CHAPTER 3
MATERIALS AND METHODS

3.1 Apparatus

3.1.1 X-ray diffractometer, Bruker AXS model D8 Discover

3.1.2 X-ray fluorescence spectrometer, Bruker AXS model S4 Pioneer

3.1.3 Fourier Transform infrared, Perkin Elmer Spectrum One and Bruker Tensor 27 FTIR spectrometers

3.1.4 Electron paramagnetic resonance, JEOL (JES-RE2X), Bruker EMX both CW spectrometers operating at X-band frequencies

3.1.5 Scanning electron microscope, JEOL model JSM-5410 LV

3.1.6 Quantachrome Autosorb-1

3.1.7 Lovibond tintometer, Pardero Model F

3.1.8 UV-visible spectrophotometer, Spectronic 21 Banusch & Lomb

3.1.9 Planetary ball mill, model Retsch S 100

3.1.10 pH meter, Delta 340 Meltler Toledo

3.1.11 Centrifuge: Labofuge 200, Heraeus, SEPATECH

3.1.12 Mechanical shaker with temperature controller, Gallenkamp

3.1.13 Oven, Memmert model UNB 500

3.1.14 Hotplate – Magnetic Stirrer, IKA model C-MAG HS7

3.1.15 Electronic Balance (4 digits)

3.1.16 Reflux condenser

3.1.17 Glassware, volumetric flask, burette, pipette

3.2 Materials and chemical reagents

3.2.1 The kaolin, from Ranong Province in southern Thailand was supplied by Had Som Pan Co., Ltd.

3.2.2 Degumed and neutralized rice bran oil was obtained from the Thai Edible Oil Co.Ltd., Thailand

3.2.3 Sulfuric acid (AR grade), Merck, Germany

3.2.4 Oxalic acid (AR Grade), Ajax Finechem, Australia

3.2.5 Hexane (AR Grade), Ajax Finechem, Australia Mallinckrod

3.2.6 Commercial bleaching clay, Taiko Clay Marketing Sdn.Bhd., Malaysia

3.3 Experimental procedures

3.3.1 Sample preparation

The kaolin from Ranong deposit, Thailand, pale yellow in color was initially washed with distilled water and dried overnight in an oven at 100 °C for 24 h. This sample was gently crushed to pass through a 0.074 mm sieve (200 mesh).

Natural kaolin samples for characterisation were prepared by drying at 80 °C. This temperature was chosen to minimize the possibility of damaging halloysite-10Å converted to halloysite-7Å by dehydration; these samples were designated NK.

3.3.2 Modification studies

1. Chemical treatment of natural kaolin (Acid activation)

Acid treatments were performed by adding dried natural kaolin (NK) to 2, 3.0, 3.7, or 5.0 M H_2SO_4; the ratio of clay:acid was 1g:50 ml. Samples were refluxed at 90 °C under mechanical stirring (using an IKA hotplate stirrer model C-MAG HS7) at about 250 rpm for 4 hours. Then the supernatants were removed and the residues washed with 2 litres of distilled water; the washing was repeating until the pH of the clay suspension was ≥3. The sample was then dried at 100 °C for 24 h, this temperature being chosen because of the planned use of the product as a vegetable oil bleaching agent. Samples were designated UGKS 2, 3, 3.7, and 5.

2. Physical treatment of natural kaolin (mechanical treatment)

Dried natural kaolin, NK, initially crushed to ≤0.074 mm as for the chemical treatments described above, was ground using a planetary ball mill (Retsch model S 100). For this physical treatment, 20 g of clay were added to a 500 ml grinding jar containing twenty 20 mm grinding balls (the weight ratio of balls to kaolin was 30:1); both the pot and milling media were stainless steel. The clay samples were ground for 1 hour at 300 rpm, this extensive grinding time was necessary preliminary step prior to acid activation. The ground product is designated as GK.

3. Combined physical and chemical treatments of kaolin

Samples of ground kaolin(GK) were treated with to 1.7, 2.0, 2.3, 2.6, 3.0 or 3.7 M H_2SO_4 as described for the preparation of the corresponding UGKS samples, and designated GKS 1.7, 2, 2.5, 3 and 3.7. Similar samples were prepared using oxalic acid instead of sulfuric acid with acid concentrations of 0.5, 0.7, and 0.9 M; these samples were designated GKO 0.5, GKO 0.7, and GKO 0.9. The optimum acid concentration for preparing modified kaolin for use in the bleaching of rice bran oil was determined on the basis of the amount of pigments that could be removed by different preparations. These samples were then further modified by suspension in dilute acid solutions at different pH values.

4. Surface modification of the products from combined physical and chemical treatments

It has been reported that kaolinite surface properties are influenced by the synthesis pH (Fialips *et al.*, 2000). The surfaces of modified kaolin samples then treated using subsequently determined optimum concentrations of sulfuric or oxalic acids (2M and 0.7 M respectively) by resuspension in solutions of the corresponding acids at pH 2.0, 2.5, 3.0, 3.5, 4.0 and 5.0. After separation by centrifugation, the kaolin samples were dried at 100°C for 24 h.

The various stages in the kaolin modification are shown in Figure 3.1.

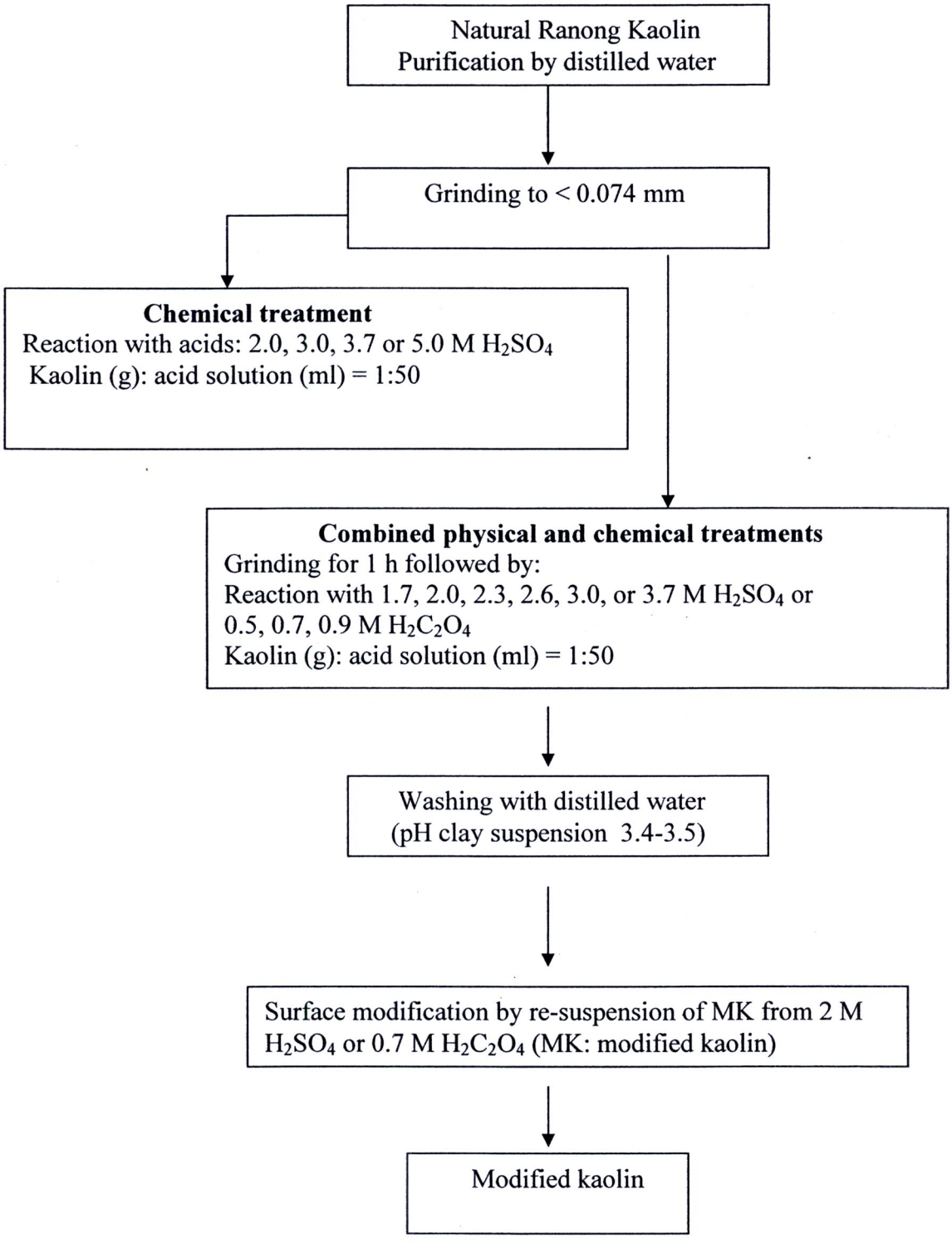

Figure 3.1 Experimental flowchart for kaolin modification

3.3.3 Characterisation of kaolin (before and after modification)

1. Mineralogical analysis by X-ray Diffraction (XRD)

A Bruker AXS model D8 Discover X-ray diffractometer employing Ni-filtered CuK_{α} radiation was used to investigate the mineralogy of the natural kaolin sample and its modified products. Kaolin powder was placed in a flat holder, with 20 mm diameter and 15×20 mm irradiated area, and diffraction patterns were then collected in the range of $2\theta = 5^{\circ}$ to 60° at a scanning speed 1° per 2θ/min, and working at 40 kV/40 mA. Mineral components in the X-ray diffractograms were identified by comparison with standards in the JCPDS Powder Diffraction File.

Additional measurements were made with oriented samples of the natural kaolin in an attempt to discriminate between halloysite-10Å, halloysite-7Å, kaolinite and illite components. These oriented samples were subjected to 4 treatments: air drying, glycolation, intercalation with formamide, and heating to 550 °C for 1 h. Structural order of natural kaolinite was estimated using the Hinckley index (HI), and the crystallinities of the kaolin minerals in the various treated products were also estimated from the widths and heights of peaks in the XRD patterns, since peak widths are inversely related to the crystallinity of the sample.

2. Chemical composition by X-ray fluorescence (XRF)

A Bruker AXS model S4 Pioneer XRF spectrometer with a scintillator detector was used to determine the chemical compositions of the kaolin samples. Wax was added to the clay samples as a binder before fusing into pellets (weight ratio of clay:wax about 7:3), and compositions are expressed as relative concentrations in the form of oxides.

3. Fourier transform infrared spectroscopy (FTIR)

Attenuated Total Reflectance Fourier Transform Infrared Spectra (ATR-FTIR) were recorded on a Bruker Tensor 27 FTIR spectrometer incorporating a 1.8 mm Ge crystal. A sample of the clay powder was placed on the Ge crystal and 1024 scans were acquired for each spectrum at a resolution of 1 cm^{-1} in the mid-IR range(4,000-600 cm^{-1}).

In addition, samples of modified kaolin before and after bleaching were prepared by mixing 1 mg of sample with 100 mg of KBr, then pressing into pellets for study by

diffuse reflectance infrared (DRIFT) spectroscopy using a Perkin Elmer (Spectrum One) Fourier Transform infrared spectrometer operating in the mid-IR spectrum range (4000-450 cm^{-1}); 16 scans were taken for each spectrum at a resolution of 4 cm^{-1}.

4. Electron paramagnetic resonance (EPR) spectroscopy

EPR spectra were recorded using various Bruker and Jeol spectrometers operating at X-band frequencies and using Gunn diodes as microwave sources. All samples were studied at room temperature (~22°C) using 100 kHz modulation frequency with other acquisition parameters determined by the linewidths and saturation properties of the component signals. Spectra were first recorded as 1^{st} derivatives of the microwave absorption over the scan range 0-5000 gauss using 10 mW microwave power and 10 gauss modulation amplitude to obtain a general overview of the signals. Free radical components were recorded as both 1^{st} and 2^{nd} derivatives using microwave powers in the range 2-10 mW and modulation amplitudes in the range 2-10 gauss over field scans ranging between 50 and 400 gauss. Receiver gain, number of scans, conversion time and time constant were adjusted individually for each spectrum depending on the intensity of the signal being characterized. g-values are expressed relative to DPPH (g = 2.0036), which was used as an external standard.

5. Scanning electron microscopy (SEM)

The surface morphology of the kaolin samples that had been dried at 80 °C and coated with gold to enhance conductivity, was investigated using a scanning electron microscope, (JEOL model JSM-5410 LV) with an accelerating voltage of 15 kV and a vacuum of 10^{-5} Pa. Samples were inserted into the SEM chamber, transferred to the path of the electron beam, and then scanned automatically. Magnifications of 10,000 and 20,000 were used to compare the textures and shapes of modified kaolins before and after treatments. In addition, chemical analyses were performed by using a link to an energy dispersive x-ray analysis system (EDS), which incorporated ISIS series 300 software.

6. Specific surface area analysis using the Brunauer-Emmet-Teller (BET) Method

The specific surface area (S_{BET}) was measured on a Quantachrome Autosorb-1 instrument by the Brunauer-Emmet-Teller(BET) method, using N_2 gas adsorption at 77.35 K, and an outgas temperature of 523 K; the relative pressure (P/P_O) ranged up to 0.3. Three porosity parameters were obtained. The BET surface area (S_{BET}) was calculated from the linear part of the BET plot according to IUPAC recommendations using the adsorption isotherm. Total pore volumes (V_p) were obtained from the adsorption maximum by the Barrett-Joyner-Halenda (BJH) method at P/P_O of ~ 0.989-0.900, and average pore diameters ($\bar{r}$) were derived from the relationship $\bar{r} = 2V_p/ S_{BET}$ and the pore size distributions were measured by applying the BJH method to the desorption branch of the nitrogen isotherm at 77 K.

7. Determination of the point of zero charge

The P_{zc} was determined by titration with different pH systems. N_2 gas was bubbled through a mixture of 10% (w/v) modified kaolin and deionised water (pH 7) for 15 minutes, and it was then shaken at 250 rpm at 30°C for 1 hour. Titration was carried out with 0.1 M NaOH and 0.1 M HNO_3. After equilibration (~10 minutes), the pH was measured using a Mettler (UK) Delta 340 pH meter. The surface charge (Q) was calculated using the equation:

$$Q = [(C_a - C_b - [H^+] + [OH^-])]/W$$

where Q = the surface charge (mol g^{-1} adsorbent dry weight)

C_a = the added acid (mol L^{-1})

C_b = the added base (mol L^{-1})

W = the dry weight of the adsorbent (g)

The point of zero charge (Q = 0) was obtained from plots of Q versus pH.

3.3.4 Adsorption (rice bran oil bleaching) studies

For the adsorption experiments, unbleached rice bran oil was placed in a flask held in an oil bath. When the temperature reached 90°C, kaolin was added to the flask (w/w = 2%), and the bleaching process carried out with stirring at 90°C for 30 min. The clay and oil were separated by centrifugation and the supernatant were filtered through filter

paper (Whatman No.41) under vauum .The various stages in the adsorption study are shown in Figure 3.2.

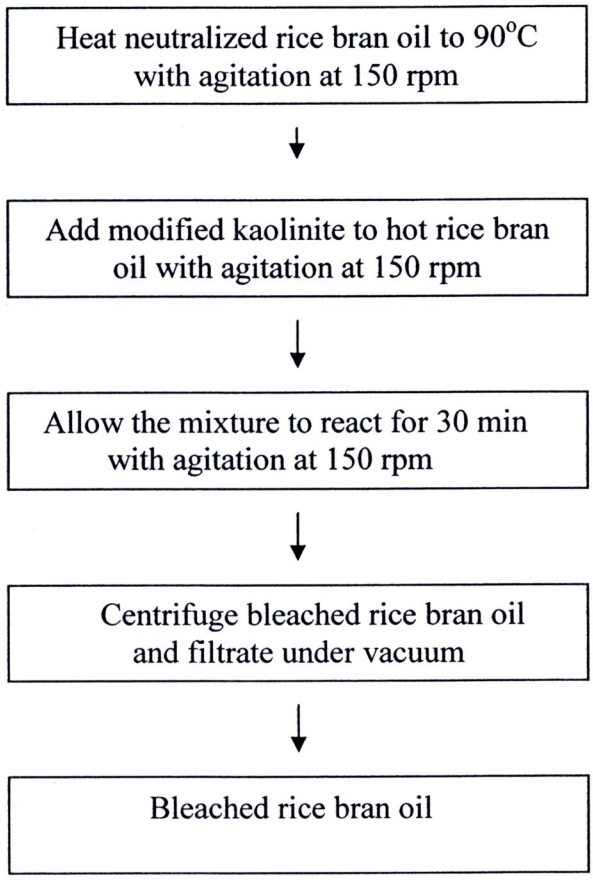

Figure 3.2 Experimental flowchart for adsorption of pigments from rice bran oil

1. Contact time determination

To study the effect of contact time, bleaching experiments were performed for 15, 30, 60 and 120 minutes using modified kaolin samples UGKS 2 and UGKS 3.7.

2. Determination of the decolorisation capacity of the modified kaolins

The amount of pigment removed (to assess the decolorisation capacity of various kaolin samples) was determined by UV-visible spectroscopy using a Bausch & Lomb Spectronic 21 spectrophotometer. Oil samples were diluted in hexane (1:4 v/v), and the absorbance measured at the maximum absorption wavelength (410 nm) using hexane as reference after having measured the full spectrum of the oil sample between 350-750 nm. The decolorisation capacity was calculated using the following formula:

$$\% \text{ decolorisation capacity} = [(A_{CRBO} - A_{SAMPLE})/ A_{CRBO}] \times 100$$

where A_{CRBO} and A_{SAMPLE} are the absorbances at 410 nm, of crude rice bran oil before and after bleaching with modified kaolin.

3.3.5 Study of mechanism of pigment adsorption

After the bleaching process, the adsorbent was washed several times with hexane with stirring in order to dissolve any rice bran oil remaining in clay sample. The clay remained green, indicating that there is a strong interaction between the pigment and the modified kaolin. In order to gain additional understanding of the adsorption process, the modified kaolin after use in the bleaching process and removal of any associated oil was investigated by DRIFT spectroscopy, scanning electron microscopy (SEM) and electron paramagnetic resonance (EPR) spectroscopy.

1. Scanning electron microscopy (SEM)

To study the adsorption of pigment in crude rice bran oil by modified kaolin, the textures of modified kaolin before and after use for bleaching rice bran oil were investigated by scanning electron microscopy.

2. FTIR spectroscopy

Samples for infrared absorption spectroscopy were prepared by mixing 1 mg of sample homogeneously with 100 mg of KBr, and then pressing the mixture into pellets. Diffuse reflectance infrared (DRIFT) spectra were measured with a Perkin Elmer (Spectrum One) Fourier Transform infrared spectrometer operating in the mid-IR spectrum range (4000-450 cm^{-1}); 16 scans were taken for each spectrum at a resolution of 4 cm^{-1}.

3. EPR spectroscopy

EPR spectra were recorded at ambient temperature using a Bruker EMX CW spectrometer operating at X-band frequencies. Most spectra were acquired in 4096 points using a sweep width of 500 mT, 10 mW microwave power, 100 kHz modulation frequency, and 1.0 mT modulation amplitude. Measurements of free radical signals, however, were performed with a 10 mT sweep width, 5 mW microwave power and 0.5

mT modulation amplitude. *g*-values are expressed relative to DPPH (g = 2.0036), which was used as an external standard.

4. Freundlich isotherm

In this work, the pigment adsorption isotherm of modified kaolin was investigated using adsorbent dosages in the range 1 - 3% (w/w). The mixture of oil and modified kaolin was stirred at 400 rpm at different temperatures (60, 75 and 90°C) for an equilibrium time of 60 minutes, then centrifuged at 4500 rpm for 10 minutes. The absorbance of the bleached oil was measured at 410 nm.

3.3.6 Physical properties of modified kaolin

1. Bulk density determination

Clay bulk density, like all density measurements, is the ratio of the mass of the dry sample to the bulk volume. The bulk volume includes the volume of solids and the pore spaces. Thus, clay that is loose, porous, or well-aggregated will have lower bulk densities than clay that is compacted or non-aggregated. This is because pore space (or air) weighs less than solid particles. Bulk density is an indirect measure of pore space and is affected primarily by texture and structure.

In this experiment clay samples were dried in an oven at 105°C for 24 hours and the weights (g) and volumes (cm^3) were then determined, and their ratios calculated.

2. Moisture determination

The following procedure was followed for determination of moisture contents of the kaolin samples. Samples were weighed accurately, then placed in a container, which was heated to 105°C overnight in an air oven. The sample was then cooled and weighed to determine the new mass of the sample. This operation was repeated until a constant mass was obtained (this was deemed to have been reached when successive mass determinations did not differ by more than 0.05%). The difference between the mass of the sample before and after heating gives the loss of moisture.

3. Loss on ignition determination

The following procedure was followed for the loss on ignition determination of the kaolin samples. Samples were weighed accurately, and spread in a container, which was

heated in a furnace at about 1000°C for 2 hours. Then it was cooled, weighed and the mass of the sample was obtained. The difference between the mass of the sample before and after heating gives the loss on ignition.

4. pH of the aqueous clay suspension

The pH of the aqueous clay suspensions (10 %W) was measured with a digital pH meter at 25°C.

5. Acidity of modified clay

The acidity of the clays was also determined by volumetric titration. In this method, 0.5 g of the clay, previously dried at 105°C for 6 h, was placed in a conical flask to which 15 mL of 0.1 N NaOH was added. After stirring for 10 min, the clay was titrated with 0.1 N HCl using phenolphthalein as indicator. Acidity was then determined as the milliequivalents of NaOH used per 100 g of clay.

3.3.7 Bleached oil properties

1. Color determination

UV-vis spectrophotoscopy

The color of oil samples was determined by UV-visible spectroscopy using a Bausch & Lomb Spectronic 21 spectrophotometer. The oil samples were diluted in hexane (1:4 v/v), and the absorbance measured at the maximum absorption wavelength (410 nm) using hexane as reference after having measured the full spectrum of the oil sample between 350-750 nm.

Lovibond tintometer

For manual color determination of rice bran oil before and after bleaching, measurements were made using a Lovibond Tintometer, Pardero Model F according to the AOCS Cc 13d – 55. This technique involves matching the color of light transmitted through a specified depth of oil with the color of light transmitted from the same source through a set of colored glass slides.

2. Peroxide value (PV) and free fatty acid value (FFA)

The peroxide value test does not specifically measure peroxides. It measures all material capable of oxidizing potassium iodide. The amount of potassium iodide oxidized in the test can be influenced by the way test is conducted. The method in this work is based on the American Oil Chemist's Society official method (AOCS Cd 8b-90).

Peroxide value determination According to the AOCS official method (Cd 8b-90), the oil 5.0 g ±0.1 g is weighed into a 250 ml stoppered conical flask, and 30 ml of acetic acid-chloroform solution added, The flask is swirled to dissolve the oil in the solvent, after which 0.5 ml of saturated potassium iodide solution is added from a 1 ml graduated pipette. The flask is then stoppered and the mixture allowed standing with occasional shaking for exactly 1 minute. After 1 minute, 30 ml of distilled water and about 5 drops of starch indicator solution are added; then the solution is titrated with 0.01 M sodium thiosulphate solution. The flask is shaken vigorously during the titration in order to extract iodine from the chloroform layer. The end point is reached when the purple colour disappears. For peroxide values >10 meq/100 g, the titration is performed with 0.1 M sodium thiosulphate. It is also necessary to perform a blank determination of the regents; the blank titration should not exceed 0.1 ml of 0.001 M sodium thiosulphate.

Calculation

$$\text{Peroxide value (meq/1000 g)} = [(A-B) \times M \times 1000] / W$$

Where A is titration value of sample (ml of thiosulphate), B is titration value of blank (ml of thiosulphate), M is molarity of sodium thiosulphate, and W is weight of sample (g of oil).

The peroxide value is expressed as milli-equivalents (meq) of peroxide per 1000 g of sample. Peroxide values of fresh oils are less than 10 meq per 1000 g; when the peroxide value is between 30 and 40 meq per 1000 g, a rancid taste is noticeable.

Free fatty acid value determination

According to the AOCS official method (Ca 5a-40), 5±0.1 g of the oil sample is weighed into an Erlenmeyer flask followed by addition of 125 ml of a solvent mixture containing equal parts by volume of isopropyl alcohol and toluene. The oil is then neutralized by titrating with 0.1 N standard potassium hydroxide using phenopthalein as indicator. The sample must be shaken vigorously whilst titrating to the first permanent reddish or pink color of the same intensity as that of the neutralized solvent before the latter was added to the sample. The color must persist for 30 seconds.

Calculations

$$\text{The acid value (mg KOH/g)} = [(A - B) \times N \times 56.1] / W$$

where A is ml of standard potassium hydroxide used in the titration, B is ml of standard potassium hydroxide used in titrating the blank, N is normality of standard potassium hydroxide, and W is grams of oil

To express free fatty acids as percent oleic acid, divide the acid value by 1.99

The chlorophyll contents of the rice bran oil before and after bleaching were determined from the absorbance at 630 nm, 670 nm and 710 nm in a 10 mm spectrometer cell. The contents of chlorophyll a pigments, expressed in ppm of pheophytin a, were calculated using the following formula:

$$C = 345.3(A_{670} - 0.5)(A_{630} - 0.5)(A_{710})/L$$

Where C is content of chlorophyll pigments in ppm of pheophytin, A is absorbance at the respective wavelength (nm), and L is thickness of the spectrophotometer cell (nm).

3.3.8 Determination of oil retention by adsorption

After the bleaching process, there are many substances retained in the filter cake, such as for example unoxidized oil, oxidized oil soap, organic color pigments (carotenoid pigments, chlorophyll, and chlorophyll derivatives), and the other organic compounds. The term **oil retention** from the refinery's standpoint includes all substances still retained in the filter cake of the bleaching clay.

For determination oil retention, weigh 5 ± 0.002 g filter cake samples into 250 ml beaker. Add ca.100 ml distilled water and stir. Add several drops of methyl orange indicator followed by concentrated hydrochloric acid dropwise with stirring until the solution is distinctly acidic, then an add a further 5 ml of acid. The beaker is then heated on a steam bath over an open hole for 30 min, stirring occasionally, after which the sample is filtered (Whatman No.42 filter paper), washed with hot distilled water, then allowed to dry overnight. When dry, transfer the contents for extraction and extract for 3 h with hexane, acetone and alcohol (equal volume of hexane, acetone and alcohol mixture) solvent. Remove the extraction flask and evaporate off the solvent on a steam bath, and continue heating on the steam bath for another 15 min after no odor of solvent can be detected. Cool flask in a desiccator and reweigh.

Calculation

% oil retention = [wt. extracted product ×100]/ wt. filter cake sample

3.3.9 Oxidation in bleaching rice bran oil process using EPR spectroscopy

Free radical signals in the oil before and after bleaching were studied by EPR spectroscopy using a Bruker EMX CW spectrometer operating at X-band frequencies. Spectra were, recorded at ambient temperature, acquired in 1024 points using a sweep width of 5 mT, 5 mW microwave power, 100 kHz modulation frequency, either 0.2 or 0.1 mT modulation amplitude, 81.92 ms conversion time and 81.92 ms time constant. Other parameters are specified in the relevant figure captions. Diphenylpicrylhydrazyl (DPPH) (g = 2.0036) was used as an external standard. Spectral interpretations were tested and parameters refined by simulation using the Bruker Simfonia software.

A summary of the experimental procedures used to investigate the rice bran oil bleaching process is presented in Figure 3.3.

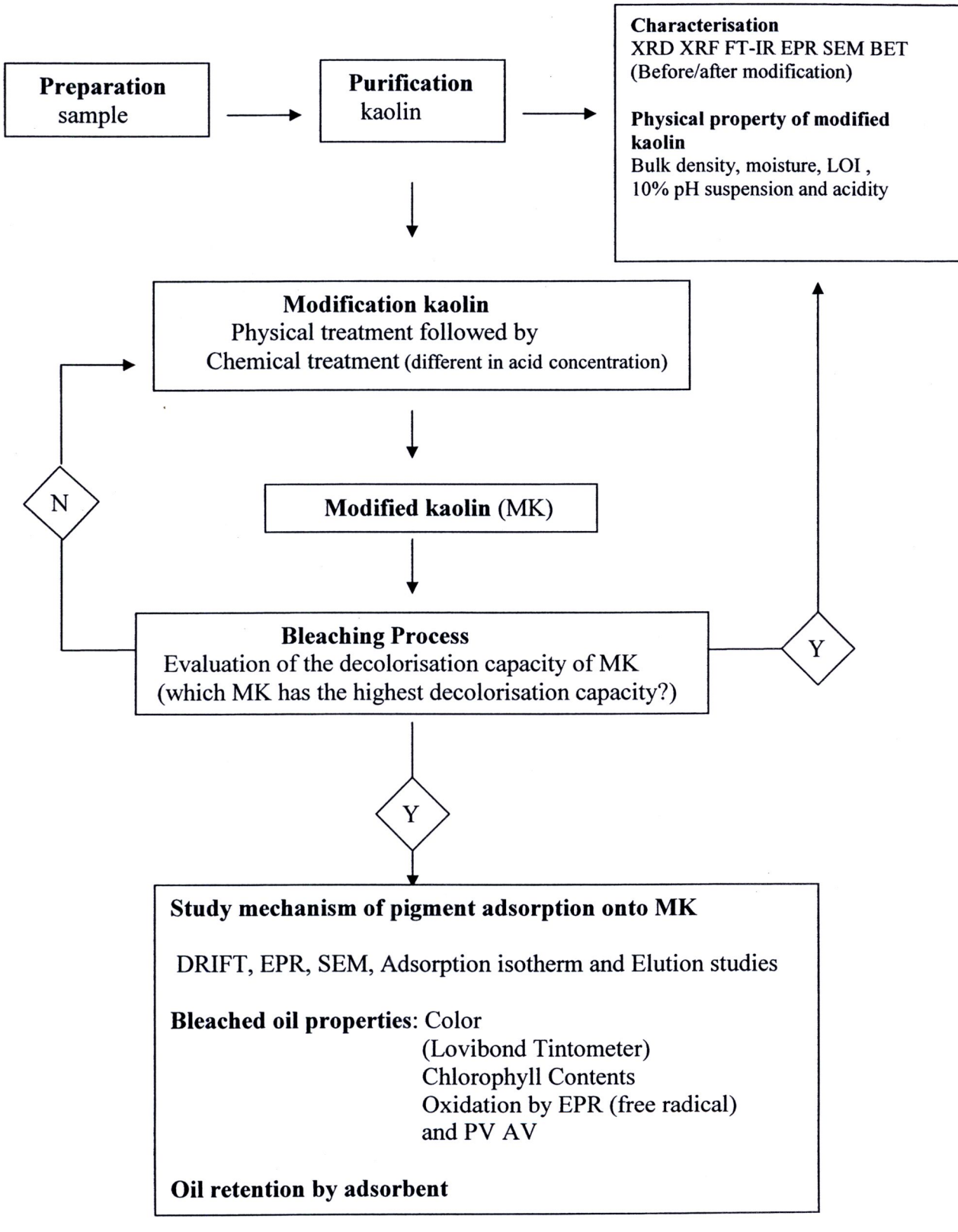

Figure 3.3 Flowchart of experimental procedure