CHAPTER IV

RESULTS AND DISCUSSION

In this study, the rice bran wax was supplied by Thai Edible Oil Co. Ltd. The separation of this wax was performed by modifying TREF with Crystaf. The objective of this research was to purify rice bran wax components and investigate physical properties of wax in each fraction. The separation was attempted twice. Therefore wax in each separation hereafter was refined as wax 1 and wax 2, respectively.

4.1 Crystallization process

4.1.1 Crystallization step with 500 µm sand

The process was heating the 500 gram inert support with 5 gram wax in 150 ml of hexane. It was started by heating the system to 72 °C and refluxing for 1hour, then cooling down at the rate of 3°C/hour until the temperature down to 20 °C. The mother liquor was removed slowly so that the rest of the system was disturbed. After the solvent was evaporated, wax is obtained.

4.2 Elution process

The elution used hexane as a solvent. The solvent tank was prepared for eluting column. Then, wax was eluted at various temperature, 30, 40 and 50°C, with hexane is obtained four fractioned waxes, respectively.

4.2.1 500 µm sand elution process with hexane solvent

The elution using hexane as solvent. After that, the inert support was packed into the column and eluted at various temperature, 30, 40 and 50°C.The flow rate of hexane solvent about 15 ml/minute. The first elution step the solvent is not clear (Color is white) until the elution and step is done when the solvent is clear.

4.3 Investigation comparison of the waxes properties of the eluting fractions.

The fractions eluting at 20°C, 30°C, 40°C and 50 °C were characterized. Replicate samples (2) from each fraction were analyzed. For comparison reference materials consisting of a wax and stearic acid samples or suitable reference (such as cyclohexene for the iodine value) were also analyzed using the same methods as the individual fraction.

Distribution of wax in the eluting fractions was determined gravimetrically after evaporating the eluting solvent and the weight of wax in each fraction was recorded.

4.3.1 The refined rice bran wax specification

Rice wax is a vegetable wax extracted from rice bran when extract rice bran oil. It is a treasure biologic wax resource in East Asia where rice is the main food. Rice wax has no odor and bleaches readily, and its impurities are easy to move. It has many applications.

Component up to now there has been many research and report concerning rice wax. The main component of rice wax is ester formed by senior fatty acid and senior mellow. The range of carbon element is limited, so the fat of this ester is mainly composed by C_{22}-C_{24}, the senior ester is C_{24}-C_{34}. DSC (differential scanning calorimeter) testing indicates that when the rice wax melts, the summit of each part is very steep. According to this we can conclude that rice wax possesses high crystallization.

Application

Long before people had used rice wax for skin maintenance, coating for furnitures and abacus, and so on. Now rice wax is used more for food (packing, insulating agents), polishing agents. It is firstly used as the material of cosmetics. Rice wax is widely used in the high technology areas such as resolvability agglutinant, plastic lubricants. The specification of refined rice wax was shown in Table 4.1.

Table 4.1 Specification of refined rice bran wax.

Product	**Product standard (typical data)**	
Refined rice bran wax*	Acid value	Under13
	Saponification value	75-90
	Iodine value	Under15
	Melting point (°C)	78-82
	Needle penetration	5
	Viscosity (cp)	14.3
	Specific gravity (kg/m^3)	0.974
	Flash point minimum (°C)	300
	Color	yellow

* Beijing cross-century science and technology Co. Ltd.

4.3.2 Physical properties of fractioned wax

The physical properties of all fractioned waxes and raw waxes were shown in Table 4.2.

Table 4.2 The physical properties of fractioned waxes.

Wax or Fractioned wax	Saponification value	Acid value	Iodine value	Drop melting point
Refined rice wax*	75-90	Under13	Under15	78-82
Raw rice wax 1	165.43	20.78	26.50	74-80
Raw rice wax 2	168.42	22.81	33.00	76-78
Fractioned wax 1 (20 °C)	252.84	25.21	52.18	69-71.5
Fractioned wax 2 (20 °C)	235.40	23.29	54.74	68.5 – 71
Fractioned wax 1 (30 °C)	160.12	13.41	44.51	75
Fractioned wax 2 (30 °C)	163.62	14.49	45.79	77
Fractioned wax 1 (40 °C)	142.89	5.06	11.25	76-80
Fractioned wax 2 (40 °C)	144.70	5.51	11.89	77-78
Fractioned wax 1 (50 °C)	129.00	15.13	8.69	75-76
Fractioned wax 2 (50 °C)	135.14	14.52	7.41	75-76

* Beijing cross-century science and technology Co. Ltd as a reference.

Saponification value, acid value, iodine value and drop melting point of all fraction eluted at 40 °C and 50 °C waxes were in the same range of the reference wax, Beijing cross-century science and technology Co. Ltd. However, the acid value of the wax in fraction eluted at 40 °C was 5.0-5.5 which lowest among all fractioned waxes was

even lower than the reference wax. This indicated that the fractioned wax at 40°C contained small amount of carboxylic acids than the other fractioned waxes. This made it more valuable for cosmetics and medicine industries. In addition, all fractioned waxes had high saponification value indicating that these waxes were ester waxes.

4.3.3 The weight distribution of rice bran wax

The distribution of wax in the eluting fractions are shown in Table 4.3

Table 4.3 The Distribution of wax in each fraction.

The Distribution of rice bran wax						
Eluted temperature (°C)	**Fractioned wax 1 gram**	**Fractioned wax 1 %**	**Fractioned wax 2 gram**	**Fractioned wax 2 %**	**Average Weight**	**Average Percent**
20	1.01	20.82 %	1.07	21.70%	1.04	21.26 %
30	1.36	28.04 %	1.33	26.97%	1.345	27.50 %
40	1.41	29.07 %	1.49	30.22%	1.45	29.64 %
50	0.75	15.46 %	0.79	16.02%	0.77	15.74 %
60	0.18	3.71 %	0.25	5.00%	0.215	4.35 %
over 60	n/a	0.00 %	n/a	0.00 %	n/a	0.00 %
Total	4.71	97.11 %	4.93	96.67%	0.9689	96.89 %
Loss	0.14	2.89 %	0.17	3.33%	0.155	3.11 %

*The raw data are shown in Appendix C

It should be seen that the distribution of wax fraction at 40 °C is the major fraction and it was about 30% of total wax.

4.3.4 FT-IR results

The IR spectra of waxes of all fractions were shown in Appendix A. The absorption bands of these fractioned waxes were also compared with the ones of raw wax.

Table 4.4 to 4.7 exhibit the absorption assignment of the waxes in fractions eluted at 20 °C, 30 °C, 40 °C and 50 °C, respectively.

Table 4.4 The absorption assignment of wax fraction at 20 °C.

Wave number (cm^{-1})		Assignment
Fractioned wax	**Raw wax**	
3450	3450	O-H Stretching
2851	2851	C-H Stretching, aliphatic
1736	1743	C=O Stretching
1463	1462	C-H Bending, aliphatic
1158	1174	C-O Stretching

Table 4.5 The absorption assignments of wax fraction at 30 °C.

Wave number (cm^{-1})		Assignment
Fractioned wax	**Raw wax**	
3450	3450	O-H Stretching
2851	2851	C-H Stretching, aliphatic
1736	1743	C=O Stretching
1463	1402	C-H Bending, aliphatic
1160	1174	C-O Stretching

Table 4.6 The absorption assignments of wax fraction at 40 °C.

Wave number (cm^{-1})		Assignment
Fractioned wax	**Raw wax**	
3450	3450	O-H Stretching
2851	2851	C-H Stretching, aliphatic
1736	1743	C=O Stretching
1463	1402	C-H Bending, aliphatic
1169	1174	C-O Stretching

Table 4.7 The absorption assignments of wax fraction at 50 °C.

Wave number (cm^{-1})		Assignment
Fractioned wax	**Raw wax**	
-	3450	O-H Stretching
2850	2851	C-H Stretching, aliphatic
1731	1743	C=O Stretching
1466	1402	C-H Bending, aliphatic
1172	1174	C-O Stretching

According to data in Table 4.4-4.7 and Appendix A, all fractioned waxes exhibit the absorption bands at 1730-1743 cm^{-1} (C=O Stretching) and 1158-1174 cm^{-1} (C-O Stretching), similar to raw wax, which are characteristics of ester wax. It could thus be anticipated that all fractioned waxes and raw wax were ester wax. It should be mentioned that the weak and broad absorption bond at 3450 cm^{-1} was observed in all spectra except for the wax in fraction eluted at 50 °C. This probably belongs to either long chain alcohol or water.

4.3.5 GC-MS results

All fractioned waxes were analyzed by GC-MS comparing with raw wax. The GC-MS spectra were shown in Appendix B. In addition, some significant GC-MS data of all fractioned waxes listed in Table 4.8-4.12.

By using GC-MS, the possible structure of each separated component could be identified. The relative quantity of each component was also shown.

Table 4.8 GC-MS result of raw rice bran wax.

Peak number	Retention time (min)	Area %	Structure
1	2.31	5.40	Benzene, 1,3-dimethyl
2	2.34	0.40	Benzene, 1,3-dimethyl

3	2.90	1.48	Decanoic acid, ethyl ester
9	7.22	1.15	Hexadecanoic acid, methyl ester
10	8.83	1.49	9 -Octadecenoic acid, methyl ester
14	12.41	5.53	Docosanoic acid, methyl ester
15	13.92	13.49	Tetracosanoic acid, methyl ester

The GC-MS results of fractioned wax at 20 °C were shown in Table 4.9 and the spectra corresponding to the individual chromatographic peaks and GC-MS profiles were shown in Figure 2B (Appendix B).

Table 4.9 The GC-MS result of fractioned wax at 20 °C

Peak number	Retention time (min)	Area %	Structure
1	2.31	5.40	Benzene, 1,3-dimethyl
2	2.34	0.40	Benzene, 1,3-dimethyl
4	3.68	9.62	Hexadecanoic acid
14	7.39	3.15	Octadecanedioic acid
20	8.85	2.30	Hexadecanedioic acid
24	10.13	0.74	n-Eicosane
25	10.25	0.99	9-Octadecenoic acid
30	11.10	1.30	Oxirane
33	11.46	1.63	Hexadecane
38	12.62	3.52	Octadecanal

The GC-MS results of fractioned wax at 30 °C were shown in Table 4.10 and the spectra corresponding to the individual chromatographic peaks and GC-MS profiles were shown in Figure 3B (Appendix B).

Table 4.10 The GC-MS result of fractioned wax at 30 °C

Peak number	Retention time (min)	Area %	Structure
1	2.25	0.83	Nonanoic acid
3	2.79	0.45	Undecenoic acid
4	2.91	0.68	Decanoic acid, ethyl ester
5	4.29	0.20	Dodecanoic acid, ethyl ester

11	7.57	9.38	Hexadecanoic acid
16	10.57	18.30	Undecanedioic acid
19	12.06	4.46	9-Octadecenoic acid
32	14.41	0.72	Nonadecane
35	15.13	1.51	n-Eicosane
40	15.78	1.63	Hexadecane
44	16.11	1.41	16-Octadecenal
47	16.49	2.24	n-Octadecane
54	17.65	1.55	Oxirane
55	18.08	0.70	Nonadecane

The GC-MS results of fractioned wax at 40 °C were shown in Table 4.11 and the spectra corresponding to the individual chromatographic peaks and GC-MS profiles were shown in Figure 4B (Appendix B).

Table 4.11 The GC-MS result of fractioned wax at 40 °C

Peak number	Retention Time (min)	Area %	Structure
1	2.30	24.10	Benzene, 1,2-dimethyl
2	2.90	4.98	Decanoic acid, ethyl ester
3	4.28	1.12	Dodecanoic acid, ethyl ester

The GC-MS results of fractioned wax at 50 °C were shown in Table 4.11 and the spectra corresponding to the individual chromatographic peaks and GC-MS profiles were shown in Figure 5B (Appendix B).

Table 4.12 The GC-MS result of fractioned wax at 50 °C

Peak number	Retention Time (min)	Area %	Structure
2	2.90	1.26	Decanoic acid, ethyl ester
3	4.28	0.30	Dodecanoic acid, ethyl ester
9	7.55	1.00	Hexadecanoic acid
11	10.55	8.55	Undecanedioic acid
13	12.03	4.79	9-Octadecenal
14	12.22	0.50	Octadecanedioic acid

18	15.01	0.12	Silicic acid
19	15.12	0.62	n-Eicosane

From Table 4.8-4.12, the major components are fatty acid and fatty acid ester. In Table 4.11, the major components are only fatty acid esters and it can be concluded that the fractioned wax at 40 °C is more purer than the others.

All spectra show the typical figures which were composed of peaks at different retention times but no peaks after retention time of 12 minute. The GC-MS temperature program was set in such a way that increasing rate of temperature was 10 °C/minute. Since no peaks appeared after retention time of 12 minutes which the temperature was around 300 °C, the rest components must be long chain ester waxes.

At low eluting temperature, small carboxylic acid, aliphatic hydrocarbon and some aliphatic aldehyde were obtained. At high eluting temperature, large molecules of these compounds as well as the ethyl ester wax were detected. However, less number of the mentioned compounds appeared in the fraction eluted at higher temperature. In the other words, higher purified wax was obtained for the fractioned wax eluted of high temperature.

These results were corresponding to the acid value measurement. For wax in fraction eluted at 40 °C, only the ethyl esters of carboxylic acids were present and the wax had acid value 5.0-5.5, which was the best fraction in this separation.