with a SC-CO<sub>2</sub> extraction apparatus. Furthermore, silica gel impregnated with AgNO<sub>3</sub> (Silica gel-AgNO<sub>3</sub>) was used as a column stationary phase and packed in the column. Silica gel-AgNO<sub>3</sub> is a highly selective stationary phase in liquid chromatography with respect to the geometry and the degree of unsaturation of fatty acid esters or related triglycerides<sup>13)</sup>.

In the present work, we report the selective extraction of fatty acid methyl esters of rice bran oil (rice bran oil ester) with SC-CO<sub>2</sub> using a column-equipped extraction apparatus. Effects of extraction pressure and column temperature on the degree of fractionation of palmitate (C16:0), oleate (C18:1) and linoleate (C18:2) were investigated. Selective fractionation of a synthetic mixture containing  $\gamma$ -oryzanol was also performed to clarify the possibility of fractionation of  $\gamma$ -oryzanol from rice bran or related substances.

## Experimental

### Materials.

Fatty acid methyl ester of rice bran oil (rice bran oil ester) was prepared in the usual manner by methylation of oil extracted with hexane from rice bran<sup>14)</sup>. The composition of fatty acid methyl esters in rice bran oil ester is listed in Table 1. Oleic acid (Sigma, 99%) and triolein (Sigma, 99%) were used as received.  $\gamma$ -Oryzanol (Wako, for biochemical use) was purified by recrystallization from acetone (m.p. 435-438 K). Commercial grade of CO<sub>2</sub> was used as received. Silica gel-AgNO<sub>3</sub> was prepared by impregnating silica gel (Gasukoro Kogyo, Unisil Q) with AgNO<sub>3</sub> aqueous solution to the 10 w/w% level, following by drying at 413 K for 4 h.

### Apparatus and procedures.

The scheme for the extraction apparatus is shown in Fig.1. This apparatus was equipped with a column (100 cm long and 1/4 inch I.D.) at the upper end of an extractor. The extraction pressure was controlled by a backpressure regulator. Temperature was controlled within  $\pm 1$  K. Ten grams of silica gel-AgNO<sub>3</sub> was packed in the rear half of a column as illustrated in Fig. 1.

A weighed sample of rice bran oil ester (or a synthetic mixture of  $\gamma$ -oryzanol) was charged in a 0.1 liter extractor containing a layer of glass wool at its bottom. Extraction temperatures and extraction pressures were in the range of 313 to 373 K and 8.2 to 21.6 MPa, respectively. The extraction solution was flashed to atmospheric pressure across a heated metering valve, and the extract was accumulated in a cooled receiver. The amount accumulated was determined by weighing, and the corresponding volume of CO<sub>2</sub> was measured with a wet-flow totalizer. The flow rate of CO<sub>2</sub> was adjusted to at 1 liter/min under atmospheric pressure by a control valve. Entrainer was supplied at a constant flow rate of 0.1 ml/min with a pump for an entrainer.

### Analysis.

Fatty acid methyl esters compositions of fractions extracted from the rice bran oil ester were determined using a Varian 3000 gas chromatograph. The stainless steel column (3 m  $\times$  1/8 inch) was packed with Silar-10C. The oven temperature was maintained at 433 K for 5 min, and then was programmed to 483 K at the rate of 5 K/min. The proportion of  $\gamma$ -oryzanol in extracts was measured by a high performance liquid chromatograph equipped with RP-18 column (25 cm  $\times$  4 mm, Merck) using acetonitrile acetone (50:50, v/v) as a mobile phase and a UV detector (Jasco UVIDEC-100), set at 206 nm<sup>15)</sup>.

## Results and Discussion

### 1. Selective fractionation of fatty acid methyl

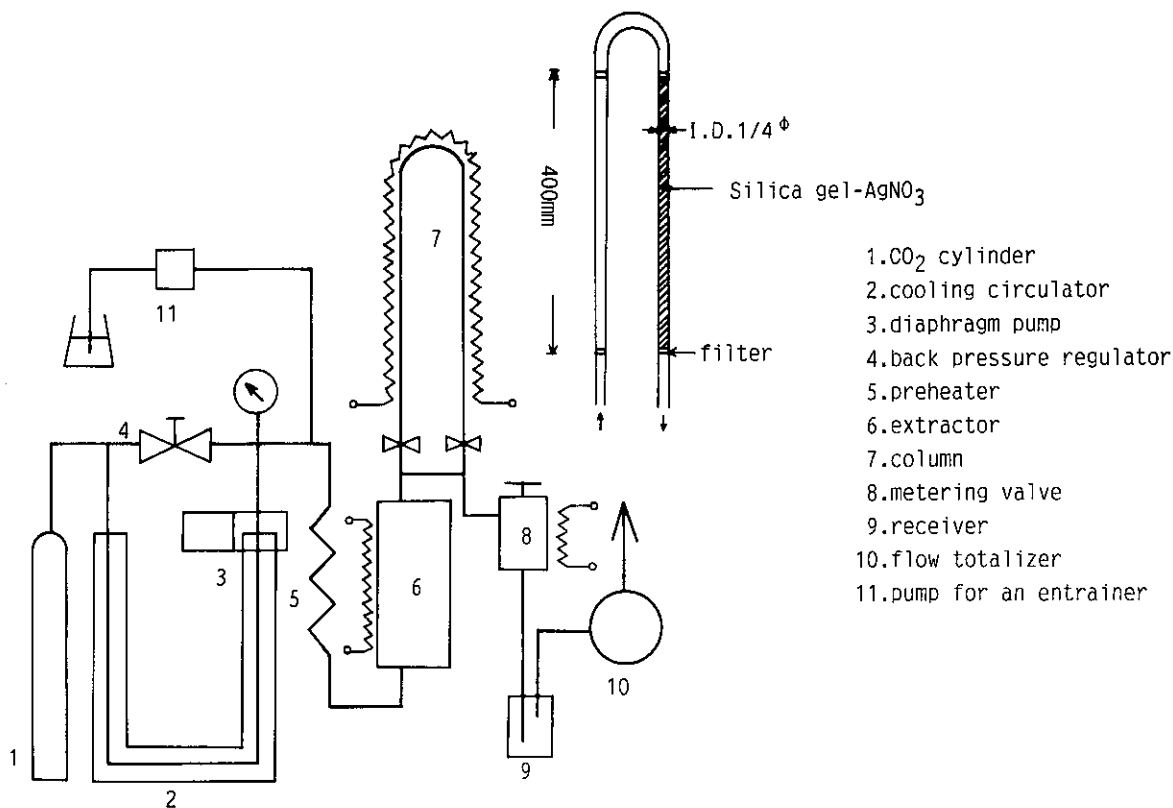


Fig.1 Extraction apparatus

esters from rice bran oil ester.

Methyl palmitate, methyl oleate, and methyl linoleate were fractionated from a 2.5 g of rice bran oil ester sample using a column. The extractor temperature was 313 K, and the column temperatures were 313, 343, and 373 K, and the pressures were in the range of 8.2–16.7 MPa. The fractionation selectivity was estimated on the basis of fatty acid methyl esters composition of the rice bran oil ester (methyl palmitate: 18.3%, methyl oleate: 41.9%, methyl linoleate: 36.0%; Table 1).

Effect of extraction pressure.

The effect of extraction pressure on the degree of fractionation of the esters from rice bran oil ester was investigated using a column without packing at 8.2 and 9.8 Mpa, and at a column temperature of 343 K. The compositions of palmitate, oleate, and linoleate in the ex-

Table 1 Composition and boiling points of fatty acid methyl esters in rice bran oil ester.

Fatty acid methyl ester	composition (%)	boiling point <sup>a</sup> (K/mmHg)
Palmitate(C <sub>16:0</sub> )	18.3	469/15
Oleate (C <sub>18:0</sub> )	41.9	485–486/15
Linoleate(C <sub>18:2</sub> )	36.0	484–485/16

<sup>a</sup> From "The dictionary of organic compounds", Oxford University press, New York, 1953.

tracts obtained from rice bran oil ester are plotted in Figure 2 as a function of the degree of extraction of rice bran oil ester at two different pressures. The proportions of palmitate at 8.2 and 9.8 MPa were in the ranges 38–20% and 28–18%, respectively, below 40% of the degree of extraction. These results indicate that the degree of fractionation was influenced moderately by extraction pressure, but

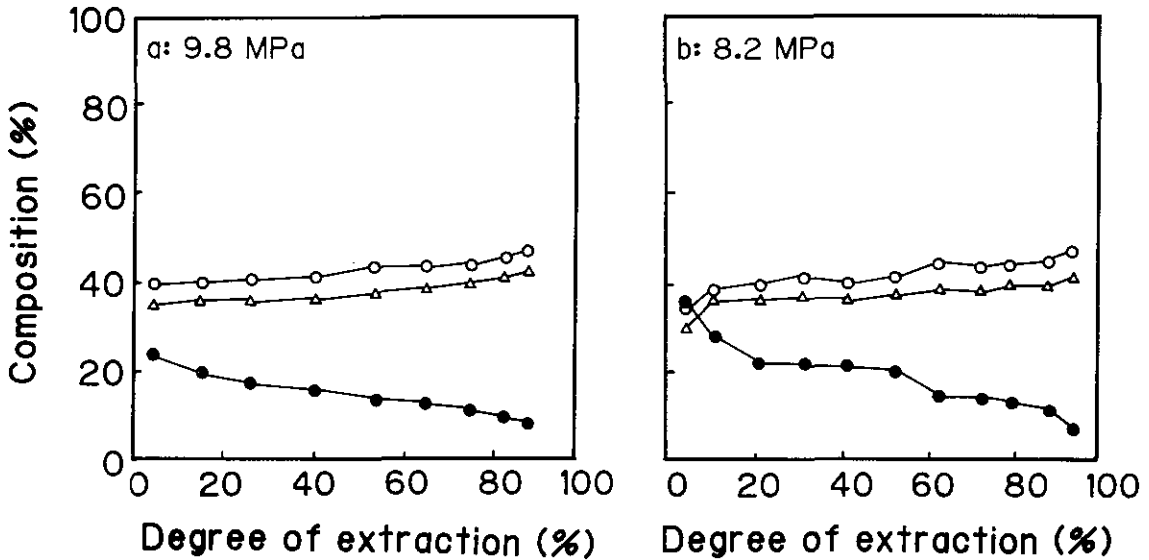


Fig.2 Influence of extraction pressure on ester compositions of extracts obtained during the supercritical CO<sub>2</sub> extraction of rice bran oil methyl ester at 8.2 and 9.8 MPa and a column temperature of 313 K. (●): C<sub>16:0</sub>; (○): C<sub>18:1</sub>; (Δ): C<sub>18:2</sub>

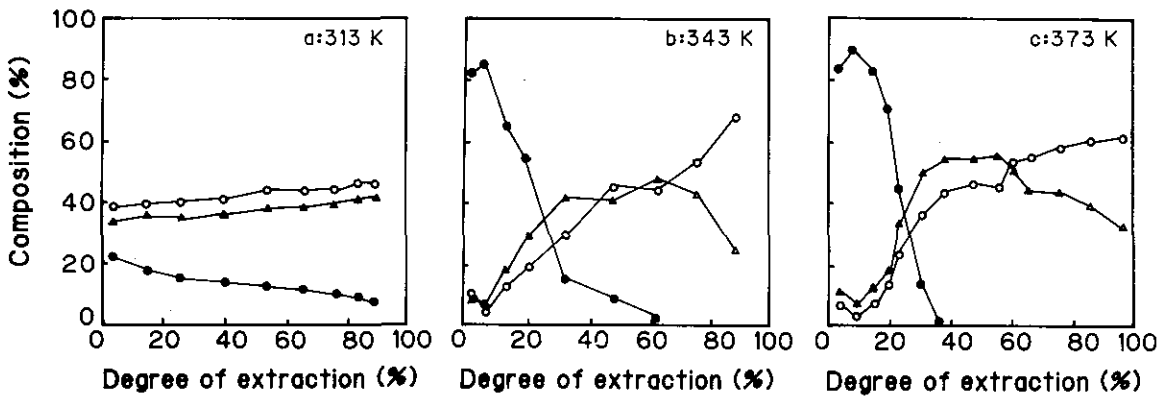


Fig.3 Influence of column temperature on ester compositions of extracts obtained during the supercritical CO<sub>2</sub> extraction of rice bran oil methyl ester at 9.8 MPa and different column temperatures. (●): C<sub>16:0</sub>; (○): C<sub>18:1</sub>; (Δ): C<sub>18:2</sub>

the effect of extraction pressure on the degree of fractionation was less than that of column temperature under the extraction condition (see Fig.3).

Effect of column temperature.

The effect of column temperature on the degree of fractionation of the esters from rice

bran oil ester was studied with using a column without packing at 9.8 MPa and at three column temperatures. Figure 3 shows that the degree of fractionation increased with column temperature. The compositions of extracts in the early fractions at a column temperature of 313 K were about the same as that of the star-

ting rice bran oil ester. At a column temperature of 343 K, however, palmitate was selectively fractionated at a ratio of 60–86% in the early fractions. At a column temperature of 373 K, the proportion of palmitate reached a maximum of 91% at a 9% of extraction, and the extraction of palmitate was completed at about 40% of extraction. Oleate and linoleate, however, could not be fractionated at all at a column temperature of 373 K. The reason is that, as the temperature increased, the solubilities of less volatile oleate and linoleate (which boil at 485–486/15 and 484–485/16 kg/mmHg, respectively; Table 1) decreased more than that of palmitate (b.p.=469/15 mmHg) with decreasing density of  $\text{CO}_2$ <sup>16</sup>. Accordingly, palmitate was concentrated selectively on passing through the column at elevated temperatures.

These results suggest that fatty acid esters having different number of carbon atoms were fractionated easily, but those having the same number of carbon atoms but different degrees of unsaturations were only barely fractionated from the rice bran oil ester, even at different column temperatures.

Effect of a column packed with silica gel- $\text{AgNO}_3$ .

To fractionate linoleate and oleate selectively, silica gel- $\text{AgNO}_3$  was used as the column stationary phase. Esters in rice bran oil ester were selectively fractionated at pressures of 11.7–16.7 MPa and a column temperature of 343 K. Chloroform was employed as an entrainer in the latter process of the extraction. The ester compositions of the extracts in Fig. 4 are expressed as a function of the degree of extraction. Fractions obtained between 5% and 12% extraction at 11.7 MPa were almost pure palmitate. After that, the proportion of palmitate decreased sharply, in contrast to the proportion of linoleate which increased sharply to a maximum 100% at a 30% of extraction. By increasing the extraction temperature stepwise, the proportion of oleate in extracts decreased gradually,

whereas the proportion of linoleate increased gradually. The proportion of linoleate in extracts

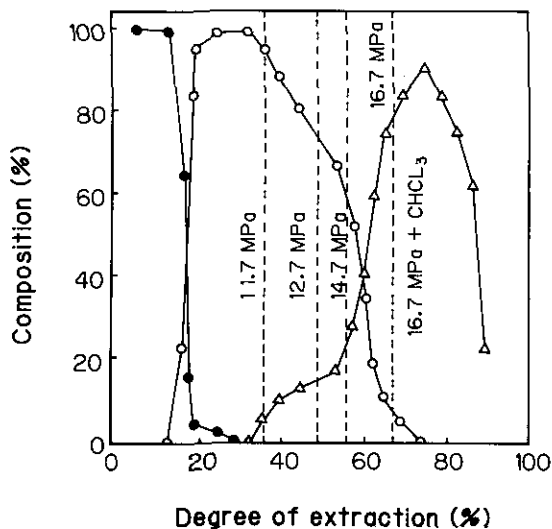


Fig. 4 Change of ester composition of extracts obtained during the supercritical  $\text{CO}_2$  extraction of rice bran oil methyl ester using a separation column packed with silica gel- $\text{AgNO}_3$  and of chloroform as an entrainer at 11.7–16.7 MPa and a column temperature of 343 K. (●) : C<sub>16:0</sub> ; (○) : C<sub>18:1</sub> ; (△) : C<sub>18:2</sub>

reached a maximum of 90% at a 72% extraction when chloroform was used as an entrainer at 16.7 MPa.

The degree of recovery of the esters is plotted in Fig. 5 as a function of the degree of extraction. The degree of recovery is expressed as a ratio of amount of the ester obtained and amount of the ester in the starting material. The curves show that palmitate and oleate were separated completely from each other, but the recovery of linoleate was less than that of either palmitate and oleate. Recoveries and purities obtained are summarized in Table 2. Palmitates, corresponding to 74% and 94% of that in the starting material, were recovered at 100% and 94% purity, respectively. 50% and 91% of oleate were recovered at 96% and 81% purities, respectively, and 38% and 67% of linoleate were recovered with purities of 84% and 48%, respectively. One reason for the low purity of the linoleate is that chloroform forced it to be

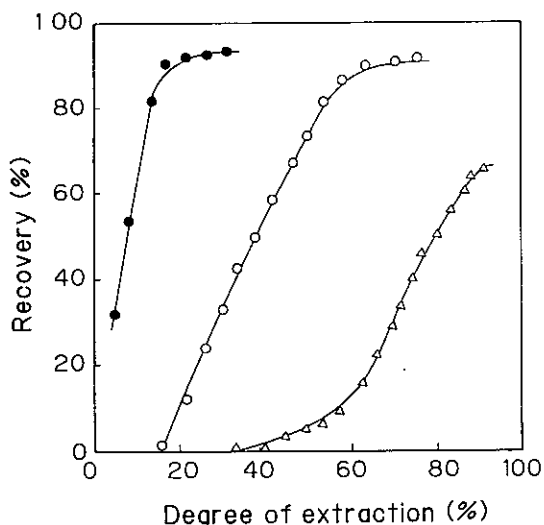


Fig. 5 Degree of recovery of esters obtained during the supercritical CO<sub>2</sub> extraction of rice bran oil methyl ester under the same conditions in Fig. 4.

(●) : C<sub>16</sub>:0 ; (○) : C<sub>18</sub>:1 ; (△) : C<sub>18</sub>:2

extracted along with the low volatile components in the starting material, and further research is necessary to fractionate oleate and linoleate more selectively. On the other hand, the SC-CO<sub>2</sub> extraction using a column packed with silica gel-AgNO<sub>3</sub> appears to be an extremely useful method of fractionating selectively fatty acid esters from natural oil resources.

Table 2 Recoveries and purities of fatty acid methyl esters fractionated from rice bran oil ester.<sup>a</sup>

Fatty acid methyl ester	Recovery(%)	Purity(%)
Palmitate (C16:0)	74 94	100 91
Oleate (C18:1)	50 91	96 81
Linoleate (C18:2)	38 67	84 48

<sup>a</sup> Extraction conditions were the same as those described in Fig. 4.

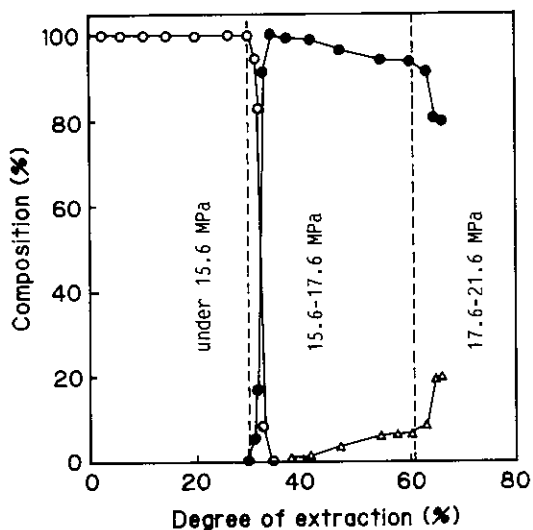


Fig. 6 Change of compositions of extracts obtained during the supercritical CO<sub>2</sub> extraction of the mixture of oleic acid, triolein, and  $\gamma$ -oryzanol using a column at 12.6-21.6 MPa and a column temperature of 343 K.

(○) : oleic acid; (●) : triolein; (△) :  $\gamma$ -oryzanol

## 2. Fractionation of $\gamma$ -oryzanol from a synthetic mixture.

A mixture of 0.85 g each of  $\gamma$ -oryzanol, oleic acid and triolein was used as a model to investigate the possibility of selective fractionation of  $\gamma$ -oryzanol from rice bran oil. Although the  $\gamma$ -oryzanol content of crude rice bran oil is usually about 3%, this equivalent mixture was used because these  $\gamma$ -oryzanol-rich samples are obtained as by-products from the refining of rice bran oil. The mixture was fractionated using SC-CO<sub>2</sub> and a column without packing at 343 K and 12.7-21.6 MPa. In Fig. 6, the compositions of oleic acid, triolein and  $\gamma$ -oryzanol in extracts are plotted as a function of the degree of extraction of the mixture. The separation of oleic acid and triolein should have been easy, because the solubilities of fatty acid methyl esters in SC-CO<sub>2</sub> are about an order of magnitude greater than those of corresponding triglycerides<sup>17)</sup>. In practice, oleic acid was fractionated first from the mixture at a purity of

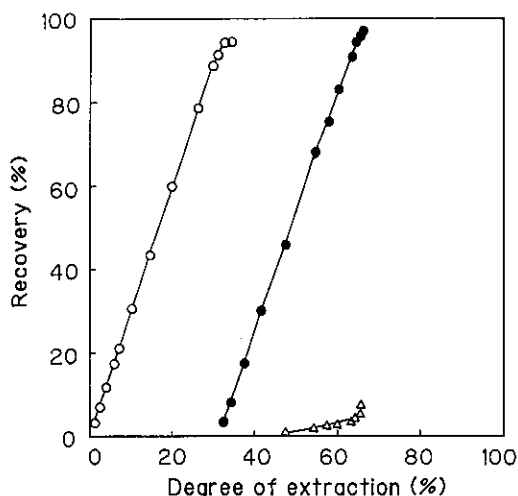


Fig.7 Degree of recovery of oleic acid, triolein, and  $\gamma$ -oryzanol during the supercritical  $\text{CO}_2$  extraction of the mixture of oleic acid, triolein, and  $\gamma$ -oryzanol under the same conditions in Fig. 6.

( $\circ$ ) : oleic acid; ( $\bullet$ ):triolein; ( $\Delta$ ):

Table 3 Recovery and purities of oleic acid, triolein, and  $\gamma$ -oryzanol.<sup>a</sup>

Ester	Recovery (%)	Purity (%)	Residue (Purity) (%)
Oleic acid	91	96	—
Triolein	95	93	—
$\gamma$ -oryzanol	10	9	86(100)

<sup>a</sup> Extraction conditions were the same as those described in Fig. 6

100% at pressures < 15.6 MPa. After the fractionation of oleic acid was nearly completed at about 32% extraction, triolein containing 0–10%  $\gamma$ -oryzanol was fractionated next at pressures of 15.6–17.6 MPa. As the fractionation of triolein was nearly complete at about 62% extraction at a pressure of 21.6 MPa, the fractionation process was complete. At this point, most of the  $\gamma$ -oryzanol could not be extracted, and it remained as a solid in a extractor. Figure 7 shows the degree of recovery of oleic acid, triolein, and oryzanol as a function of the

degree of extraction of a mixture. The curves also show that oleic acid and triolein were fractionated highly selectively, but triolein and oryzanol were not. As shown in Table 3, the recovery of oleic acid was 91% at a purity of 96%, and the recovery of triolein was only 10%, but  $\gamma$ -oryzanol (100% purity) corresponding to 86% of that in the starting mixture was obtained as a solid residue left in the extractor.

One of the reasons for the low recovery of  $\gamma$ -oryzanol (m.p.=435–438 K), is probably that it did not melt in the mixture at the extraction temperature of 343 K; thus, its solubility in  $\text{SC-CO}_2$  was much less than that of triolein. These results suggest that  $\text{SC-CO}_2$  extraction with the use of a column has a potential for selective separation of  $\gamma$ -oryzanol from  $\gamma$ -oryzanol-rich substances such as by-products obtained from the refining of rice bran oil.

#### Conclusion

An increase of column temperature enhanced the fractionation selectivity of fatty acid esters having different numbers of carbon atoms. The use of a column packed with silica gel- $\text{AgNO}_3$  was effective in the selective fractionation of fatty acid esters having same number of carbon atoms but different degrees of unsaturations.

From our studies of a synthetic mixture, it appears that  $\gamma$ -oryzanol can be fractionated rice bran oil or its related substances.

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