CHAPTER III

EXPERIMENTAL

3.1 Apparatus

1. Fourier-Transform Infrared Spectrophotometer : Model Impact 410; Nicolet
2. Water circulating system : Model F33; Julabo
3. Rotary evaporators : Model R244; Buchi
4. Gas Chromatography Mass Spectometer : Model HP 6890 GC-MS :Agilent

3.2 Chemicals

1. Hexane: analytical grade; Lab-Scan
2. Dichloromethane: analytical grade; Lab-Scan
3. Acetone: analytical grade; Lab-Scan
4. Mixed-Xylene : analytical grade; Merck
5. Cyclohexene: analytical grade; Fluka
6. Carbon tetrachloride : analytical grade; Merck
7. Ethanol : analytical grade; Merck
8. Potassium hydroxide: analytical grade; Merck
9. Potassium bromide: analytical grade; Fluka
10. Potassium dichromate: analytical grade; Merck
11. Potassium iodide: analytical grade; Merck

12. Potassium permanganate: analytical grade; Merck
13. Phenolphthalein indicator : analytical grade; Merck
14. Iodine : analytical grade; Merck
15. Sodium thiosulfate: analytical grade; Fluka
16. 96% Sulfuric Acid: analytical grade; Lab-Scan
17. 37% Hydrochloric acid: analytical grade; Merck
18. Glacial Acetic Acid: analytical grade; Merck
19. Chloroform-D1: NMR spectroscopy grade; Merck
20. Stearic acid : analytical grade; Merck
21. Rice Wax; Thai Edible Co., Ltd.

3.3 Procedure

3.3.1 Preparing inert support

Inert support was prepared by sand. First of all, sand 1500 gramof sand, 150 ml of conc. sulfuric acid and 1500 ml of DI water were added into beaker, stirred a mixture for 24 hours. The mixture was filtered off, and sand residue was washed with excessive amount of DI water. The sand residue was dried in the oven at 110 °C for 10 hours. After this time, a sand residue was sieved (Retsch) on 710 μm and 500 μm, respectively.

3.3.2 Wax crystallization process with Crystaf

The crystallization step was taken as a model from TREF technique. The temperature was heating and cooling by the Julabo circulating system the condition using 500 μm sand as an inert support rice bran wax 5 g to dissolve in hexane 300ml and reflux at 72°C for 1 hour. The temperature was gentle reducing at 1°C /hour between 72 °C to 20

°C. Then, remove the upper solvent and evaporation solvent to get the 20 °C wax fraction from crystaf technique.

3.3.3 Wax elution process by TREF technique

The elution step was also taken as a model from TREF technique. The rate of elution step about 15 cc/ min from the glass column and using hexane as the solvent flow in each fraction using hexane about 1500 cc. The extraction of each fractionation used the rotary evaporator (BUCHI) after, the solvent was dried we get the wax fractions (10 °C per fraction).

3.4 Characterization and determination of wax properties

The properties of wax were measured by the following test method as shown in Table 3.1

Table 3.1 The properties of wax

Testing method	Standard
Saponification Number	ASTM D 1387
Acid Number	ASTM D 1386
Iodine Value	ASTM D 5554
Drop Melting Point	ASTM D 127

3.4.1 Determination of saponification number (ASTM D1387) [11]

The test method covers the determination of the saponification number of synthetic waxes and natural waxes. In term of saponification number is the number of milligrams of potassium hydroxide required to hydrolyze 1 g of the sample and is a measure of the amount of saponifiable matter present.

Procedure

Transfer the sample, weighed to the nearest 0.001 g to 250ml Erlenmeyer flask. Added 40 ml of xylene and few boiling chip, glass bead, dissolve by heating on the hot plate 30 min. Remove from the hot plate and add 50.0 ml of 0.1 *N* ethanolic KOH solution from buret and reflux with reflux condenser for 3 hour using hot plate.

Remove the condenser from the flask and added 5 drops of phenolphthalein solution and titrate the sample with 0.5 *N* HCl until the pink color disappears. Reheat the sample to boiling point and titration until the pink color does not reappear.

Calculation the saponification number

Calculate the saponification number as follows:

Saponification number = (B-A) *N* x 56.1 /C

A = milliliters of HCl solution required for titration of the sample.

B = milliliters of HCl solution required for titration of the blank.

C = grams of sample used.

N = normality of HCl Solution.

3.4.2 Determination of acid number (ASTM D1386) [12]

The test method covers the determination of the acid number of synthetic waxes and natural waxes. The number is obtained by direct titration of the material and indicates the amount of free acid present.

Procedure

Transfer the sample, weighed to the nearest 0.001 g to 250ml Erlenmeyer flask. Added 40 ml of xylene and few boiling chip, glass bead, dissolve by heating on the hot

plate 30 min. Remove from the hot plate and add added 5 drops of phenolphthalein solution and titrate with ethanolic potassium hydroxide, Standard solution (0.1*N*) the hot solution to the first persistent pink color. The end point is taken when the pink color remains for at least 10 sec. Swirl the flask vigorously during the titration, reheat the sample. The titration should be carried out as quickly as possible. Record the number of milliliters of standard alkali solution used.

Calculation the acid number

Calculate the acid number as follows:

Acid number = (A*N* x 56.1) / B

A = milliliters of alkali solution required for titration of the sample.

B = gram of the sample used.

N = normality of alkali solution solution.

3.4.3 Determination of the iodine value of fats and oils (ASTM D5554) [13]

The iodine value is a measure of unsaturation and is expressed as the number of g of iodine absorbed, under the prescribed conditions, by 100 g of the test substance.

Wijs solution

Dissolve 13 g of resublimed iodine in 1,000 ml of glacial acetic acid. Pipet 10.0 ml of this solution into a 250-ml flask, add 20 ml of potassium iodide TS and 100 ml of water, and titrate with 0.1 N sodium thiosulfate adding starch TS near the endpoint. Record the volume required as A. Set aside about 100 ml of the iodine-acetic acid solution for future use. Pass chlorine gas, washed and dried with sulfuric acid, through the remainder of the solution until a 10.0-ml portion requires not quite twice the volume

of 0.1 N sodium thiosulfate consumed in the titration of the original iodine solution. A characteristic colour change occurs when the desired amount of chlorine has been added. Alternatively, Wijs solution may be prepared by dissolving 16.5 g of iodine monochloride, ICl, in 100 ml of glacial acetic acid. Store the solution in amber bottles sealed with paraffin until ready for use, and use within 30 days.

Total halogen content

Pipet 10.0 ml of Wijs Solution into a 500-ml Erlenmeyer flask containing 150 ml of recently boiled and cooled water and 15 ml of potassium iodide TS. Titrate immediately with 0.1 N sodium thiosulfate, recording the volume required as B.

Halogen ratio

Calculate the I/Cl ratio by the formula A/(B - A). The halogen ratio must be between 1.0 and 1.2. If the ratio is not within this range, the halogen content can be adjusted by the addition of the original solution or by passing more chlorine through the solution.

Procedure

The appropriate weight of the sample, in g, is calculated by dividing the number 25 by the expected iodine value. Melt the sample, if necessary, and filter it through a dry filter paper. Transfer the accurately weighed quantity of the sample into a clean, dry, 500-ml glass-stoppered bottle or flask containing 20 ml of carbon tetrachloride, and pipet 25.0 ml of Wijs Solution into the flask. The excess of iodine should be between 50% and 60%

of the quantity added, that is, between 100% and 150% of the quantity absorbed. Swirl, and let stand in the dark for 30 min. Add 20 ml of potassium iodide TS and 100 ml of recently boiled and cooled water, and titrate the excess iodine with 0.1 N sodium thiosulfate, adding the titrant gradually and shaking constantly until the yellow colour of the solution almost disappears. Add starch TS, and continue the titration until the blue colour disappears entirely. Toward the end of the titration, stopper the container and shake it violently so that any iodine remaining in solution in the carbon tetrachloride may be taken up by the potassium iodide solution. Concomitantly, conduct two determinations on blanks in the same manner and at the same temperature.

Calculation

Calculate the iodine value by the formula:

Calculate the iodine value = (B - S) x 12.69 *N* / W

B - S represents the difference between the volumes of sodium thiosulfate required for the blank and for the sample respectively,b///

N is the normality of the sodium thiosulfate,

W is the weight, in g, of the sample taken.

3.4.4 Determination of drop melting point (ASTM D127) [14]

Melting point of rice bran waxes in this research was studied by following ASTM D 127. The waxes were deposited on two thermometer bulbs by dipping chilled thermometers into melting waxes. The thermometers bearing the waxes were placed in test tubes and heated by means of water bath until the waxes melts and first drop fell

from each thermometers bulb. Average of temperatures at which these drops fall was the drop melting point of wax

3.4.5 Determination of wax by GC-MS [11]

Gas Chromatography (GC) is a powerful analytical tool used for the separation and detection of organic compounds. A typical GC system utilizes a carrier gas (usually helium or hydrogen) to transport the analytes from the injection port, through the column (which is located in a temperature-programmable oven, where separation occurs) and on to the detector where responses are observed as the specific compounds pass.

Separation of multiple analytes is done by taking advantage of the differences in boiling points of the compounds in solution, as well as the differences in their affinities toward the analytical column through which they pass (e.g., polarity may influence affinity). The higher the boiling point, the later the compound comes off, or elutes. The solution to be analyzed is introduced to the system by either injection or purging of the solution. At this point all the compounds of interest are in a liquid state on the head of the column with carrier gas rushing by and the internal temperature is less than the boiling points of the compound to be analyzed.

At the end of the column is a detector (or detectors) which observe compounds based on their specific properties. Qualitative peak identification is based upon the retention times of the components in an unknown solution as compared to the retention times in a known standard. Quantitation is achieved by analyzing a known amount of a substance in a series of standards and obtaining a response factor. The magnitude of the detector response of the unknown is then compared to the calibration curve and the concentration calculated

Procedure followed by CIF condition

A Hewlett Packard 6890 GC equipped with a MSD (Mass Spectrometer Detector). A capillary column model number Agilent 19091S-433 with a nominal length of 29.8 meters was used and installed for the front inlet. The maximum recommended temperature for this column is 350 °C. The initial temperature for injection inlet was set to 325 °C using the split mode setting of the split/splitless inlet. The split ratio was 5:1. The pressure of the helium carrier gas was set 13.19 psi with a total flow rate of 8.7 ml/min and split flow of 5:0 ml/min.

3.4.6 Determination of wax by FT-IR

KBr pellets were prepared with samples of the waxes from each fraction and anhydrous KBr. A Nicolet Model Impact 410 FT-IR spectrometer with a KBr pellet press holder and the supplied pellet making apparatus was used to record the IR spectrum of each wax fraction. The IR spectra of waxes were shown in appendix A. The important absorption bands of waxes were listed in table 4.4 to 4.8, respectively.

3.5 Choose the inert support

In this study, 4 inert supports in this experiment need the suitable support. The support are glassbead 2 mm and 4 mm, siliga gel 0.063-0.200 mm and the sand 500 µm, respectively.

Because of using glassbeads and siliga gel can't control the crystallization and elution methods as much as possible glassbeads have not enough area surface in the other hand siliga gel has too much surface area and in this study don't have the high pressure

pumping for feeding the solvent into column. So, in this research using sand 500 μm as an inert support.