Treatment of Rice Bran with Supercritical Carbon Dioxide

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Three kinds of rice bran, Aka, Naka, and Shiro brans were defatted by extraction with supercritical carbon dioxide (SC-CO₂). More than 80% of crude fat of the Aka bran was extracted with SC-CO₂ at 200 atm and 40°C. The oil extracted with SC-CO₂ was lighter in color and contained less phosphorus than that extracted with hexane. Oryzanol was found to be extracted from rice bran with SC-CO₂.

Supercritical carbon dioxide (SC-CO₂) extraction has been investigated and recognized as a novel and powerful technique for separating lipophilic and thermally unstable compounds. The characteristics of SC-CO₂ are based on larger diffusivity and smaller viscosity as compared with those of liquid, and higher density than that of gas under atmospheric pressure and ambient temperature¹⁾²⁾.

The use of SC-CO₂ as a solvent for natural products in food industry has a number of advantages over other organic solvents, because CO₂ is harmless to human beings, non-reactive, noncorrosive, cheap and readily available in large quantity and high purity. A number of examples for the application of extraction with SC- and liquid CO₂ has been reported for the treatment of natural products such as coffee, hops, spices and aroma compounds of fruits³⁾⁴⁾.

With plant seed oils, SC-CO₂ extraction is considered to be a promising alternative to current extraction using hexane as the solvent. In our previous work⁵⁾, extraction of wheat germ oils with SC-CO₂ was investigated. Not only operating conditions for effective extraction but also analytical characterization of the oil obtained by extracting with SC-CO₂ were reported as compared with those by other extraction methods. In the present work, extraction of rice bran oil with SC-CO₂ was carried out with a view of utilizing the residue

after the extraction for alcohol production as well as evaluating the quality of the oil extracted.

Materials and Methods

Materials

Three kinds of rice bran were used: Aka, Naka and Shiro brans were produced when unpolished rice was refined up to the weight ratio of 90%, up to 80% from 90%, and up to 70% from 80%, respectively. The total amounts of extracts (crude fat) obtained from Aka, Naka, and Shiro by diethyl ether extraction with Soxhlet apparatus for 16 h were 18.4, 9.1, and 2.6 wt% of dry matter, respectively. Liquid CO₂ of commercial grade was used. All other chemicals were of guaranteed grade.

Extraction apparatus and procedures

A flow diagram of the SC-CO₂ extraction apparatus made by Mitsubishi Kakoki Co. Ltd. and the extraction procedures were previously described⁶⁾. The amount of CO₂ consumed was usually 0.7 kg/h.

Saccharification test

The rice bran defatted with SC-CO₂ was saccharified with Koji-extracted solution according to the standard method⁸⁾. Koji-extracted solution was prepared as follows: Ten parts of 0.5%-NaCl solution was added to Koji. After incubation of the suspension with shaking at 20°C for 3 h, the suspension was

centrifuged and filtrated through a glass filter (G4). The supernatant obtained was used as the Koji-extracted solution. Prior to saccharification, rice bran defatted with SC-CO₂ was wetted by adding water at a weight ratio of 2 (rice bran) to 3 (water) and subsequently steamed in autoclave at 105°C for 1 h. Steamed rice bran (25 g) was saccharified by addition of 10 ml Koji-extracted solution and subsequent incubation of the mixture with shaking at 55°C for 20 h. After incubation, the mixture was centrifuged at 12 000 rpm for 10 min and filtrated through a glass filter (G4) to remove unsolubilized substances. The supernatant was analyzed for reducing sugar, amino acid and color intensity as described below.

Analytical methods

The amount of oil extracted with liquid and SC-CO₂ was determined by subtracting the amount of crude fat of the residue after the extraction with CO₂ from that of crude fat of native rice bran. The ratio of oil extracted was expressed as the ratio of the amount of oil extracted with CO₂ to that of crude fat extracted with diethyl ether per gram of dry rice bran. Oils were also extracted by recycling hexane in a Soxhlet apparatus for 16 h in order to compare with the properties of oils extracted with CO₂. Oils containing complex lipids were prepared by three times-extraction with 85% methanol at 85°C according to the method of NISHITANI *et al.*⁷⁾

General properties such as acid, iodine and saponification values of the oils extracted with SC-CO₂ or hexane were determined according to the standard method⁸⁾. The amount of phosphorus was measured according to the method of BARTLETT⁹⁾. Color intensity was expressed as the absorbance at 430 nm of 1% oil solution in chloroform or the saccharified solution of the defatted rice bran.

The composition of fatty acids of oils extracted was determined by gas chromatography using a glass column (2 m × 3.0 mm) packed with Unisole 400. The content of oryzanol was estimated from the absorbance at 315 nm of the oil solution in *n*-heptane¹⁰⁾. The amounts of reducing sugar and amino acid in the saccharified solution of rice bran were determined by the dinitrosalicylic acid method¹¹⁾

and the ninhydrin method¹²⁾, respectively.

Results and Discussion

Conditions for oil extraction

Effect of operating conditions on the extraction ratio of crude fat was examined for Aka bran with respect to pressure, temperature and time for extraction. Moreover, the three kinds of rice bran were treated with SC-CO₂ in order to prepare the defatted bran for the saccharification test as well as to obtain oils for the comparison of the properties with hexane-extracted oils.

Figure 1 shows the effect of pressure on the extraction ratio of crude fat at 40°C for 2 h. The extraction ratio of the oil depended primarily upon pressure and no oil was extracted by CO₂ at 60 atm. Sharp increase for the oil extraction was observed between 100 atm and 200 atm. At pressure of more than 200 atm, slight gradual increase in the amount of the oil extracted was observed.

Figure 2 shows the effect of temperature when the pressure and extraction time was kept constant at 200 atm and 2 h, respectively. The ratio of the oil extracted was independent of the temperature at pressure of 200 atm.

Figure 3 shows a time course of the extraction with SC-CO₂ at 40°C and 200 atm. Oil was extracted linearly in the early stage of the

Fig. 1 Effect of pressure on the extraction of rice bran (Aka) oil with supercritical CO₂

Fig. 2 Effect of temperature on the extraction of rice bran (Aka) oil with supercritical CO_2

Fig. 3 Time course of extraction of rice bran (Aka) oil with supercritical CO_2

Fig. 4 Time courses of extractions from different kinds of rice bran with supercritical CO_2

Extraction conditions: 300 atm, 40°C

extraction. Mass flow rate of CO₂ used was about 0.65 kg per hour. Under the conditions used in the present work, the height (about 15 cm) of Aka bran packed in the extractor is regarded to be sufficiently high that the concentration of oil soluble in SC-CO₂ could reach saturation at the outlet of the extractor as described previously⁶⁾. Therefore, the solubility of Aka bran oil in SC-CO₂ at 200 atm and 40°C could be estimated to be 0.38 wt% from the slope of the straight line in the early stage of the extraction. This value was very similar to that (0.35 wt%) of wheat germ oil as described previously⁶⁾. The ratio of crude fat extracted increased at first linearly with the amount of CO₂ used. Then, it decreased gradually as shown in Fig. 3, which may be due to intraparticle diffusion limitation of oil within rice bran.

Figure 4 shows time courses of extraction of bran oils with SC-CO₂ at 300 atm and 40°C. In this experiment, the amount of CO₂ used was about 8.5 kg/h. Total extraction time was 7, 6 and 4 h for Aka, Naka and Shiro brans, respectively. The extraction process was also divided into two stages for Aka and Naka brans. From the slope of the initial straight lines, solubility of the oil in SC-CO₂ at 300 atm and 40°C was estimated to be 1.0 wt%, which is 2.6-times higher than that at 200 atm and 40°C and comparable value (0.95 wt%) to solubility of vegetable oil at 300 atm and 40°C reported by FILIPPI¹³⁾. Extraction

ratio of oil sharply decreased at about 10 kg-CO₂/kg-rice bran. Through the extraction, water was also extracted. Solubility of water was also estimated to be 0.17 wt% from the slope of the straight lines in Fig. 4(a) and (b), which corresponds to 0.18 wt% (300 atm, 25°C) reported by EVELEIN *et al.*¹⁴⁾. As solubility of water is lower than that of oils, longer early stages were observed for extraction of water. In Shiro bran, the early stage was not observed.

Properties of defatted rice bran by SC-CO₂ extraction

Table 1 shows the compositions of rice bran residues obtained at the end of SC-CO₂ extractions shown in Fig. 4. The contents of crude fat in the residues decreased to considerable extent. If the rice bran was extracted for longer time, crude fat contents will become lower. Water contents also decreased in proportion to the amount of CO₂ used per weight of the bran. Composition and content of remaining fatty acids in each rice bran defatted with SC-CO₂ were compared with those of each corresponding native sample. To determine the amounts of total fatty acids including constituents of complex lipids such as glycolipids and phospholipids, lipids were thoroughly extracted by 85% methanol at 85°C⁷⁾. In the case of rice bran oil, a large portion of the fraction C_{18:0+18:1} is oleic acid (C_{18:1})¹⁵⁾. In this context, oleic acid was relatively easily extracted and palmitic acid

Table 1 Properties of rice bran defatted by extraction with supercritical CO₂

Rice bran		Crude fat* (%)	Water content** (%)	Fatty acid**** (mg/g·dry sample), () %					
				C _{14:0}	C _{16:0}	C _{18:0+18:1}	C _{18:2}	C _{18:3}	Total
Aka	Native	18.4	12.4	0.7 (0.5)	26.6 (18.9)	56.7 (40.3)	54.1 (38.4)	2.7 (1.9)	140.8
	Defatted***	1.88	9.2	0.1 (0.9)	2.9 (27.4)	3.2 (30.2)	4.3 (40.6)	0.1 (0.9)	10.6
Naka	Native	9.14	11.9	0.2 (0.6)	7.9 (23.3)	11.7 (34.5)	13.7 (40.4)	0.4 (1.2)	33.9
	Defatted***	0.58	10.3	0.1 (1.0)	3.1 (30.0)	2.4 (23.3)	4.5 (43.7)	0.2 (2.0)	10.3
Shiro	Native	2.64	11.8	0.1 (0.7)	4.5 (31.9)	3.5 (24.8)	5.8 (41.1)	0.2 (1.4)	14.1
	Defatted***	0.12	11.0	0.1 (1.2)	3.2 (38.6)	1.4 (16.9)	3.4 (41.0)	0.2 (2.4)	8.3

* Crude fat was determined by diethyl ether extraction with Soxhlet apparatus for 16 h.

** Water content was measured by drying at 135°C for 3 h.

*** Rice bran was defatted with SC-CO₂ at 300 atm and 40°C.

**** Fatty acid was obtained by 85% methanol extraction at 85°C and hydrolysis with 1 N-NaOH at 30°C for more than 3 h.

(C_{16:0}) was less extracted. Ratios of other fatty acids were fairly invariable between native bran and defatted bran. Protein and carbohydrate in rice bran were not extracted (data not shown).

Properties of hydrolyzate of rice bran residues

Table 2 shows properties of hydrolyzates of the three kinds of rice bran residue after extraction with SC-CO₂ at 300 atm and 40°C. The three kinds of native rice bran and 30% polished rice were also solubilized for comparison. Defatted rice brans had higher content of carbohydrate than the corresponding native one, because crude fat was extracted. Thus, the reducing sugar produced by solubilization with the Koji-extracted solution also increased in the sample of defatted rice bran except in Naka bran. Concentrations of amino acids in hydrolyzate of defatted rice brans were almost comparable to those of native ones. These results show no occurrence of undesir-

able changes in solubilization of rice bran residue during SC-CO₂ extraction. The hydrolyzates of the rice brans defatted with SC-CO₂ showed significantly less color intensity. This is favorable change when the rice bran residue is used for raw material for alcohol production.

Properties of extracted oils

Table 3 shows a comparison of the yields and properties of the rice bran oils extracted with hexane and with SC-CO₂. In this case, oils extracted by SC-CO₂ at 300 atm and 40°C for 2 h were compared with oils extracted by hexane for 16 h. With all the rice brans, the overall yields of the oil obtained by SC-CO₂ extraction on the basis of dry sample were lower than those by hexane extraction. The oil yield in SC-CO₂ extraction was increased with longer extraction time as shown in Figs. 3 and 4. However, it will be still less than that in hexane extraction, because complex lipids are hardly extracted with SC-CO₂. Acid

Table 2 Properties of hydrolyzate of rice bran defatted by extraction with supercritical CO₂

Sample	Reducing sugar (mg/ml)		Amino acid (mg/ml)		Coloration A ₄₃₀	
	A*	B**	A*	B**	A*	B**
Aka	86	100	3.48	3.64	2.24	0.56
Rice bran Naka	186	181	1.85	1.78	0.37	0.19
Shiro	207	238	1.46	1.40	0.14	0.11
70% polished rice	210		1.55		0.06	

*A: Native sample.

**B: Sample was defatted with SC-CO₂ at 300 atm and 40°C.

Table 3 Comparison of extracted crude rice bran oils

Rice bran	Extraction* methods	Yield [%]	Acid value [mg/g]	Phosphorus [μg/g]	Color** intensity	γ-oryzanol [%]
Aka	SC-CO ₂	16.5	39.1	1.2	0.075	1.21
	Hexane	17.9	82.3	508.9	0.309	3.18
Naka	SC-CO ₂	8.6	12.2	0.6	0.067	0.42
	Hexane	9.2	24.0	419.2	0.143	0.57
Shiro	SC-CO ₂	2.5	8.2	2.9	0.032	0.47
	Hexane	2.6	23.0	3.4	0.076	0.71

* Oils were obtained by SC-CO₂ extraction for 4~7 h at 300 atm and 40°C or by hexane extraction in a Soxhlet apparatus for 16 h.

** E_{1%}^{1cm}, CHCl₃, 430 nm

values of oils extracted with SC-CO₂ were considerably lower than those with hexane. However, there was no significant difference in the saponification and iodine values between two kinds of oils obtained by different extraction methods (data not shown). The oil extracted with SC-CO₂ showed less color intensity and much less phosphorus content than hexane-extracted oil, similarly to the extraction of wheat germ oil described previously⁵⁾. Therefore, extraction with SC-CO₂ is expected to eliminate or simplify the processing steps such as degumming and decoloration for refining crude oil⁶⁾. Oryzanol, mixture of esters of triterpenealcohols and ferulic acid (3-methoxy 4-hydroxy cinnamic acid), is recognized as a natural substance possessing many pharmaceutical and biochemical actions such as promotion of growth and anti-oxidation. SC-CO₂ was found to be able to extract oryzanol from rice bran.

With SC-CO₂ extraction, solvent-free defatted rice bran was easily obtained by removing CO₂ under atmospheric pressure and ambient temperature. Solvent-free defatted rice bran is favorable in using as a raw material for production of alcoholic beverage, confectionery and seasonings and expected to be effectively utilized in a number of new fields.

In the view of effective oil extraction with SC-CO₂, other important potential factors than temperature and pressure which affect ability of SC-CO₂ to dissolve oil of rice bran are moisture content and morphology of the sample. Moreover, addition of entrainer such as water or alcohol has been known to improve the solubility of low volatile substances in SC-CO₂¹⁷⁾. Based on the results obtained in the present work, improvement and optimization of extraction conditions are necessary together with taking care of quality of the residue.

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超臨界二酸化炭素による米糠の処理

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3種類の米糠を 超臨界二酸化炭素により処理を行なった。200 気圧、40°C の抽出条件において、赤糠から 80%

以上の粗脂肪が抽出できた。粗脂肪の抽出率は、抽出圧力に大きく影響された。超臨界二酸化炭素により抽出された油は、ヘキサン抽出油に比較して着色度が低く、リ

ン含量も低かった。オリザノールも超臨界二酸化炭素により抽出できた。また、超臨界二酸化炭素により脱脂した糠を麴抽出液で可溶化した。