



G 1254-0882

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**Development of Technology on Purification
and Isolation of Octacosanol and
-Oryzanol in Refining Byproducts
from Rice Bran Oil Processing**

“ ” ,

1997. 12. 20

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:
:
:
:
:
:
:

I.

,

II.

-

10%(50 60)

20%

,

.

-

80%

,

-

, , , ,

10%

가

가

50%

.

-

.

-

(octacosanol)

(-oryzanol)

.

-

.

III.

1. (octacosanol)

가.

.

.

2. (-oryzanol)

가. Dark oil crude -oryzanol

. Dark oil crude -oryzanol

. (-oryzanol) ,

1) dark oil

2) dark oil pitch

3) -oryzanol

4) -oryzanol

5)

6) dark oil ,

,

IV.

1. (octacosanol)

가.

10

toluene , 10°C

8.10% 가

· toluene 가

5 가 30 4C
17.5%

가 가 40 가
4C
17.5%

2. (-oryzanol)

가. Dark oil

Dark oil

7가
가 35% 30%

crude -oryzanol

hexane pentane

, pentane hexane 가

hexane

. Hexane

crude -oryzanol

가

1:1

2

1:20

. Dark oil crude -oryzanol
 Soap stock -oryzanol dark oil ,
 hexane dark oil dark oil -oryzanol
 가 , dark oil .
 Dark oil -oryzanol -oryzanol
 , hexane . 1
 crude -oryzanol hexane 가 , dark oil
 : hexane = 1 : 1 (W/V) 32.4% 가 , 31.6%
 . 2 crude -oryzanol : hexane = 1 : 20 (W/V)
 -oryzanol 75.6% 26.2% -oryzanol
 . -oryzanol , -oryzanol
 methanol , -oryzanol : MeOH = 1 : 30
 (W/V) 91.5% -oryzanol dark oil 24%

. (-oryzanol) ,

1) dark oil
 Soap stock
 가 ,
 dark oil 가 10% KOH
 가 dark oil

2) dark oil pitch
 10% KOH dark oil

180C, 400 milli torr , 1% steam pitch
 -oryzanol dark oil 1.6 .
 pitch -oryzanol
 73.17% -oryzanol .

3) -oryzanol
 dark oil 461g 40 , 5000psi
 pitch -oryzanol 37.2% .
 pitch -oryzanol ,
 90.17% .

4) -oryzanol
 , pitch, crude -oryzanol,
 -oryzanol pitch 24-methylene
 cycloartenyl ferulate -sitoseryl ferulate
 , cycloartenyl ferulate
 24-methylene cycloartenyl ferulate . -sitoseryl
 ferulate campesteryl ferulate가 ,
 24-methylene cycloartenyl ferulate > cycloartenyl ferulate > campesteryl
 ferulate, -sitoseryl ferulate .

5)
 24-methylene cycloartenyl ferulate, cycloartenyl ferulate, campesteryl
 ferulate, -sitoseryl ferulate 24-methylene cycloartenyl
 ferulate -sitoseryl ferulate가 ,
 24-methylene cycloartenyl ferulate가 .

6) dark oil

dark oil 24-methylene
cycloartenyl ferulate cycloartenyl ferulate ,
cycloartenyl ferulate 24-methylene cycloartenyl
ferulate가 , -sitosteryl ferulate가

.

가, 가

가 .

Summary

I. Title

Development of technology on purification and isolation of octacosanol and -oryzanol in refining byproducts from rice bran oil processing

II. Purpose and Significance of the Study

Rice brans of 50 - 60 metric ton were produced as the byproducts of a rice processing every year. Only ten percentages of them were used as a raw materials for a production of the rice bran oil and the others were treated as the waste. A processing of rice bran oil was consisted of dewaxing, deguming, acidifying and decolorizing steps. The dewaxing materials and soap stock were obtained at the refining steps such as dewaxing and acidifying during the processing of a rice bran oil. Octacosanol and -oryzanol were presented in dewaxing materials and soap stock. Octacosanol and -oryzanol have been used as a additives of a functional foods and exported from Japan.

The purpose of this research was to develop the technology on a purification and isolation of octacosanol and -oryzanol in the refining byproducts from a rice bran oil processing.

III. Scope and Content of the Study

1. Final objective at the end of study

Development of technology on purification and isolation of octacosanol and -oryzanol in refining byproducts from rice bran oil processing

2. Scope and Content

1) The area of octacosanol

- A. Isolation of octacosanol by solvent fractionation
- B. Decolorization and recrystallization of octacosanol
- C. Flow sheet of purification and isolation of octacosanol

2) The area of γ -oryzanol

- A. Isolation of crude γ -oryzanol from dark oil by solvent fractionation
- B. Flow sheet for isolation of crude γ -oryzanol from dark oil by hexane
- C. Purification and isolation of γ -oryzanol by resaponification
 - a. Preparation of dark oil by resaponification
 - b. Preparation of pitch from dark oil by steam distillation
 - c. Isolation of γ -oryzanol by supercritical carbon dioxide extraction
 - d. γ -Oryzanol compositions at purification steps
 - e. Properties of γ -oryzanol in fatty acid fraction by SCDE
 - f. Isolation of γ -oryzanol by hexane fractionation after preparation of dark oil obtained by resaponification

IV. Results and Recommendation

1. The area of octacosanol

A. Isolation of octacosanol by solvent fractionation

Ten kinds of solvents were used for the isolation of octacosanol by solvent fractionation. When dewaxing materials were fractionated by toluene at 10°C, octacosanol contents were 8.10% and then octacosanol contents increased as toluene extracted after saponification.

B. Decolorization and recrystallization of octacosanol

Unsaponifiables extracted by hot hexane were decolorized by the fuller's earth (dewaxing material : fuller's earth, 1:5, W/W) at a boiling temperature for 30 min. Purity of octacosanol was 17.5% after decolorization. This purity was similar to content of a commercial octacosanol exported from Japan

C. Flow sheet of purification and isolation of octacosanol

The insoluble dewaxing materials in hexane were saponified by sodium hydroxide and then the unsaponifiables were extracted by hot hexane. The unsaponifiables extracted by hot hexane were decolorized by fuller's earth at boiling temperature for 30 min. Crystallization of octacosanol in hexane extract was conducted at 4°C. Purity of octacosanol was 17.5% after a decolorization and crystallization.

2. The area of γ -oryzanol

A. Isolation of crude γ -oryzanol from dark oil by solvent fractionation

Seven kinds of solvents were used for the isolation of γ -oryzanol by solvent fractionation. The suitable solvent was hexane. Optimum ratio for isolation of γ -oryzanol were dark oil : hexane = 1:1 (w/v) at the 1st isolation step and their ratio were 1:20 (v/w) at the 2nd isolation step.

B. Flow sheet for isolation of crude γ -oryzanol from dark oil by hexane

Optimum ratio for purification and isolation of γ -oryzanol were dark oil : hexane = 1:1 and 1:20 (v/w) at the 1st and 2nd isolation steps, respectively. Optimum ratio for a final crystallization of γ -oryzanol were crude γ -oryzanol : methanol = 1:30 (v/w) and their purity and yield were 91.5 and 24%, respectively.

C. Purification and isolation of γ -oryzanol by resaponification

a. Preparation of dark oil by resaponification

The remaining triglycerides and phospholipids in soap stock were resaponified by 10% potassium hydroxide. The dark oil was prepared from the resaponified soap stock containing sulfuric acid.

b. Preparation of pitch from dark oil by steam distillation

The dark oil from the soap stock resaponified by 10% potassium hydroxide was conducted under 180°C, 40 milli torr and 1% steam by the distillation method. The purity of γ -oryzanol was 73.17% after the distillation method and the hexane fractionation described above.

c. Isolation of γ -oryzanol by supercritical carbon dioxide extraction

The dark oil from the soap stock resaponified by 10% potassium hydroxide was treated under 40°C and 5000 psi by supercritical carbon dioxide. The purity of γ -oryzanol was 90.17% after supercritical carbon dioxide extraction (SCDE) and hexane fractionation described above.

d. γ -Oryzanol compositions at purification steps

Major compositions of γ -oryzanol in the pitch by supercritical carbon dioxide extraction (SCDE) were 24-methylene cycloartenyl ferulate and β -sitosterol ferulate, but major compositions of γ -oryzanol in SCDE pitch by hexane fractionation were cycloartenyl ferulate and 24-methylene cycloartenyl ferulate. β -Sitosterol ferulate and campesterol ferulate were lost during SCDE and hexane fractionation. The ratio of composition were, decreasing order, 24-methylene cycloartenyl ferulate > cycloartenyl ferulate > campesterol ferulate, β -sitosterol ferulate.

e. Properties of α -oryzanol in fatty acid fraction by SCDE

Compositions of α -oryzanol in the fatty acid fraction by SCDE were 24-methylene cycloartenyl ferulate, cycloartenyl ferulate, campesteryl ferulate and β -sitosteryl ferulate. 24-methylene cycloartenyl ferulate and β -sitosteryl ferulate were lost at the initial stage, but 24-methylene cycloartenyl ferulate was lost at the final stages under SCDE.

f. Isolation of α -oryzanol by hexane fractionation after preparation of dark oil obtained by resaponification

Major components of dark oil were 24-methylene cycloartenyl ferulate and cycloartenyl ferulate but major components of α -oryzanol by hexane fractionation were cycloartenyl ferulate and 24-methylene cycloartenyl ferulate. β -Sitosteryl ferulate decreased as purification step progressed.

For the industrial production of octacosanol and α -oryzanol, the production cost, processing step and the required machinery should be reestimated in pilot plant.

Summary

1

2

1 (octacosanol)

1. (octacosanol)

2. GC (octacosanol)

3. (standard curve)

4.

5. Toluene

6. (octacosanol)

7.

2 (-oryzanol)

1.

2. Soap stock dark oil

3. Dark oil crude -oryzanol

가. -oryzanol

. Hexane crude -oryzanol

4. -oryzanol

5.

가. dark oil

·
1) dark oil pitch

·
1)
2) -oryzanol

가) pitch
) pitch -oryzanol ,

3)

4)

3

1 (octacosanol)

1.

2. Toluene

3. (octacosanol)

4.

5.

2 (-oryzanol)

1. Soap stock dark oil

2. Dark oil crude -oryzanol

가. -oryzanol

. Hexane crude -oryzanol

3. -Oryzanol

4. (-oryzanol) ,

가. Soap stock

1) dark oil

2) dark oil pitch

5. -oryzanol

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 - b. Solvent ratio for recovery of γ -oryzanol by hexane
 - C. Crystallization of γ -oryzanol
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 - a) Preparation of dark oil by resaponification
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a. Preparation of pitch by SCDE

b. Purification and isolation of γ -oryzanol from pitch extracted SCDE

c. Properties of γ -oryzanol in fatty acid fraction by SCDE

F. Isolation of γ -oryzanol by solvent fractionation after preparation of dark oil obtained by resaponification

. References

1

- 500 - 600
10% 50 - 60 20%
, .
- 80% ,
, , , ,
10 % 가 가 50%
.

-
.
-
(octacosanol) (-oryzanol)
.
-
.
-
.

2

1 (octacosanol)

1. (octacosanol)

. , ethanolic
KOH 가 . Heating mentle
(melting point) wax .
wax TMS GC wax
TMS pyridine 1mL 20mg vial
pyridine , BSA 0.1mL 가 capping , 60C
20 , 가 pyridine 가 chloroform
GC .

2. GC (octacosanol)

GC ramp rate
, GC 1 .

3. (standard curve)

GC
 $Y = 49337.6X + 3152.4$, $r^2 = 0.99877$. 2

squalane

Table 1. Conditions for analysis of octacosanol by GC

GC	Hewlett Packard series II
Column	Supelco SPB 1
Oven temperature	200
Oven max temperature	300
Injection temperature	250
Detector temperature	300
Initial time	5 min
Final time	25 min
Final temperature	300
Rate	6 /min
Split ratio	150:1
Injection volume	0.4µL

4.

10 22°C
10:1(v/w),
16 . acetone(AT), pentane(PT), toluene(TO),
isopropyl alcohol (IPA), petroleum ether(PE), heptane(HI), diethyl
ether(DE), methylethyl ketone (MEK), ethyl acetate(EA) .

5. Toluene

, toluene octacosanol

가 toluene .
 , toluene 0, 10, 20, 30°C ,
 toluene 10:1(v/w) .

6. (octacosanol)

. TO, DE, EA, MEK,
HX, PT, HT 7 ,

7.

10, 20, 30, 40, 50 60% 가 30 .
가 (Minolta, Chroma meter CR-200)

300 mL 가 4°C

L: 97.43, a: -0.23, b: 1.49 .

2 (-oryzanol)

1.

chloroform 318nm soap stock dark
oil (E 1%,
1cm) 358 .

$$\text{-oryzanol (\%)} = a \times 100 / b \times c$$

a : absorbance

b : sample wt. (g)

c : absorbance coefficient, 358

2. Soap stock dark oil

Soap stock hexane
, 2가 dark oil
-oryzanol , .

Soap stock 100g 20ml 가 , hexane 100ml
가 hexane hexane , dark oil

Soap stock 100g 20ml 가 , 25 , 10,000rpm
15 (dark oil) .

3. Dark oil crude -oryzanol

가. -oryzanol
Dark oil 7가 (acetonitril, methanol, acetone,
chloroform, ether, hexane, pentane) 1:2(W/V) 4

20hrs. , 4 , 10,000rpm 10
-oryzanol .

. Hexane crude -oryzanol
Dark oil hexane 1: 1, 4, 8, 12, 16, 20, 24 (W/V)
4 20hrs. , 4 , 10,000rpm 10
-oryzanol .

4. -oryzanol

Hexane crude -oryzanol methanol
1: 5, 10, 15, 20, 25, 30, 35 (W/V) 80
, . methanol Whatman
No. 1 -oryzanol .

5.

가. dark oil
97 2 3 soap stock
-oryzanol , dark oil
1 dark oil ,
1 . Soap stock soap
stock 가
, dark oil 가
가 , ()
.

1) dark oil pitch

10% KOH dark oil 2

180°C, 400 milli torr, 1% steam

pitch -oryzanol

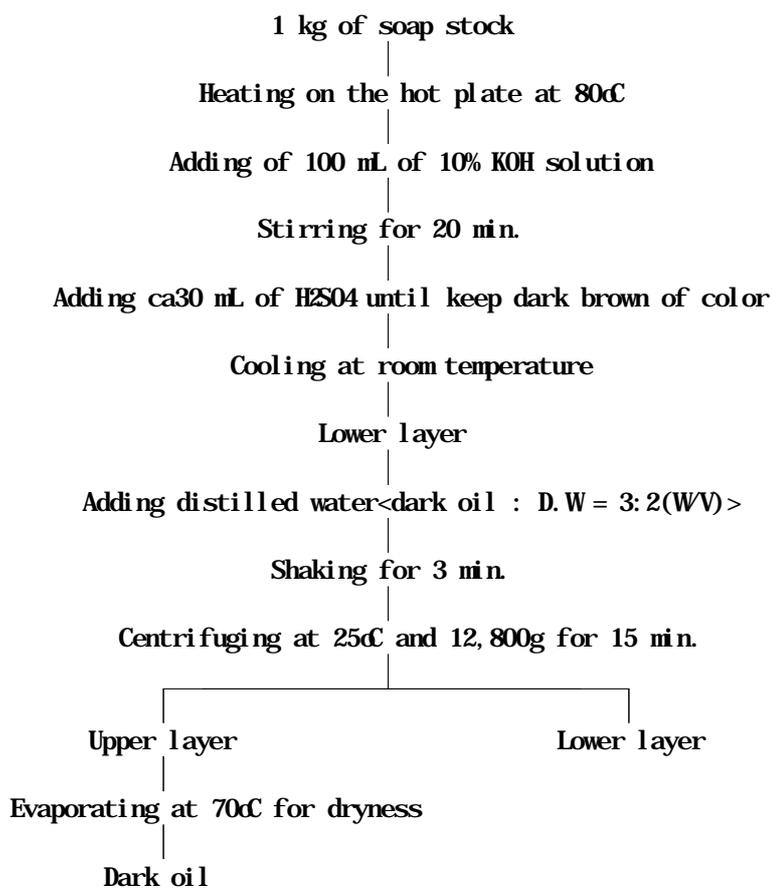


Fig. 1. Flow sheet of resaponification method for preparation of dark oil

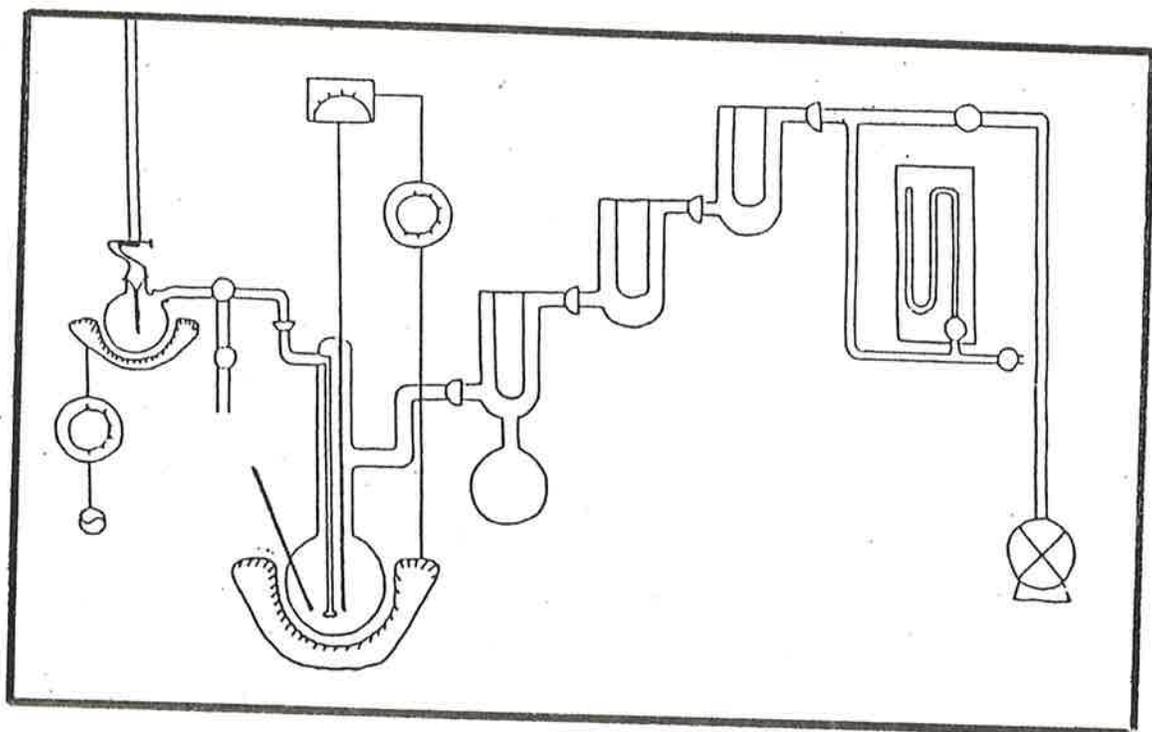


Fig. 2. Steam distillation apparatus for preparation of pitch

1)

oil -oryzanol , dark
3 .
Auto Clave Engineers Inc(Erie,
Pennsylvani a) system setting
. CO2 CO2 check valve 5um filter liquid
pump(Model No. M5110, Haskel Energy System Ltd., Sunderland, UK)
. CO2 pressure relief valve
, CO2 가 가
가 .
, CO2
. CO2 flow rate roatameter ,
CO2 CO2가 totalizer .

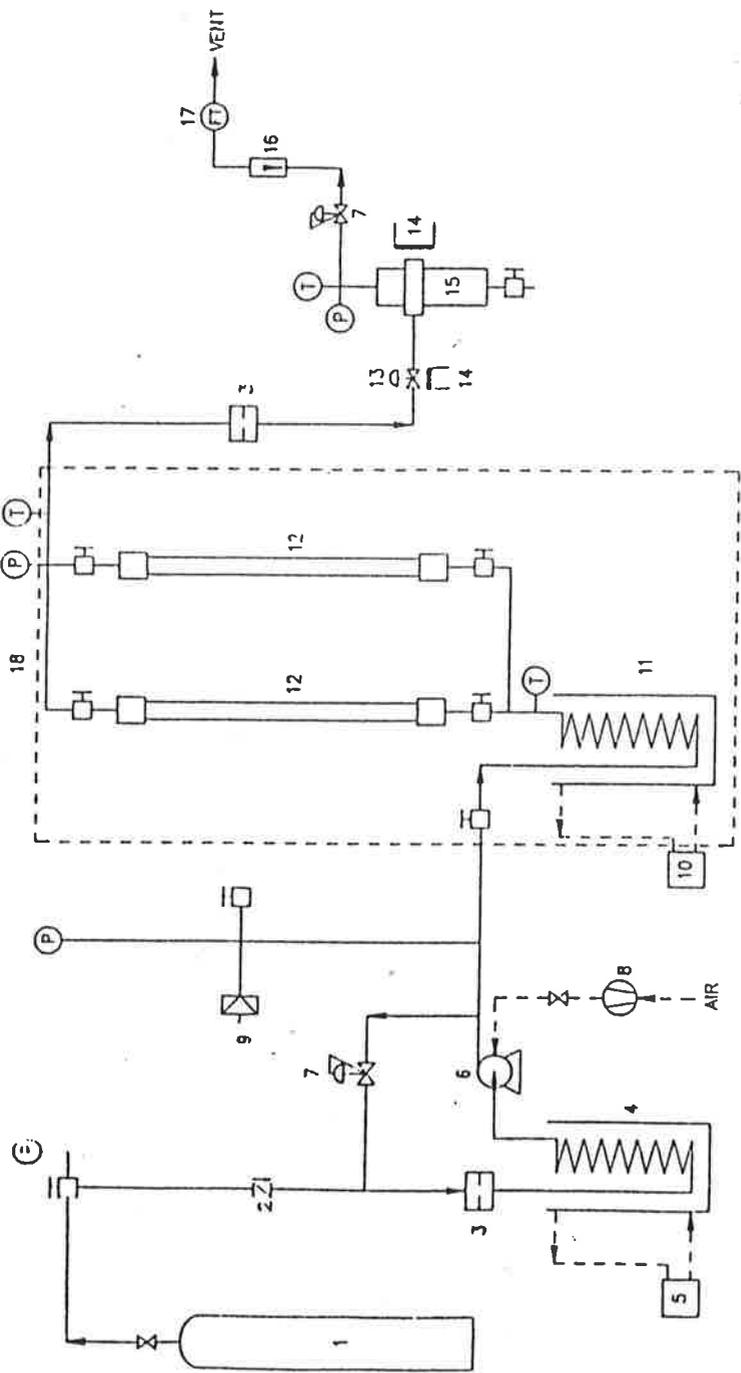
2) -oryzanol

가) pitch

dark oil 461g 40 , 5000psi
pitch -oryzanol , .

) pitch -oryzanol ,

dark oil pitch , 1



- | | | | |
|-----------------------------|----------------------------|---------------------|--------------------|
| 1. CO ₂ Cylinder | 6. Pump | 11. Preheater | 16. Rotameter |
| 2. Check Valve | 7. Back Pressure Regulator | 12. Extractor | 17. Flow Totalizer |
| 3. Filter | 8. Air Compressor | 13. Metering Valve | 18. Air Bath |
| 4. Subcooler | 9. Rupture | 14. Electric Heater | P: Pressure Gauge |
| 5. Refrigerated Circulator | 10. Heating Circulator | 15. Separator | T: Thermocouple |

Fig. 3. Diagram of the supercritical fluid extraction apparatus

-oryzanol , .

3)

pitch, crude -oryzanol, chloroform
-oryzanol
0.1% TLC plate spotting . TLC plate benzene :
ethyl acetate(9:1, v/v) 가 chamber
iodine vapor -oryzanol fraction
HPLC . HPLC 2가
1 : : : (45: 45: 5: 5,
V/V/V/V) , 2 : : (50: 45: 5,
V/V/V) , gradient 0 - 6 1 1.5 mL/min , 6 -
10 1 2 1.5 mL/min , 10 -
30 2 1.5 mL/min , 30 - 40 2 1
1.5 mL/min HPLC(JASCO Model) .
UV (JASCO Model) 325nm
SYMMETRYRC18(3.9×15mm, Waters Co. Ltd) .

4)

1(1+2), 2(3+4), 3(5+6),
4(7+8) 5(9) silica gel(25g, 70 230 mesh, column chromatography ,
Merk) column , n-hexane 200ml, n-hexane : ether (8 : 2)
150ml, n-hexane : ether (5 : 5) 150ml, ether 150ml
TLC -oryzanol spot . n-hexane, n-hexane : ether (8 :
2) -oryzanol spot , n-hexane : ether (5 :

5), ether -oryzanol spot . , n-hexane :
ether (5 : 5), ether HPLC

3

1 (octacosanol)

1.

10 22℃
10: 1(v/w) ,
16 . acetone(AT), pentane(PT), toluene(TO),
isopropyl alcohol (IPA), petroleum ether(PE), heptane(HI), diethyl
ether(DE), methylethyl ketone (MEK), ethyl acetate(EA) .
2 .
toluene, heptane, acetone isopropyl alcohol
, toluene octacosanol 7.01% 가 .
toluene .

2. Toluene

, toluene octacosanol
7.68% 가 toluene
. , toluene 0, 10, 20,
30℃ , toluene 10: 1(v/w) ,
16 .
3 .

3. (octacosanol)

HX, PT, HT 7 , TO, DE, EA, MEK, 4

Table 4. Octacosanol contents of extracts in the extracting solvents after saponification

Solvent	Octacosanol contents(%)
Pentane	1.02
Toluene	8.15
Heptane	1.82
Diethyl ether	2.75
Methyl ethyl ketone	2.00
Ethyl acetate	1.25
Hexane	3.90

TO 8.15% 가
 TO , DE
 3.0 TO
 10C toluene
 가 .

4.

10, 20, 30, 40, 50, 60% 가 30
 가 (Minolta, Chroma water CR-200)
 300 mL 가 4C
 가
 5

Table 5. Octacosanol yield, contents and color properties of hexane extracts obtained by the addition of Fuller's earth

The amount of Fuller's earth(g)	Yield(g)	octacosanol content(%)	color properties		
			L	a	b
20	0.57	14.9	82.30	3.38	14.30
30	0.95	15.1	91.00	0.39	10.79
40	1.14	16.4	92.25	0.41	11.71
50	1.42	17.9	93.87	-0.24	10.77
60	1.21	17.4	91.49	0.62	11.54

가 가 (L)가 가
 50% 가 가 가
 50% 가 17.9% 가 가
 5

5.

1 2

4 .

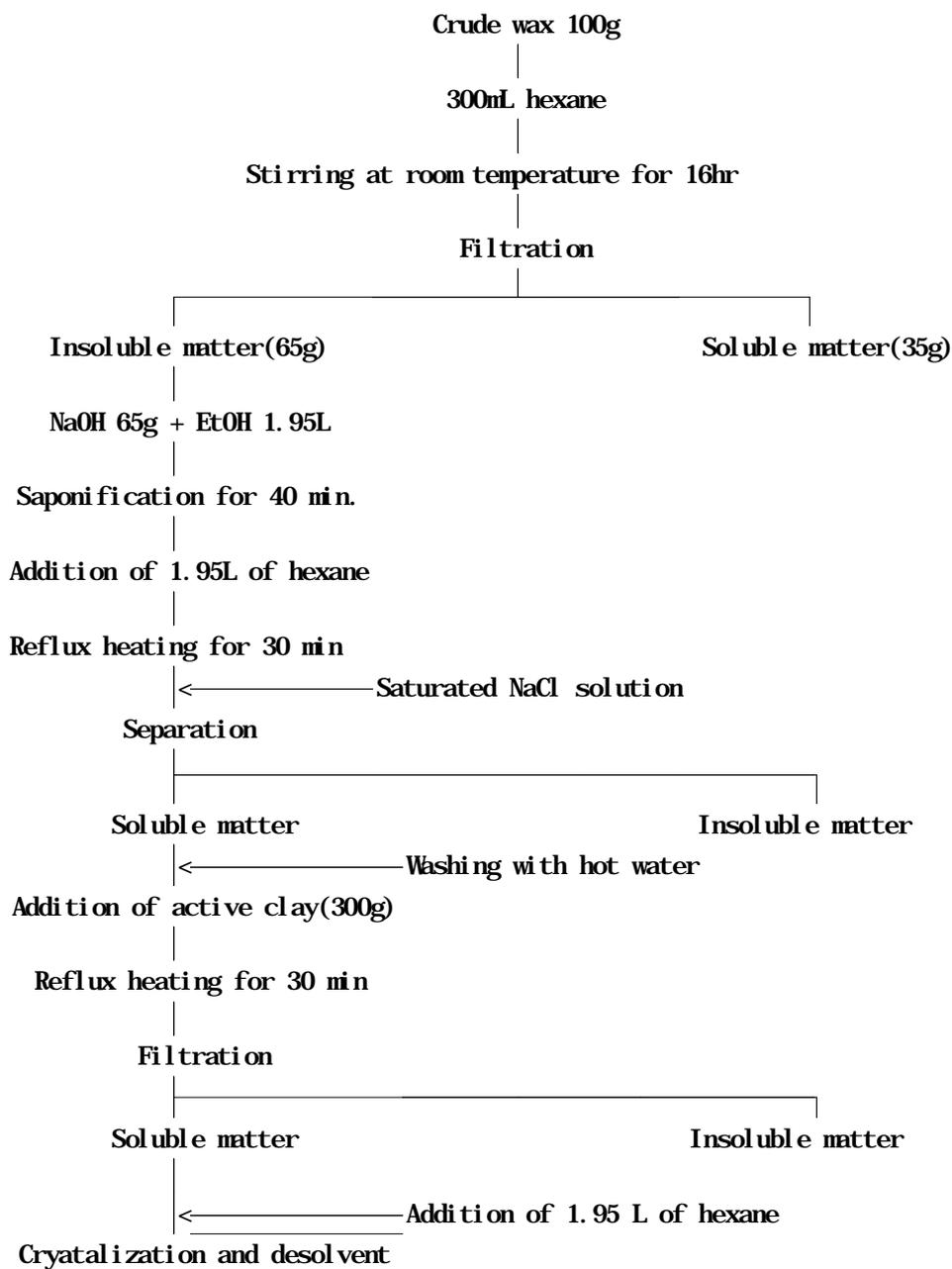


Fig. 4. Flow sheet of purification and isolation of octacosanol in dewaxing materials from rice bran oil processing

가 가 40 가
 4c
 17.9%

2 . (-oryzanol)

1. Soap stock dark oil

가 soap stock hexane
 dark oil -oryzanol
 , soap stock 100g 20ml 가
 , hexane 100ml 가 hexane hexane
 , dark oil , soap stock
 100g 20ml 가 , 25 , 10,000rpm 15
 (dark oil)
 dark oil 6
 , dark oil . hexane
 가
 dark oil .
 soap stock dark oil dark oil
 7 . Soap stock 250g
 dark oil , 가

가 , 70°C dark oil
49% .

Table 6. The comparison of concentration and recovery yield of -oryzanol in the dark oils by different separating methods

		Wt. (g)	water concentration(%)	-oryzanol		
				concentration(%)	wt. (g)	recovery(%)
Soap stock		100	58.24	5.88	5.88	100
D. oil	A	39.06	0.24	12.57	4.90	83.33
	B	37.84	4.70	12.87	4.87	82.82

(A: dark oil by hexane-extracting from soap stock, B: dark oil by centrifugal method from soap stock)

soap stock dark oil -oryzanol 70°C
가 soap stock 70°C

Table 7. The comparisons of concentration and recovery yield of -oryzanol in the dark oils from soap stock at different temperatures

	temp. ()	Wt. (g)	H2SO4 consum- ption(ml)	final temp. ()	-oryzanol		
					conc. (%)	wt. (g)	recovery(%)
Soap stock	—	250	—	—	6.03	15.08	100
D. oil	30	42.7	38.5	55.0	12.07	5.15	34.15
	50	30.9	26.0	61.5	11.20	3.46	22.94
	70	62.8	22.0	70.0	11.88	7.46	49.47
	75	61.3	20.0	75.0	11.96	7.33	48.61

2. Dark oil crude -oryzanol

가. -oryzanol

Dark oil 7가 (acetonitril, methanol, acetone, chloroform, ether, hexane, pentane) 1:2(W/V) 4
20hrs. , 4 , 10,000rpm 10

-oryzanol 5 .

Chloroform , hexane
pentane 가 35% 30% ,
pentane hexane 가
hexane .

. Hexane crude -oryzanol

Dark oil hexane 1:1, 4, 8, 12, 16, 20, 24 (W/V)
4 20hrs. , 4 , 10,000rpm 10

-oryzanol 6

. Hexane crude -oryzanol
hexane 2 7 . 6

dark oil hexane 가
, 1:12 가 ,

1:1 dark oil ,

1 가 1:1

. 7 2 1:1

1:16 , 1:20 .

2 1:16 1:20

가 1:20 .

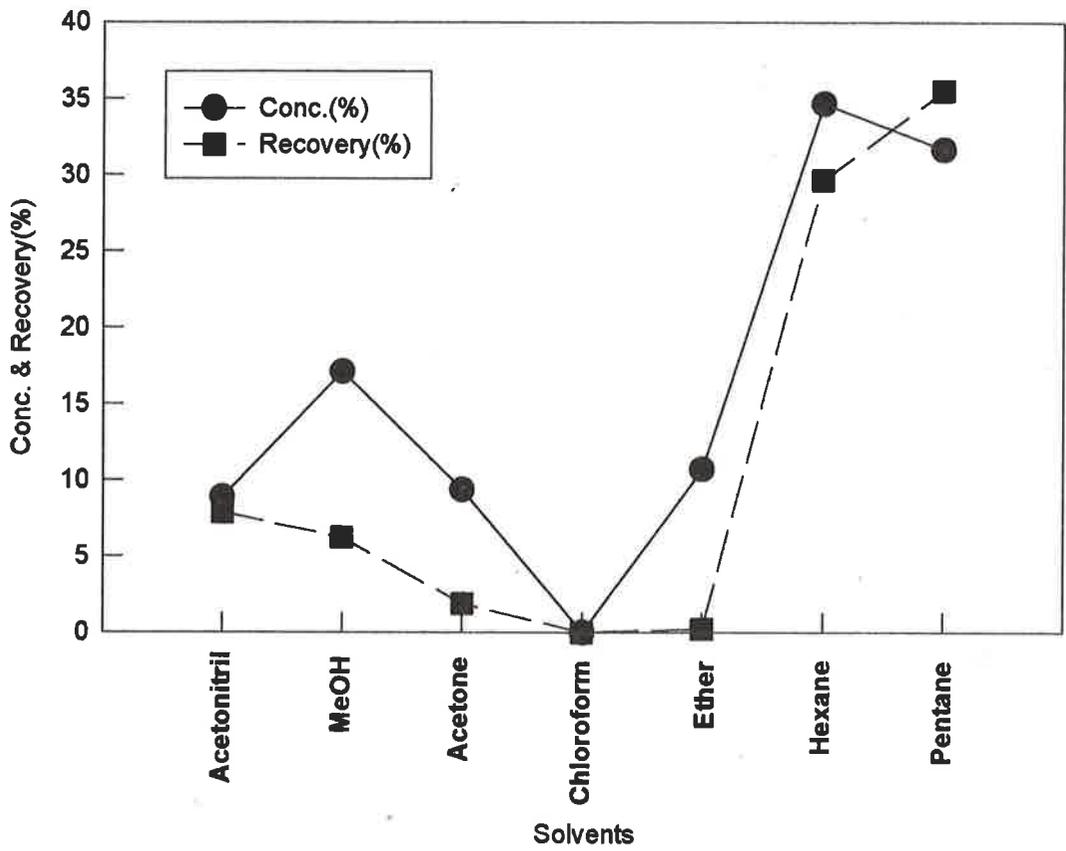


Fig. 5. The changes of concentration and recovery yield of *r*-oryzanol in the precipitate by various solvents

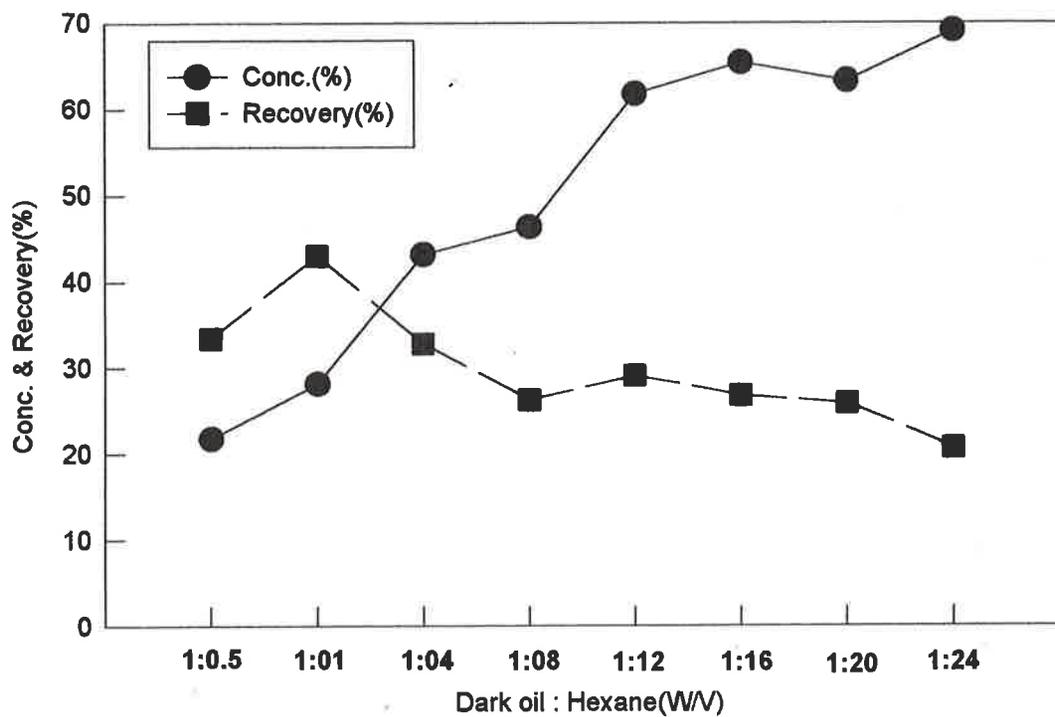


Fig. 6. The changes of concentration and recovery yield of crude *r*-oryzanol from dark oil

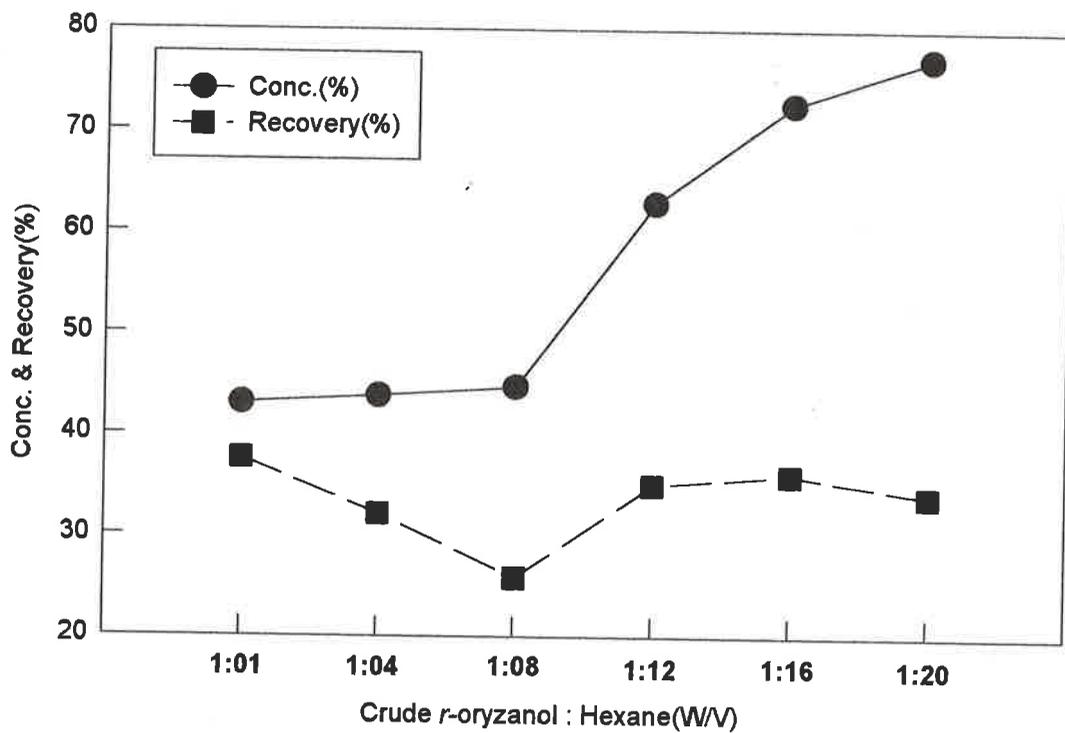


Fig. 7. The changes of concentration and recovery yield of the partial purified *r*-oryzanol from the crude *r*-oryzanol

3. -oryzanol

Hexane crude -oryzanol methanol
 1: 5, 10, 15, 20, 25, 30, 35 (W/V) 80
 , . methanol Whatman
 No. 1 -oryzanol
 8 .
 9 8 .
 -oryzanol ,
 가 ,
 soap stock -oryzanol , .
 Soap stock -oryzanol dark oil
 , hexane dark oil dark oil
 -oryzanol 가 , dark oil
 . Dark oil -oryzanol
 -oryzanol ,
 hexane . 1 crude -oryzanol
 hexane 가 , dark oil : hexane = 1 : 1 (W/V)
 32.4% 가 , 31.6% . 2
 crude -oryzanol : hexane = 1 : 20 (W/V) -oryzanol
 75.6%, 26.2% -oryzanol .
 -oryzanol , -oryzanol
 methanol , -oryzanol : MeOH = 1 :
 30 (W/V) 91.5% -oryzanol dark oil
 24% .

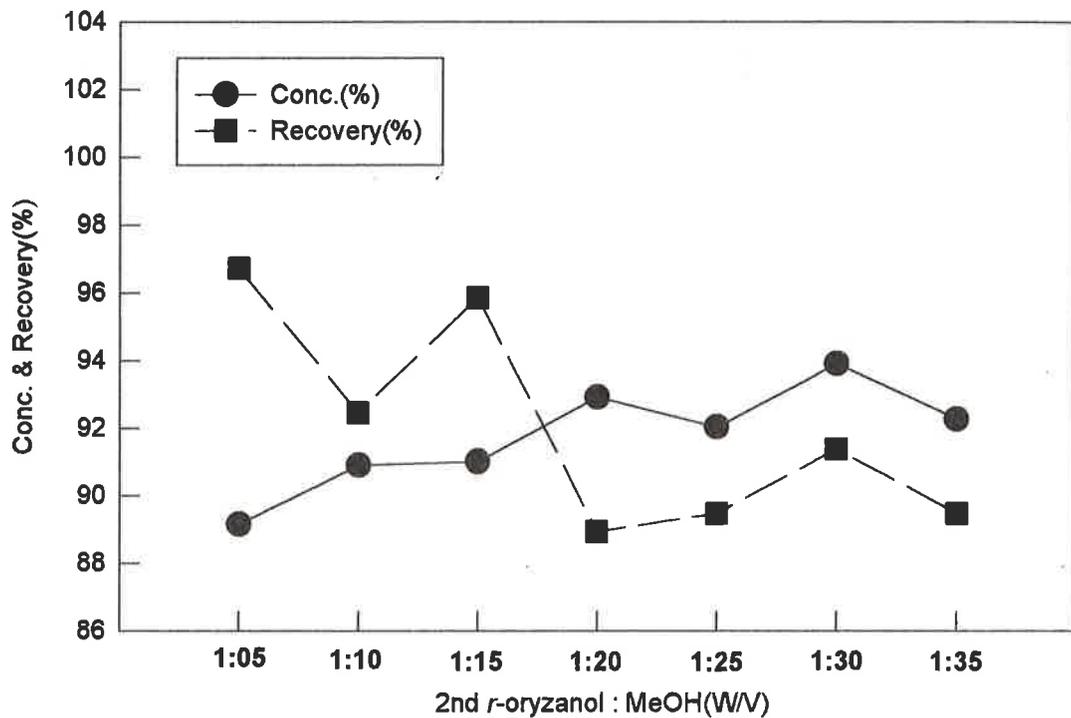


Fig. 8. The changes of concentration and recovery yield of pure *r*-oryzanol from the partial purified *r*-oryzanol from the partial purified *r*-oryzanol by amount of methanol

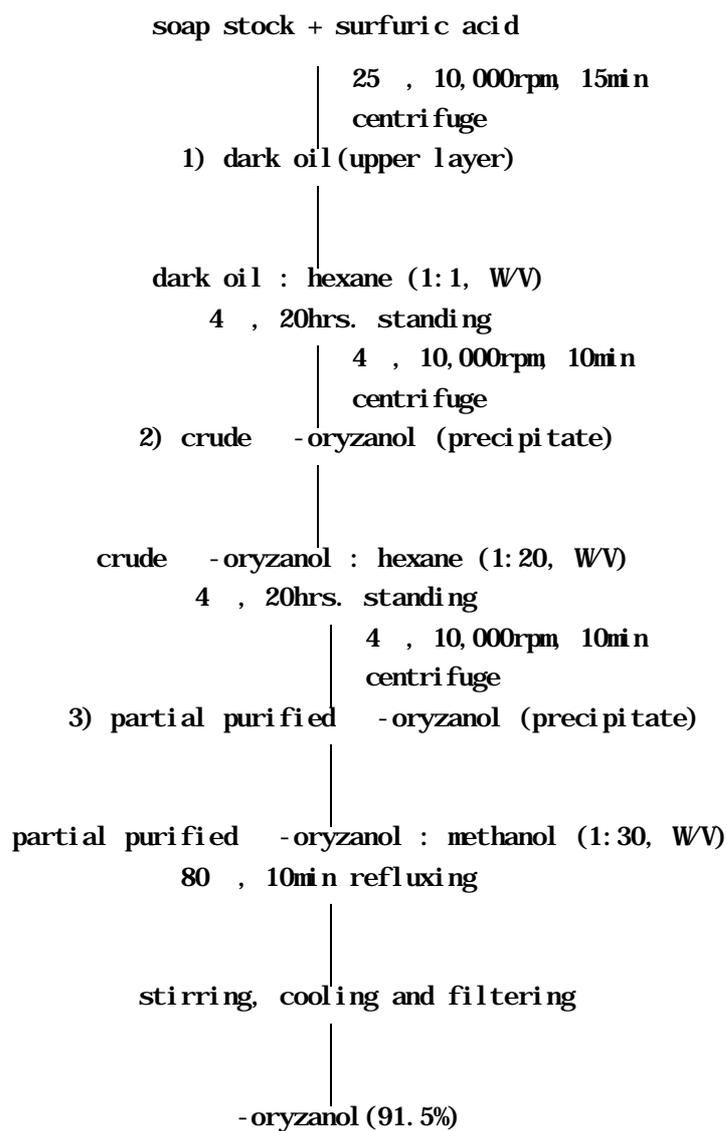


Fig. 9. The flow sheet for isolation of -oryzanol by solvent fractionation

Table 8. The change of concentration and recovery yield of -oryzanol in the products from isolation process

	-oryzanol		
	conc. (%)	recovery(%)	wt. from dark oil (g)
1) dark oil	13.46	100	13.46
2) crude -oryzanol	31.67	32.40	4.23
3) partial purified			
2nd cru. -oryzanol	75.60	26.26	3.44
4) final -oryzanol	91.54	24.00	3.14

4. (-oryzanol) ,

가. Soap stock

1) dark oil

97 2 3 soap stock
-oryzanol , dark oil

1 dark oil ,

10 . 10% KOH dark oil

9 . 1

soap stock 가 dark oil

13% , 10% KOH 1

dark oil . soap stock

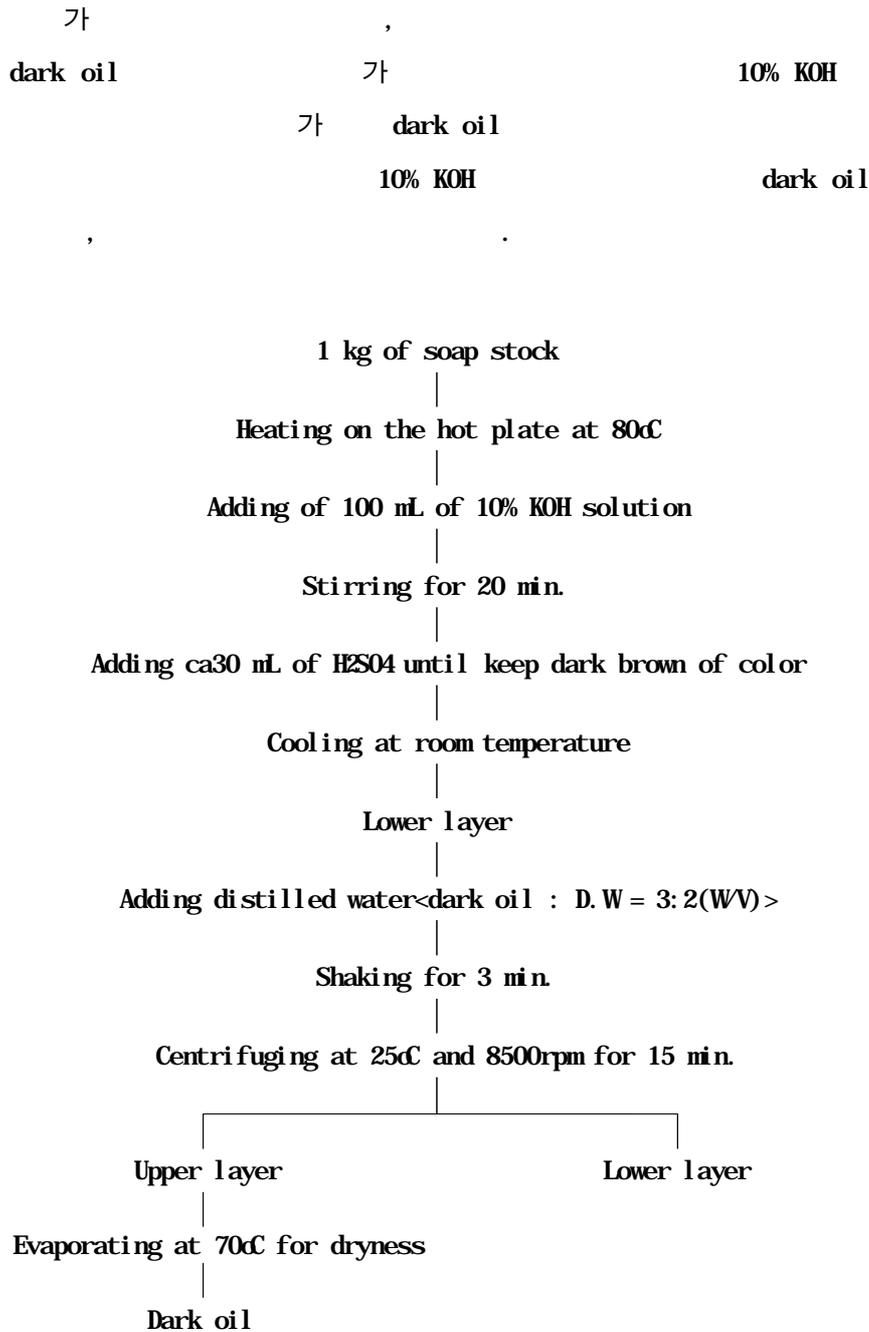


Fig. 10. Flow sheet of resaponification method for preparation of dark oil

Table 10. -Oryzanol contents of pitch prepared by steam distillation method

Samples	Yield(g)	-oryzanol content(%)
Dark oil	433.3g	11.62
Distilled fatty acids	127.0g	0.19
Pitch	325.0g	19.00
Isolated -oryzanol after DM*		73.17

* DM: steam distillation method

5. -oryzanol

가. pitch

dark oil 461g 40 , 5000psi
pitch , pitch 87.6g , -oryzanol
37.2% .
pitch 2 pitch
. 11
-oryzanol 11 .
5000psi 40c dark
oil dark oil , .

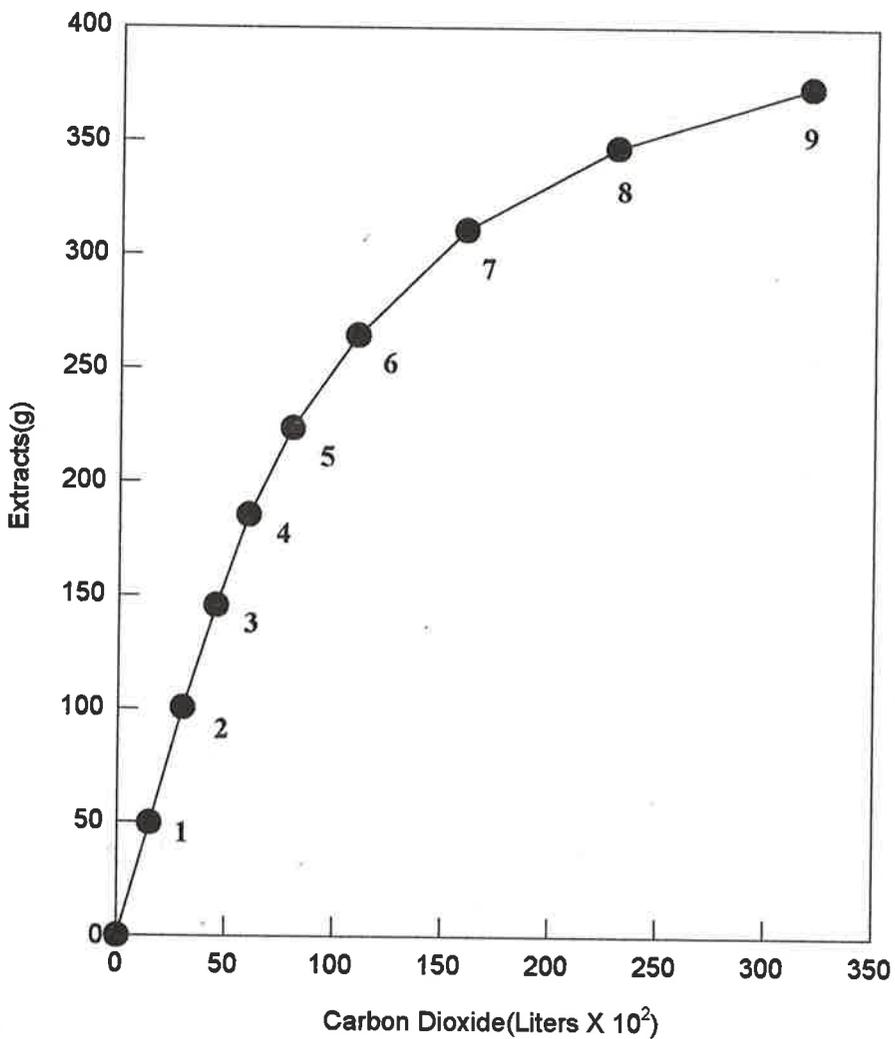


Fig. 11. The extraction curve of the fatty acid extracted for a preparation of the pitch from the dark oil by supercritical carbon dioxide

Table 11. -Oryzanol contents of fatty acid fractions from dark oil using supercritical carbon dioxide at 40 °C under 5000psi

Fractions	Consumed CO2 (L)	Fatty acid (g)	Oryzanol contents (%)
Fr. 1	1500	49.6	1.67
Fr. 2	1500	50.8	1.83
Fr. 3	1500	45.3	2.11
Fr. 4	1500	40.0	2.42
Fr. 5	2000	38.2	2.76
Fr. 6	3000	41.0	3.26
Fr. 7	5000	46.2	4.46
Fr. 8	7000	36.1	6.76
Fr. 9	9000	26.6	11.52
pitch	-	-	37.17
total	32000	373.8	

pitch -oryzanol ,
 dark oil pitch , 1
 -oryzanol , 90.17%
 -oryzanol

12

Table 12. Content and yield of -oryzanol obtained in saponification steps from pitch treated by supercritical carbon dioxide extraction

	-oryzanol (%)	-oryzanol (g)	recovery rate(%)
SCDE pitch	37.17	19.72	100
crude oryzanol	79.84	5.37	27.23
-oryzanol	90.17	2.47	12.53

* supercritical carbon dioxide extraction

pitch, crude -oryzanol, -

-oryzanol chloroform

0.1% TLC plate spotting . TLC plate benzene :

ethyl acetate(9:1, v/v) 가 chamber

iodine vapor -oryzanol fraction

HPLC chromatogram

13 .

pitch 24-methylene cycloartenyl ferulate

-sitoseryl ferulate ,

cycloartenyl ferulate 24-methylene cycloartenyl ferulate .

-sitoseryl ferulate campesteryl ferulate 가

, 24-methylene cycloartenyl ferulate >

cycloartenyl ferulate > campesteryl ferulate, -sitoseryl ferulate

Table 13. Changes or -oryzanol compositions of pitch obtained by supercritical carbon dioxide extraction at purification steps

unit : Area%(relative %)

	cycloartenyl ferulate	24-methylene cycloartenyl ferulate	campesteryl ferulate	-sitoseryl ferulate
SCCD pitch	9.22(14.36)	19.53(30.41)	16.01(24.93)	19.46(30.30)
Crude oryzanol	19.64(30.69)	30.69(47.96)	7.59(11.86)	6.07(9.49)
-oryzanol	27.74(34.01)	38.98(47.79)	7.58(9.29)	7.27(8.91)

11 1(1+2),
 2(3+4), 3(5+6), 4(7+8) 5(9) silica gel(25g, 70 230 mesh, column
 chromatography, Merck) column, n-hexane 200ml, n-hexane
 : ether (8 : 2) 150ml, n-hexane : ether (5 : 5) 150ml, ether 150ml
 TLC -oryzanol spot, n-hexane,
 n-hexane : ether (8 : 2) -oryzanol spot,
 n-hexane : ether (5 : 5), ether -oryzanol spot
 , n-hexane : ether (5 : 5), ether HPLC
 14

24-methylene
 cycloartenyl ferulate, cycloartenyl ferulate, campesteryl ferulate,
 -sitosteryl ferulate 24-methylene cycloartenyl ferulate
 -sitosteryl ferulate가,
 24-methylene cycloartenyl ferulate가.

Table 14. Compositions of -oryzanol in fatty acid removed by supercritical carbon dioxide extraction

unit : Area%(relative %)

fraction	cycloartenyl ferulate	24-methylene cycloartenyl ferulate	campesteryl ferulate	-sitosteryl ferulate
Fr. 1	15.0(20.9)	19.8(27.7)	15.3(21.4)	21.5(30.0)
Fr. 2	13.4(18.7)	19.1(26.7)	15.4(21.5)	23.6(33.0)
Fr. 3	14.7(20.0)	20.0(27.2)	15.9(21.6)	22.9(31.2)
Fr. 4	19.3(23.4)	23.0(27.9)	18.0(21.8)	22.2(26.9)
Fr. 5	18.0(24.2)	22.1(29.7)	17.8(24.0)	16.4(22.1)

6. dark oil

1 soap stock 15 . Dark oil

11.3% 95.7%

Table 15. Content and yield of -oryzanol obtained from isolation steps

	-oryzanol (%)	-oryzanol (g)	recovery rate(%)
Dark oil	11.30	16.95	100
Crude oryzanol	35.71	7.42	43.8
2nd purified oryzanol	89.44	4.90	28.9
-oryzanol	95.77	4.10	24.2

dark oil, crude oil, 2nd purified -oryzanol, -oryzanol
HPLC 16 . dark oil

24-methylene cycloartenyl ferulate cycloartenyl ferulate
, cycloartenyl ferulate

24-methylene cycloartenyl ferulate가 ,
-sitosterol ferulate가 .

Soap stock dark oil

1 . 1

2 ,

-oryzanol dark

Table 16. Changes of -oryzanol compositions at purification step of -oryzanol from dark oil by solvent fractionation

unit : Area%(relative %)

	cycloartenyl ferulate	24-methylene cycloartenyl ferulate	campesteryl ferulate	-sistosteryl ferulate
Dark oil	19.59(27.15)	26.18(36.29)	14.37(19.92)	12.01(16.65)
Crude oryzanol	21.89(29.83)	27.95(38.09)	13.88(18.92)	9.66(13.16)
2nd purified oryzanol	19.36(29.16)	28.74(43.28)	11.08(16.69)	7.22(10.87)
-oryzanol	22.60(31.65)	30.57(42.82)	11.13(15.59)	7.10(9.94)

oil
 24-methylene cycloartenyl ferulate cycloartenyl ferulate
 , cycloartenyl ferulate 24-methylene cycloartenyl
 ferulate 가 .
 campesteryl ferulate가 .
 pitch 2가 ,
 95% 가 .

3

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