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# SEPARATION OF CAROTENOIDS FROM PALM OIL BY ADSORPTION ON CLAYS



Miss Winunta Poopunpanich

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วินันทา พุพันธ์พานิช : การแยกแคโรทีนอยด์จากน้ำมันปาล์มโดยการดูดซับบน

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ได้ศึกษาการแยกแคโรทีนอยด์จากน้ำมันปาล์มโดยการดูดซับบนดินเหนียวชนิดต่างๆที่มีในประเทศไทย 6 ชนิด ได้แก่ เบนโทไนต์ ไดอะตอมไมต์ แทลคัม ดินขาวจังหวัดลำปาง ดินขาวจังหวัดระนอง และบอลเคลย์ ที่ผ่านการปรับสภาพดินด้วยการกระตุ้นด้วยกรด และการแลกเปลี่ยนไอออน โดยทำการศึกษาเบื้องต้นเกี่ยวกับปัจจัยที่มีผลต่อการสลายตัวของเบตา-แคโรทีน ได้แก่ สารป้องกันการเกิดออกซิเดชัน พบว่า การใช้ดินเหนียวที่ไม่ผ่านการบำบัดด้วยสารป้องกันการเกิดออกซิเดชันในการดูดซับสารแคโรทีนจากน้ำมันปาล์ม มีผลทำให้เกิดการสลายตัวของสารเบตา-แคโรทีน จากนั้นทำการศึกษาปัจจัยที่มีผลต่อการดูดซับและนำกลับเบตา-แคโรทีน ได้แก่ ชนิดของดินเหนียว อุณหภูมิ อัตราส่วนระหว่างดินเหนียวกับน้ำมันปาล์ม และระยะเวลาในการดูดซับ และปัจจัยที่มีผลต่อการชะเบตา-แคโรทีน ได้แก่ ชนิดของตัวชะ

จากผลการทดลองพบว่า ภาวะที่เหมาะสมในการดูดซับเบตา-แคโรทีนบนดินเหนียว คือ การใช้เบนโทไนต์ที่ผ่านการปรับสภาพดินด้วยกรด และผ่านการบำบัดด้วยสารป้องกันการเกิดออกซิเดชัน เป็นตัวดูดซับ โดยใช้อัตราส่วนระหว่างดินเหนียวกับน้ำมันปาล์ม เท่ากับ 1:5 โดยน้ำหนัก ภายใต้อุณหภูมิในการดูดซับ 70 องศาเซลเซียส เป็นระยะเวลา 70 นาที โดยสามารถดูดซับเบตา-แคโรทีนได้ 99.78 เปอร์เซ็นต์ สำหรับภาวะที่เหมาะสมในการชะเบตา-แคโรทีนออกจากดินเหนียวคือ ทำการชะที่อุณหภูมิห้อง โดยใช้เตตระไฮโดรฟูราน เป็นตัวชะ ภายใต้ภาวะดังกล่าวสามารถสกัดสารเบตา-แคโรทีน ได้ผลิตผลกลับคืนเท่ากับ 73.73 เปอร์เซ็นต์ จากภาวะข้างต้นสามารถนำเบตา-แคโรทีนกลับคืนมาได้จากน้ำมันปาล์มดิบ คิดเป็น 73.56 เปอร์เซ็นต์โดยน้ำหนักของปริมาณแคโรทีนเริ่มต้นในน้ำมันปาล์ม

หลักสูตร...ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์..... ลายมือชื่อนิสิต.....  
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Separation of carotenoids from palm oil was carried out by adsorption on six types of Thai clays, namely bentonite, diatomite, talcum, china clay (Lampang), china clay (Ranong) and ball clay. The efficiencies of acid activated clays and ion-exchanged clays for  $\beta$ -carotene adsorption were investigated. The preliminary study of factors affecting degradation of  $\beta$ -carotene extraction in palm oil were antioxidant, which was found that using activated clay without antioxidant to adsorb carotene resulted in degradation of  $\beta$ -carotene. Then, different types of clays, adsorption temperature, ratios of clay to palm oil, adsorption time and types of eluting solvents were investigated to determine the effect on the percentage of  $\beta$ -carotene adsorption and  $\beta$ -carotene recovery, respectively.

Results showed that the optimum conditions for  $\beta$ -carotene adsorption on clays were carried out by using acid activated bentonite which was treated with antioxidant at a ratio of 1:5 by weight of clay to palm oil, at 70°C adsorption temperature for 70 minutes could adsorb 99.78 %  $\beta$ -carotene. The suitable condition for  $\beta$ -carotene desorption from clay was carried out at room temperature by using tetrahydrofuran as an eluting solvent resulted in 73.73 %  $\beta$ -carotene desorption. From the above conditions, overall  $\beta$ -carotene recovery from crude palm oil corresponding to 73.56 % by weight of carotenoids in crude palm oil.

Program...Petrochemistry and Polymer Science.....Student's signature.....

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จุฬาลงกรณ์มหาวิทยาลัย

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## LIST OF ABBREVIATIONS

$\beta$	:	Beta
$\alpha$	:	Alpha
$\gamma$	:	Gamma
$\lambda_{\max}$	:	Absorption maxima
$^{\circ}\text{C}$	:	Degree Celsius
$\text{cm}^{-1}$	:	Unit of wave number
XRD	:	X-ray Powder Diffractometer
FTIR	:	Fourier Transform Infrared Spectrophotometer
SEM	:	Scanning Electron Microscope
UV	:	Ultraviolet
Meq	:	Millimolequivalent
min.	:	Minute (s)
mL	:	Milliliter (s)
mg	:	Milligram (s)
nm	:	Nanometer
ppm	:	Parts per million
% wt	:	Percent by weight
THF	:	Tetrahydrofuran
BHT	:	Butylated Hydroxytoluene
China L	:	China clay (Lampang)
China R	:	China clay (Ranong)
CPO	:	Crude Palm Oil

## CHAPTER I

### INTRODUCTION

#### 1.1 Statement of problems

Palm oil is a fruit flesh fat derived from the species *Elaeis guineensis* and owes its distinctive orange-red colour to its relatively high content of natural carotenoids (500-700 ppm) [1]. The major carotenoids of palm oil are  $\alpha$ - and  $\beta$ -carotene; together they constitute more than 80% of the total carotenoids in palm oil [2]. Carotenes, in particular  $\beta$ -carotene and, to a lesser extent,  $\alpha$ -carotene, are known for their provitamin A activities, as they are transformed into vitamin A *in vivo*. Comparing to other sources of natural carotenoids, palm oil has 15 times more retinol equivalents than carrots and 300 times more than tomatoes. Recent studies have also strongly associated  $\beta$ -carotene with the prevention of certain types of cancer, such as oral, pharyngeal, lung and stomach cancers [3].

Most of the carotenoids in palm oil are destroyed and discarded in the present refining process to produce the light coloured to colourless oil, as per the demand from major consumers. This represents a loss of a potential source of natural carotenoids. Since the world production of refined palm oil amounts to about  $20 \times 10^6$  tons, every year  $1.2 \times 10^4$  tons of carotenoids are destroyed [4]. However, in the conventional process of extraction, when compared to mechanised process, palm oil retains about 3 times more carotene. If recovered, the value of the carotene concentrate would be close to that of the oil itself, excluding the processing costs [5]. The importance of carotenoids is well documented, and various methods of carotenoid recovery from palm oil have been developed. These include extraction by saponification [6], selective solvent extraction [7], and transesterification, followed by both phase separation and distillation of the esters [8]. Transesterification is the

only commercially viable process. This unique method of carotenoid recovery from palm oil has already been developed by Lion Corporation (Tokyo, Japan). It involves the interesterification of triglycerides with methanol to yield methyl esters, followed by phase separation, resulting in a carotene-rich layer and a decolorized methyl ester layer. The carotene-rich layer is further concentrated by molecular distillation and chromatographic methods. A similar process, involving transesterification of palm oil, followed by molecular distillation of the esters, to recover the carotenoids has been reported [9], but an edible oil was not obtained because crude palm oil has to be converted to methyl esters, which are not edible. The known processes as above have been found that the yield of carotene is too low as a result of thermal and chemical decomposition or poor selectivity or that, after the process has been carried out the palm oil or the palm oil derivative is no longer suitable for the production of fatty alcohol. Therefore, carotenoid extraction by adsorption without a chemical conversion of palm oil has been reported. A number of investigations have been carried out to examine adsorbents in adsorption process. These adsorbents are zeolite [10], activated carbon [11,12], synthetic polymer [13], rice hull ash [14]. However, these above adsorbents are alternatives to conventional bleaching clays such as fuller's earth [15], bentonite [5] and local Nigerian clays [16] since their activities in bleaching palm oil are less than commercial bleaching clays and their procedures are more complicated.

In the present study, separation of carotenoids from palm oil by adsorption on adsorbents was studied in easier procedure. These adsorbents are 6 clays : ball clay, china clay (Lampang), china clay (Ranong), talcum, bentonite, diatomite. They are available in many parts of Thailand and low cost. Thus, this research can use domestic resources which save cost in the refining of palm oil because bleaching clay dosages of 0.5-1% are generally used every year. Most clays are imported from foreign countries. Moreover, this work also evaluate recovery method for palm carotene which may be suitable as an edible palm oil pretreatment process due to its efficient mass recovery of a valuable bioresource.

## 1.2 Objectives

The objectives of this research are i) to study appropriate clays in Thailand as adsorbents in the separation of carotenoids from palm oil, and ii) to determine the optimum conditions for carotene adsorption on adsorbents and desorption of adsorbed carotene from adsorbents.

## 1.3 Scope of the investigation

1.3.1 Preparing various Thai clays by using acid activation and ion exchange process and then characterize them by :

- Fourier Transform Infrared Spectrometer (FTIR)
- Scanning Electron Microscope (SEM)
- X-ray Powder Diffractometer (XRD)
- Acidity characterization

1.3.2 Separating carotenoids from palm oil by adsorption on prepared clays.

1.3.3 Studying the effect of these following parameters for adsorption step :

- processes for treatment of clay (acid activation, non acid activation and ion-exchange)
- type of adsorbents (ball clay, china clay (Lampang), china clay (Ranong), talcum, bentonite, diatomite)
- adsorption temperature
- adsorption time
- ratios of clay to palm oil

1.3.4 Studying the effect of this following parameter for desorption step :

- type of eluting solvents (hexane, toluene, tetrahydrofuran, ether)

1.3.5 Studying the effect of antioxidant to carotene degradation.

1.3.6 Determining the optimum conditions for adsorption and desorption process.

1.3.7 Summarizing the results.

## CHAPTER II

### THEORY AND LITERATURE REVIEWS

#### 2.1 Palm Oil

##### 2.1.1 Production of palm oil

The oil palm, *Elaeis guineensis*, along with the coconut palm are the two most important oil trees in the world. The oil palm fruit is unusual in that it yields two distinct oils : palm kernel oil is obtain from the kernel and palm oil from the pulp (about 50% oil). Palm oil is by far the more important of the two [17]. Of all oil-bearing plants, the oil palm produces the most oil per unit area, with current production amounting to 5 to 7 tonnes of oil per hectare. In 1980, palm oil became the world's second most important vegetable oil after soybean. Production had increased in a spectacular manner, more than double during the decade from 1970 to 1980 (Table 2.1). Palm kernels are also an important oil source, with a composition similar to coconut oil [18].

**Table 2.1** World palm kernel and palm oil production by region and major production countries.

Region or country	Palm kernels (tonnes)			Palm oil (tonnes)		
	1969-71	1979-81	1985	1969-71	1979-81	1985
World	1178651	1781655	2659420	1983034	5048105	7578121
Africa	731005	724202	781570	1108647	1341362	1474900
Nigeria	287100	343333	370000	528333	666667	770000
N. and C.America	21464	16001	15950	31037	36364	46200
S. America	249489	303711	305500	46752	137285	255021
Asia	177683	715922	1503000	796583	3470580	5642000
China	28333	41333	50000	114333	188000	230000
Indonesia	48980	127343	214000	217900	720487	1148000
Malaysia	98996	540587	1210000	457298	2529455	4130000
Thailand	-	4251	20000	-	20221	100000

Palm products possess relatively high levels of carotenoids and vitamin E. The potential of extracting these natural substances has increased with the recent jump in palm oil production in Southeast Asia [19].

In 1977, 78% of the palm oil produced came from Southeast Asia (Malaysia and Indonesia); 17% from Africa and 5% from South America. More recently, Malaysia and Indonesia have accelerated the areas being planted to oil palm. This evolution will result in a distinct increase in palm oil production in the coming years. Palm oil is approximately 12 to 15 times higher in terms of productivity (expressed in kilograms per hectare per year) than many seed oils (Table 2.2).

**Table 2.2** Productivity of various oils in Southeast Asia

Oil	Productivity (kilograms per hectare per year)
Palm oil	3900
Sunflower oil	589
Rapeseed oil	409
Soybean oil	319
Corn oil	254

The major palm-producing nations are Malaysia, Indonesia, Thailand, Nigeria, Ivory Coast, Columbia, Venezuela and Papua New Guinea. Malaysia produces 4.5 to 5.0 million metric tons (MT) of palm oil annually. Today Malaysia processes more than 98% of its crude palm oil domestically and exports 90% of its processed oil. Then by the year 2000, global palm oil production will reach 18 million MT annually, with Malaysia accounting for ten million MT. There are indications that palm oil production will continue to be concentrated in Southeast Asia.

## **2.1.2 Refining of Palm Oil**

A refining process must be applied to crude oil to obtain the purity characteristics (acidity, color, etc.) desirable in an edible oil [4]. Refining of palm oil divide into 2 processes : Physical refining and Chemical refining.

### **2.1.2.1 Physical refining**

Physical refining was originally introduced because it has a lower loss factor associated with the removal of free fatty acid (FFA) and because the environmental pollution problem and the splitting of soap-stock does not arise [20].

For this process, firstly the crude feedstock is subjected to pretreatment and then to deodorization and deacidification. The pretreatment comprises a degumming step and an earth bleaching step which together remove certain nonvolatile impurities by filtration. Volatile and thermally labile components are removed during the conditions of steam distillation under vacuum, which originally gave the process its name of steam refining. Crude palm oil is first mixed with about 0.1% of concentrated phosphoric acid. After holding, about 1 to 2% activated bleaching earth is added under vacuum and at a temperature of 80 to 130 °C. After a suitable contact time the spent earth is removed by filtration. This degummed and bleached oil is first deaerated before steam stripping under a vacuum of 2 to 6 mm Hg at a temperature between 240 and 270 °C. Careful cooling and a polishing filter then yields the refined bleached and deodorized product (RBDPO). Curiously, during degumming nothing is removed, and reduction of color is unimportant during earth bleaching.

The initial treatment with phosphoric acid should be thought of as a conditioning treatment assisting the subsequent removal of insolubilized impurities at the time of earth filtration. The color of good quality crude palm oil is visually dominated by its carotenoid content. This also is true of partially earth-bleached product. Now carotenoids are known to be efficiently thermally bleached under the conditions of deacidification and deodorization. Thus the important action of earth



bleaching, which is one of adsorptive cleasing, is not seen until the color and quality of the fully refined product.

This means that adsorptive cleasing has to be described in chemical terms so that an optimum performance for an adsorptive clay may be defined and it must reduce the trace metal content, particularly iron, copper and phosphorus compounds to an acceptable level of less than 5 ppm. Oxidation products in the oil should also be reduced. Bleaching does mean reduction of those pigments or color-precursors which would remain after deodorization.

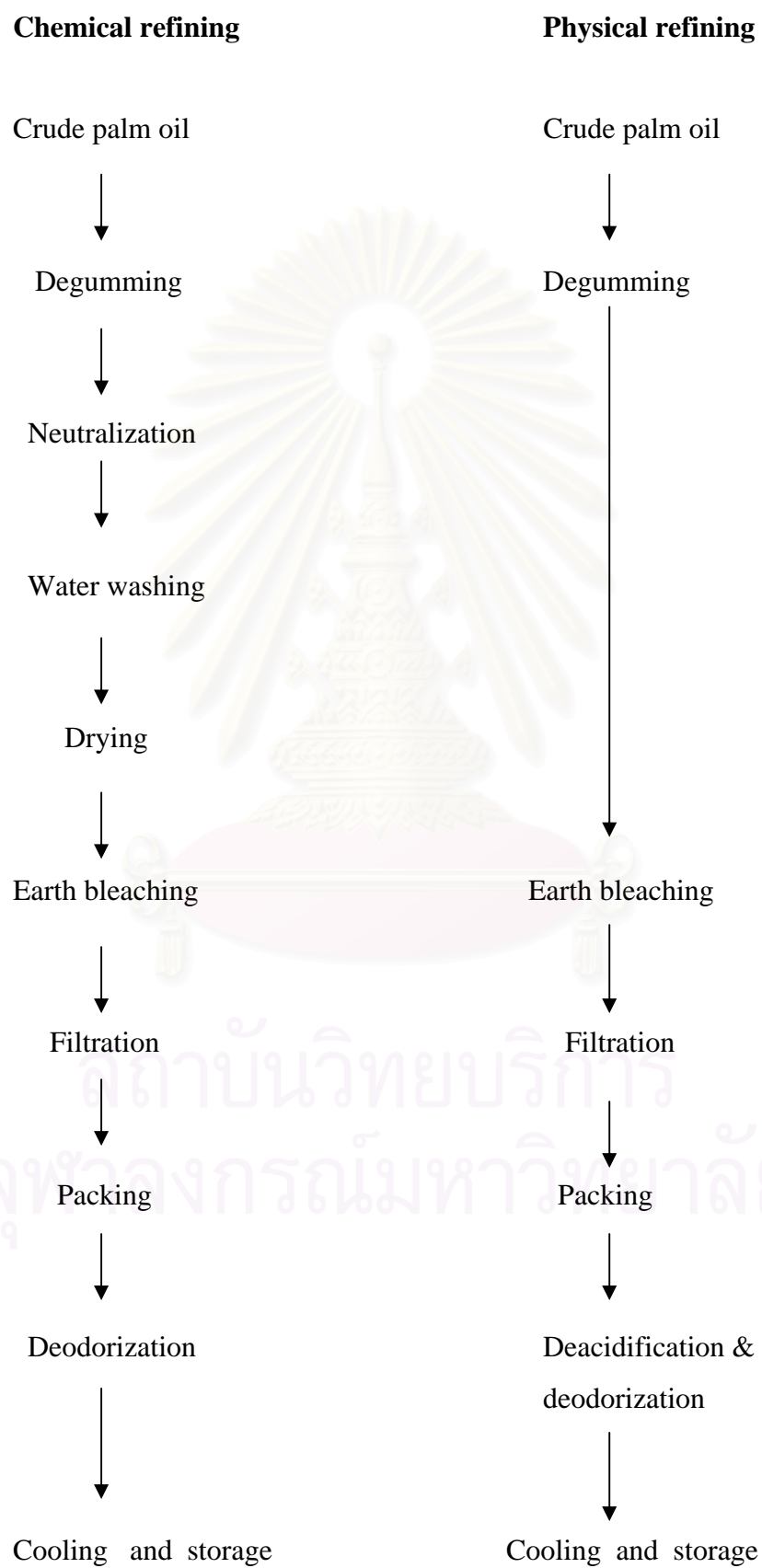
The process of deodorization and deacidification, as the process is well described in terms of its physical chemistry. Carotenoid is sufficiently thermally labile to be completely destroyed under conditions of steam refining. However, not all decomposition products are volatile.

#### **2.1.2.2 Chemical refining**

Chemical refining, where neutralization with alkali is used after phosphoric acid mediated gum conditioning, is still a considerable small proportion of the refining capacity. With the removal of fatty acid in the soap stock comes a considerable extent of removal of the impurities necessary to process palm oil. However, an efficient alkali refining plant has been found to purify crude palm oil so efficiently that it can be deodorized directly.

Normally, chemical refining includes a stage of earth bleaching between the neutralization and the deodorization steps. For palm oil, between 1 and 2% acid activated clay is used. If the crude palm oil feedstock is of bad quality, higher levels of the earth are required. Even then it is not possible to produce a fully refined product with the lowest color specification.

With the right pretreatment process physical refining of palm oil is not only much more economical than chemical refining in connection with stripping stream deodorization, but also causes much less pollution by waste water and exhaust air [21].

**Figure 2.1** Chemical refining and physical refining [22]

Crude palm oil (CPO) contains approximately 1% of minor components including carotenoids, tocopherols, sterols, triterpenes, phospholipids, glycolipids, terpenic and aliphatic hydrocarbons and other trace impurities. Carotenes, which impart a distinctive orange-red color to palm oil, are the precursors of vitamin A and tocopherols (vitamin E) contribute to the stability and nutritional value of the oil. To a great extent these and other minor constituents determine the quality characteristics of palm oil [23].

## 2.2 Carotenoids

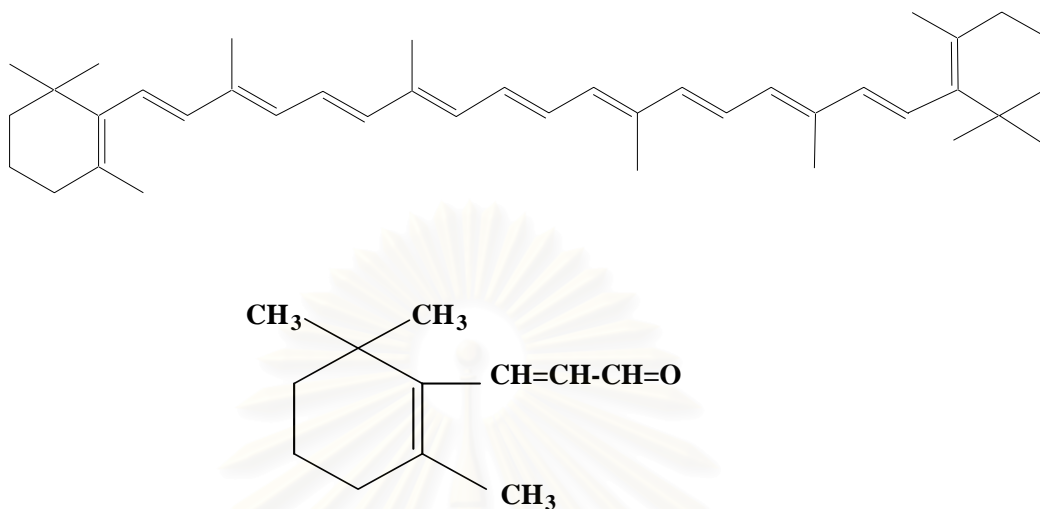
### 2.2.1 Introduction

Carotenoids, which are  $C_{40}$  tetraterpenoids, are an extremely widely distributed group of lipid-soluble pigments, found in all kinds of plants from simple bacteria to yellow-flowered composites. These pigments are also present in mango, papaya, peach, apricot, tomato, red pepper, carrot, squas, etc. In plants, carotenoids have two principal functions : as accessory pigments in photosynthesis and as colouring matters in flowers and fruits [24].

The carotenoid pigments are of importance from the standpoint of human and animal nutrition because of the conversion of some of them into vitamin A. The carotenoid pigments found largely in plants along with chlorophyll are polyenes belonging to four principal groups given below [25] :

1. The carotenes,  $C_{40}H_{56}$ , which include  $\beta$ - , $\alpha$ - and  $\gamma$ -carotene and lycopenes.
2. The xanthophylls and oxy and hydroxy derivatives of the carotenes which include among others, cryptoxanthin ( $C_{40}H_{55}OH$ ), and lutein [ $C_{40}H_{54}(OH)_2$ ]
3. The xanthophyll esters, which are esters of xanthophylls with fatty acid.
4. The carotenoid acids, which are carboxyl derivatives of the carotenes.

Of the several vitamin A precursors found in nature, the better known ones are  $\beta$ - , $\alpha$ - and  $\gamma$ -carotene.  $\beta$ -carotene contains two  $\beta$ -ionone rings and is capable of splitting into two molecules of vitamin A, whereas the others possess only one  $\beta$ -ionone ring and, therefore, have less activity.



**Figure 2.2** Structure of  $\beta$ -carotene and  $\beta$ -ionone ring

In recent years there have been many reports to suggest that  $\beta$ -carotene may also be important in affording protection against cancer, heart disease and it may have anti-ageing benefits. It is usually considered that the beneficial protective actions of  $\beta$ -carotene are due to its antioxidant properties [26].

### 2.2.2 Carotenoids in palm oil

In CPO, the carotenoid content varies between 500 and 700 ppm. A typical analysis of the composition of the carotenoids shows that  $\beta$ - and  $\alpha$ -carotene are the major components (91%) and the rest are  $\gamma$ -carotene, lycopene and xanthophylls which are represented more or less equally (Table 2.3). This group of compounds is responsible for the deep orange red colour of CPO [27].

**Table 2.3 Carotene compositions in Palm oil [27]**

Compound	Quantity
$\beta$ -Carotene	62%
$\alpha$ -Carotene	29%
$\gamma$ -Carotene	4%
Xanthophylls	3%
Lycopene	2%

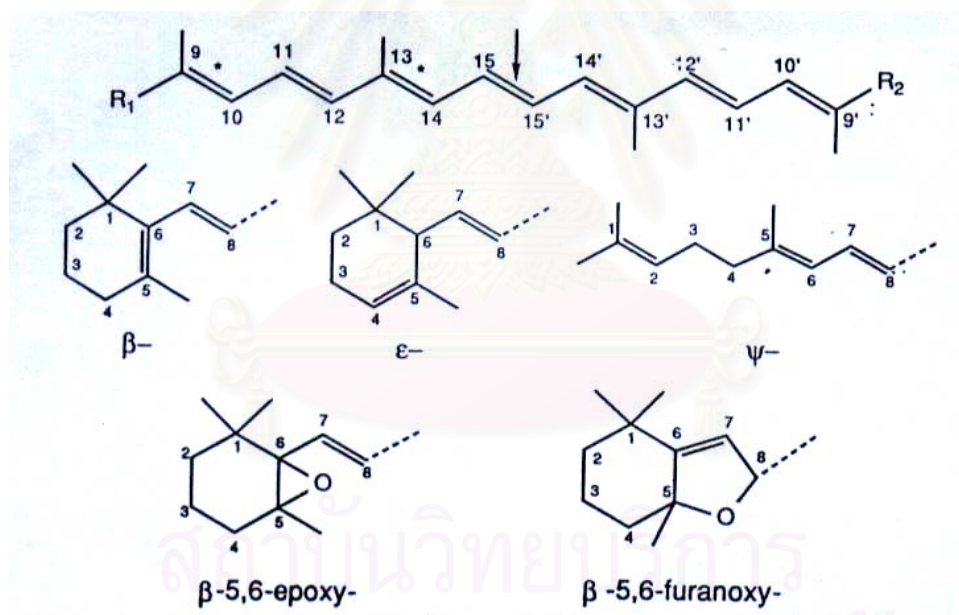
It has been generally accepted that 'carotene' equates to  $\beta$ -carotene, because  $\beta$ -carotene has occupied almost all markets for carotene. However, new and natural sources of carotene containing a considerable level of  $\alpha$ -carotene (palm oil) revealed some interesting physiological effects. It is expected that this will significantly expand the carotene market.

The following characteristics of palm oil carotenoids can be made from these comparisons :

- Palm oil contains the highest known concentration of agro-derived carotenoids.
- The preponderant palm oil pigments are  $\beta$ - and  $\alpha$ -carotene. The major carotenoids in palm oil are the same as in carrot oil, but the latter contains at least three intermediate amounts of other carotenoids.
- Fewer *cis* isomers of carotenoids are identified in palm oil. Generally, *cis* carotenoids have decreased or no vitamin A activity.
- Palm oil has the highest carotene-derived vitamin A activity. This is primarily due to  $\beta$ -carotene and, secondarily, to  $\alpha$ -carotene. For example, red palm oil has 15 times more vitamin A activity than carrots and 300 times more than tomatoes.

### 2.2.3 Structure and nomenclature

Chemically, carotenoids are conjugated hydrocarbons that may be further classified as carotenes (without an oxygen molecule) and xanthophylls (with one or more oxygen molecules). The polyene backbone of the pigment is characteristically connected to any two of the seven 9-carbon recognized end-groups. A semi-systematic nomenclature includes two Greek letters indicating the structures of the two end-groups. Therefore the semi-systematic nomenclature of common names  $\beta$ -carotene and  $\alpha$ -carotene are  $\beta,\beta$ -carotene and  $\beta,\epsilon$ -carotene, respectively. In palm oil, only  $\beta,\epsilon$  and  $\psi$  end-groups have been identified. An illustrative list of 40-carbon carotenoid structures (up to 8 isoprene units) is shown in Figure 2.3.



**Figure 2.3** Representative list of palm oil carotenoid structures [19].

$R_1$  and  $R_2$  contain one of the three ( $\beta,\epsilon$  and  $\psi$ ) side groups. In  $\beta$ -carotene, cleavage to vitamin A occur at 15,15' (arrow  $\downarrow$ ) and *cis*-isomer usually occurs at position 9 or 13 (asterisks \*)

### 2.2.4 Solubility of $\beta$ -carotene [28]

- Insoluble in water, acids or alkalies
- Very sparingly soluble in methanol, ethanol, acetonitrile
- Moderately soluble in dichloromethane, toluene, chloroform and cyclohexanone
- More soluble in tetrahydrofuran (THF)

$\beta$ -Carotene could be dissolved in nonpolar organic solvents. Solubility in many organic solvents was shown in Table 2.4.

**Table 2.4** Solubility of  $\beta$ -carotene in many organic solvents

Type of Solvents	Solubility (mg/L)
Acetonitrile	10
Methanol	10
Ethanol	30
Ethylether	1000
Cyclohexane	2000
Cyclohexanone	2000
Chloroform	2000
Toluene	4000
Benzene	4000
Dichloromethane	6000
Tetrahydrofuran	10000

Besides solubility of  $\beta$ -carotene in the solvent, we must also consider about toxicity of solvent, cost and stability of  $\beta$ -carotene in the solvent. The study in stability of  $\beta$ -carotene in various solvents found that  $\beta$ -carotene has the most stability in THF which has antioxidant (butylated hydroxytoluene : BHT).

**Table 2.5** Degradation of  $\beta$ -carotene in various solvents [28]

Type of Solvents	% Absorption of $\beta$ -carotene			
	Period of time (days)			
	1	3	6	10
Acetonitrile	99	96	93	92
Methanol	97	92	89	88
Ethanol	98	94	92	91
Ethylether	94	78	70	69
Cyclohexane	98	98	93	91
Cyclohexanone	86	67	45	32
Chloroform	97	91	92	91
Toluene	90	80	76	71
Dichloromethane	77	59	47	34
Tetrahydrofuran+ BHT	99	98	99	97

## 2.2.5 Factor affecting carotenoid degradation [29]

### 2.2.5.1 Oxidation

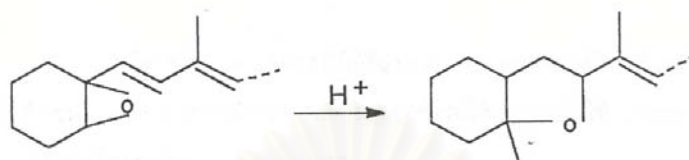
The major cause of carotenoid losses in vegetables is due to oxidation of the highly unsaturated carotenoid structure. Oxidation may occur by (a) autooxidation, which is a spontaneous, free-radical chain reaction in the presence of oxygen, (b) photooxidation produced by oxygen in the presence of light. These oxidation reactions may result in carotene bleaching, which is a consequence of formation of colourless end-products. Colour bleaching leads to changes or loss of the colour.

- *Autooxidation*

During autooxidation of the carotenoids, alkylperoxyl radicals are formed. These radicals mainly attack the double bonds resulting in formation of epoxides. The



products being formed during autooxidation of  $\beta$ -carotene were identified as a mixture of products with epoxy, hydroxy and carbonyl groups.



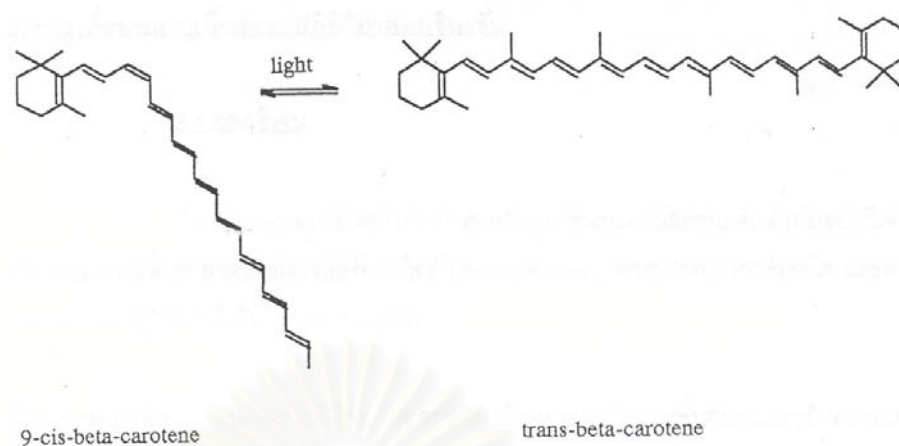
**Figure 2.4** Formation of epoxide [22].

- *Photooxidation*

Carotenoids are sensitive to light and may be subjected to photooxidation in the presence of oxygen. The rate of decolouration of the carotenoids by photooxidation is enhanced by the presence of sensitizers and the intensity of illumination by fluorescent light. In general, photooxidation of  $\beta$ -carotene results in formation of *cis*-isomers of  $\beta$ -carotene and its 5,6- and 5,8-epoxides.

#### 2.2.5.2 *Cis-trans*-isomerisation

Different *cis*-isomers are formed during oxidation. The carotenoid structure makes a *cis-trans*-isomerisation possible around the double bonds. In vegetables, the carotenoids are predominantly the all-*trans* isomers, which may be converted to the *cis*-isomers which are less stable and have reduced vitamin A activity. *Cis-trans*-isomerisation results in colour changes in vegetable products as the spectral properties of the *cis*-carotenoids are different from the corresponding *trans*-carotenoids.



**Figure 2.5** *cis-trans*-isomerisation [22].

Acids, light and heating promote isomerisation of all-*trans*-carotenoids to *cis*-forms. In general, the rate of *cis*- isomerisation increases with enhanced temperatures. Additionally, adding antioxidant (BHT) results in minimal carotenoid oxidation.

## 2.2.6 Uses of carotenoids

### 2.2.6.1 Direct use in food coloration [26]

The main use of carotenoids is for the direct coloration of food. Natural extracts are used, but the major market is for synthetic carotenoids in either oil-based or water-dispersible formulation.  $\beta$ -carotene is the main carotenoid used in oil suspensions and solutions, and can provide yellow-orange and orange-red colours, respectively, depending on concentration.

The oil-based preparations, especially of  $\beta$ -carotene, are widely used for colouring butter, margarine, cheese, cooking fats, industrial egg products, bakery products, pasta, salad dressings, dairy product substitutes, popcorn, potato products and many others.

Water-dispersible forms of  $\beta$ -carotene is used extensively for colouring soft drinks (especially 'orange juice'), ice-cream, desserts, sweets, soups and meat products.

### 2.2.6.2 As additives [30]

The carotenoid pigments are major natural food colorant additives, and their advantages have been summarized as having :

- A high tinctorial potency, and a desirable range of yellow-orange-red colours.
- Lipid solubility, and applicability of their emulsion and colloidal forms to make water-soluble pigments.
- A natural connotation, and non-corrosive character.
- Possession of vitamin A activity in some carotenoids : to this can be added the reported role of  $\beta$ -carotene in the prevention of cancer.
- Relative stability under reducing conditions.
- Relative stability over the acid and neutral pH range of food products.

However, they have a limited colour range, are sensitive to light and oxidative degradation, and are more expensive than azo dyes. Carotenoid additives are available either as extracts from plant sources, probably containing mixtures of pigments, or in a manufactured pure form called *nature identical* .

### 2.2.6.3 Animal feeds : feed for cattle, birds and fish.

### 2.2.6.4 Medical and health products

The beneficial role of  $\beta$ -carotene as provitamin A has long been known.  $\beta$ -Carotene is also now used successfully to alleviate the symptoms of light-sensitivity diseases that are characterized by extreme irritation of the skin when exposed to strong light.  $\beta$ -Carotene, administered at a high level (around 180 mg/day), is deposited in the skin and then quenches the triplet state of the sensitizer and prevents formation of singlet oxygen. The increasing reports that  $\beta$ -carotene may be a useful antioxidant that can afford protection against cancer and other diseases have led to the appearance of a great number of  $\beta$ -carotene preparations on the

health market, including crystalline carotene, extracts of natural sources such as carrots and algae, carotene drinks and even dried algae cells. There seems little doubt that the demand for  $\beta$ -carotene and perhaps other carotenoids as beneficial health products will increase. Carotenoids are also used simply for coloration purposes in health products such as pills and capsules.

### 2.3 UV-visible light absorption spectroscopy

UV-visible light absorption spectroscopy is the basic spectroscopic method used as a first identification criterion for carotenoids. Both the position of the absorption maxima ( $\lambda_{\max}$ ) and the shape or fine structure of the spectrum are characteristic of the chromophore of the carotenoid molecule. It is stressed, however, that the spectrum provides information only about light-absorbing chromophore and not about other structural features of the molecule, such as the presence of functional groups [26]. The spectra of carotenoids are quite characteristic between 400 and 500 nm, with a major peak around 450 nm and usually two minor peaks either side. For example,  $\beta$ -carotene and  $\alpha$ -carotene have  $\lambda_{\max}$  at 425, 450, 478 nm and 437, 462, 494 nm, respectively. Also, there are overall spectral shifts according to the solvent used [24].

### 2.4 Clay mineral

#### 2.4.1 Introduction

Clay minerals have long been recognized as efficient material for the promotion of many organic reactions. They are highly versatile, affording both industrial (petroleum, etc.) and laboratory catalysts with excellent product-, regio- and stereo-selectivity. Clay catalysts have distinct advantages over homogenous catalyst as the work-up of the reaction mixture is often very simple, i.e. the clay is removed by filtration.

Clays are powerful adsorbents with extremely high surface areas, especially when acid activated, and industrially they are used in such diverse applications as the co-reactant for carbonless copying paper development and the decolorizing of vegetable oils [31].

The term “bleaching earths” refers to clays that in their natural states or after chemical or physical activations, have the capacity to adsorb coloring matters from oil. Bleaching earths are finely crystalline silicates of aluminum and/or magnesium with variable amounts of lime, alkalis and iron with a large proportion of water of hydration.

Bleaching clays are often classified as fuller’s earths and Bentonites according to their inherent properties. The geological term “Bentonites” refer to any devitrification of volcanic ash. Mineralogically, bentonites are 75 percent or more of montmorillonites with fragments of kaolinite, illite feldspar and traces of other minerals.

The best known property of bleaching earth is its high adsorption capacity, which can be enhanced by acid treatment.

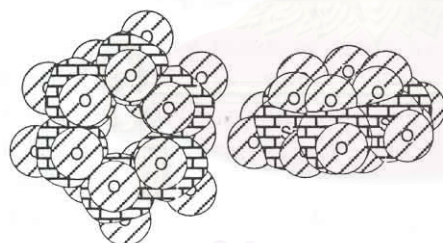
Bleaching clays in powder or granular form were used for the refining of petroleum products using two main processes: contact and percolation. In the contact process, recovered oil, paraffin wax or liquid paraffin is previously treated with sulfuric acid to remove acid tar, and after its decantation the bleaching earth is added. In the percolation process, the solvent to be treated is passed through a tower containing a bed of bleaching clay granules [32].

#### **2.4.2 The structure of clay minerals**

Clay minerals are the most abundant sedimentary mineral group. They predominate in the colloidal fractions of soils, sediments, rocks and waters and are classified as phyllosilicates or hydrous aluminosilicates.

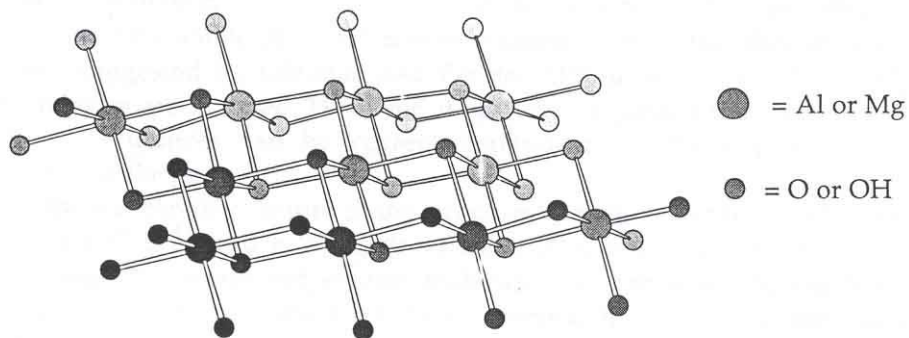
In geology the word clay is used in two ways: firstly as a rock classification which generally implies an earthy, fine-grained material that develops plasticity on mixing a limited amount of water. Secondly, it is used as a particle size term, which describes clay as materials which have particle sizes  $<4 \mu\text{m}$ , although the modern tendency is to define clays as having particle sizes  $<2 \mu\text{m}$  as fractions of this size generally give the very pure mineral.

Clay minerals are characterized by an extremely fine particulate construction and by a composition which is based primarily on oxygen and hydrated silicates of aluminum, iron and magnesium. They have been shown by X-ray diffraction studies to be crystalline, even in their finest particles, although the presence of traces of amorphous materials have been verified in some clay samples. Most of the important clay minerals are made up of combinations of two basic types of layers, the first consists of sheets of  $[\text{SiO}_4]^{4-}$  tetrahedra arranged in hexagonal ring sub-units, show in Figure 2.6. Each silica ring is combined with the two adjacent rings so as to form another 12-membered ring.



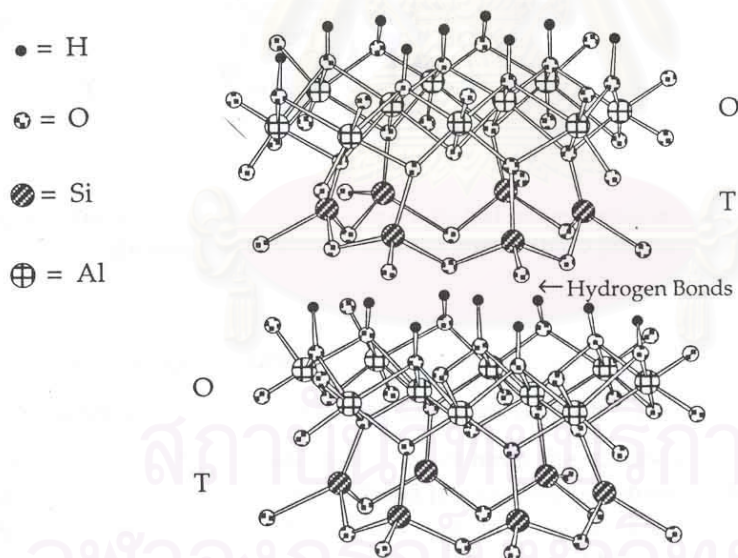
**Figure 2.6** Silica sub-units of the tetrahedral sheets.

The second type of layer is usually composed of either gibbsite  $[\text{Al}_2(\text{OH})_6]$  or brucite  $[\text{Mg}_3(\text{OH})_6]$  units which form octahedrally coordinated sheets, with the octahedra arranged so that each oxygen atom is shared with two neighboring metal atoms, show in Figure 2.7.



**Figure 2.7** The arrangement of atoms in a dioctahedral layer.

There are two dominant patterns of combining the layers. The first has alternating tetrahedral and octahedral sheets and is described as a T:O or 1:1 arrangement. This group includes kaolinite ( $\text{Al}_4\text{Si}_4\text{O}_{10}(\text{OH})_8$ ) – the main constituent of china clay – and cristobalite ( $\text{Mg}_4\text{Si}_4\text{O}_{10}(\text{OH})_8$ ). The main bonding forces between layers are hydrogen bonds between  $-\text{OH}$  on one layer and a bridging  $-\text{O}-$  on the next.



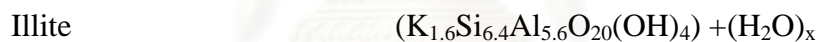
**Figure 2.8** The idealised structure of kaolinite.

The second, and perhaps chemically more important type, has repeating units of tetrahedral:octahedral:tetrahedral layers and is described as a T:O:T or 2:1 arrangement. If the octahedral layer consists of  $\text{M}^{2+}$  cations then all of the octahedral sites must be occupied to maintain electrical neutrality. However, if an  $\text{M}^{3+}$  cation is used then only  $2/3$  of the octahedral sites need be occupied to maintain neutrality. The

former mineral type is thus known as trioctahedral and the latter as dioctahedral. Some of the most common minerals in this class are presented in Table 2.6 [33].

**Table 2.6** Classification of 2:1 type clay minerals

Mineral group (Layer charge)	Subgroups	
	Dioctahedral	Trioctahedral
Pyrophyllite-Talc ( $x \approx 0$ )	Pyrophyllite $[Al_2](Si_4)O_{10}(OH)_2$	✓ Talc $[Mg_3](Si_4)O_{10}(OH)_2$
Smectite ( $0.25 < x < 0.6$ )	✓ Montmorillonite $M_x[Al_{2-x}Mg_x](Si_4)O_{10}(OH)_2$ Beidellite $M_x[Al_2](Si_{4-x}Al_x)O_{10}(OH)_2$	Hectorite $M_x[Mg_{3-x}Li_x](Si_4)O_{10}(OH)_2$ Saponite $M_x[Mg_3](Si_{4-x}Al_x)O_{10}(OH)_2$
Vermiculite ( $0.6 < x < 0.9$ )	Dioctahedral vermiculite $M_x[Al_2](Si_{4-x}Al_x)O_{10}(OH)_2$	Trioctahedral vermiculite $M_x[Mg_3](Si_{4-x}Al_x)O_{10}(OH)_2$
Mica ( $x \approx 1$ )	Muscovite $K[Al_2](Si_3Al)O_{10}(OH)_2$	Phlogopite $K[Mg_3](Si_3Al)O_{10}(OH)_2$ Taeniolite $M[Mg_2Li](Si_4)O_{10}F_2$
		Fluor-tetrasilicic mica $M[Mg_{5/2}](Si_4)O_{10}F_2$
Brittle mica ( $x \approx 2$ )	Margarite $Ca[Al_2](Si_2Al_2)O_{10}(OH)_2$	Clintonite $Ca[Mg_2Al](Si_4)O_{10}(OH)_2$

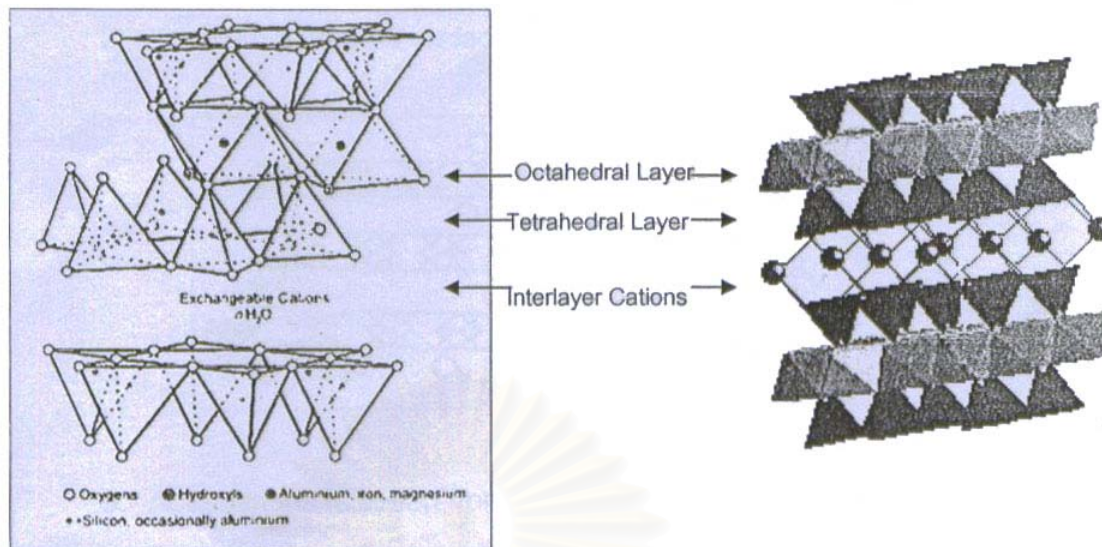


The formulae given here are idealised general formulae and large variations in composition are encountered within each sub-group of minerals; for instance most montmorillonites have at least a few percent of  $Fe^{3+}$  cations replacing the octahedral  $Al^{3+}$  cations and  $Ca^{2+}$  cations often replace the interlayer  $Na^+$

One of the most useful groups of clays is the smectite (swelling) clays, this group includes montmorillonite and hectorite.

Montmorillonite is the main constituent of bentonites and fuller's Earth which are noted for their excellent swelling properties when wetted. The most important bentonite clay is the montmorillonite group of minerals. Figure 2.9, at the atomic level, montmorillonite possesses the layered sheet structural that is typical of most minerals. Each sheet consists of a three-layer sandwich of ions in octahedral form. The central layer consists of aluminium ions surrounded by oxygen ions in tetrahedral form.





**Figure 2.9** Structure of montmorillonite

### 2.4.3 Ion exchange of clays

Clay minerals have the property of sorbing certain anions and cations and retaining these in an exchangeable state; i.e., they are exchangeable for other anions or cations by treatment with such ions in a water solution (the exchange reaction also takes place sometimes in a nonaqueous environment). The exchange reaction is stoichiometric. The exchangeable ions are held around the outside of the silica-alumina clay mineral structural units, and the exchange reaction generally does not affect the structure of the silica-alumina packet [34].

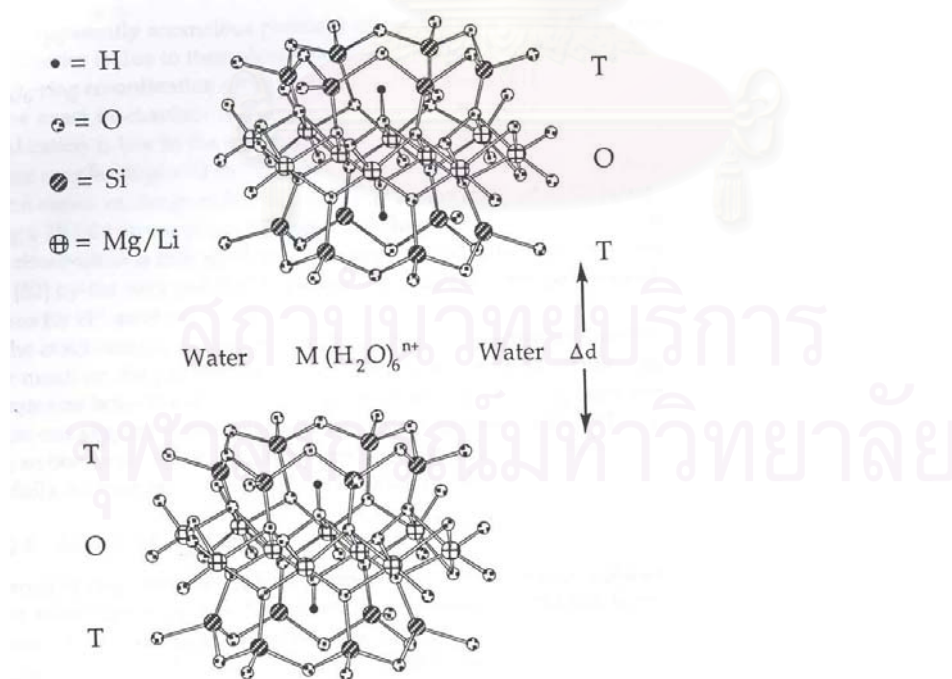
More information is available regarding cation exchange than anion exchange. In clay minerals the common exchangeable cations are calcium, magnesium, hydrogen, potassium, ammonium, and sodium, frequently in about that order of general relative abundance. The common anions in clay minerals are sulfate, chlorine, phosphate, and nitrate. The general relative abundance of the anions is not yet known.

The property of ion exchange is of great fundamental and practical importance in the investigation of the clay materials. In the application of clay mineralogy it is important because the nature of the exchangeable ion may influence substantially the physical properties of the material. Thus, a clay material carrying sodium frequently has very different plastic properties than material the same in every way except that calcium is exchangeable cation.

### 2.4.3.1 Cation exchange clays

To a greater or lesser degree, many clays have the ability to adsorb and exchange cation from solution and it is this cation 'storage' that makes clays such an important component of many soils. A typical montmorillonite can exchange over 100 millimoles of  $M^+$  cations per 100 g of clay, whilst a kaolinite only adsorbs a few tens of millimoles.

The ideal structures of the clay minerals depicted above are deviated from in a number of ways which introduce charge imbalances into the structure. The main causes of these charge imbalances are isomorphous exchange of  $Al^{3+}$  cations for  $Si^{4+}$  in the tetrahedral layers (e.g. in true montmorillonites), and of  $Li^+$  for  $Mg^{2+}$  or  $Mg^{2+}$  for  $Al^{3+}$  cation in the octahedral layer and crystal defects (e.g. in true kaolinites), usually at the crystallite edge. Natural minerals will usually combine each of these types of exchange sites. The layers therefore have an overall negative charge which is balanced by adsorption of metal cations into the interlayer region of the clay mineral in Figure 2.10.



**Figure 2.10** The idealised structure of a trioctahedral smectite clay showing the interlayer aqueous metal cations.

A typical montmorillonite will have  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  or  $\text{Mg}^{2+}$  cations in the interlayer space. These cations are hydrated, usually hexa(aqua), and the remainder of the interlayer is filled by a variable amount of water which can swell the clay. The interlayer cations are much less strongly bound than the layer cations and will thus easily exchange with cations from an aqueous solution .

Very small cations such as  $\text{Li}^+$  initially occupy the interlayer region, but they can migrate on heating through the hexagonal cavities in the silica layer to occupy unfilled octahedral sites. This decreases the ability of the mineral to adsorb cations.

The position in which these adsorbed cations reside depends upon the hydration of the clay, the cation and whether edge or layer charges are involved.  $\text{Na}^+$  and  $\text{Ca}^{2+}$  cations sit in the hexagonal cavities of the silica in dry montmorillonite, but they move into the interlayer region as the clay is hydrated. In contrast, kaolinite, whose charge imbalance is due mainly to edge defects, adsorbs the metal ions at the edges of the particles. Excess water can separate the metal cation from the surface by imposition of extra aquation spheres.

When a solution of a metal cation is used to exchange the interlayer cations of a clay. It has been observed that the smaller the size of the exchange cation and the higher the charge on the more powerful that cation is at replacing the interlayer exchangeable cations. Similarly the ease of replacement of interlayer cations follows the reverse trend. Thus the following series can be constructed:



Increasing exchange power  
(Decreasing ease of exchange)

**Figure 2.11** The exchange properties of cations with clays.

There are three causes of cation-exchange capacity of the clay minerals [34]:

1. Broken bonds around the edges of the silica-alumina units would give rise to unsatisfied charges which could be balanced by adsorbed cations. The number of broken bonds, hence the exchange capacity due to this cause, would increase as the particle size decreased. In kaolinite mineral broken bond is the major cause of exchange capacity. In montmorillonites broken bond is responsible for a relatively small portion (20 percent) of the cation-exchange capacity.

2. Substitutions within the lattice of trivalent aluminum for quadrivalent silicon in the tetrahedral sheet and of ions of lower valence, particularly magnesium, for trivalent aluminum in the octahedral sheet result in unbalanced charges in the structural units of some of the clay minerals. In montmorillonite substitutions within the lattice cause about 80 per cent of the total cation-exchange capacity.

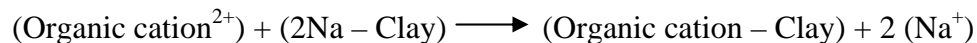
3. The hydrogen of exposed hydroxyls may be replaced by a cation which would be exchangeable. Some hydroxyl groups would be exposed around the broken edges of all the clay minerals, and cation exchange due to broken bonds would, in part at least, be replacement of the hydrogens. There is considerable doubt that this factor is a substantial cause of the cation-exchange reaction since it seems quite certain that the hydrogen of the hydroxyl would not normally be replaceable under the conditions of the exchange reaction.

In the clay minerals in which cation exchange results from broken bonds, the exchangeable cations are held around the edges of the flakes and elongate units. In the clay minerals in which the exchange reaction is due to lattice substitutions, the cations are held mostly on the basalplane surfaces.

Therefore, in kaolin minerals, some compounds can intercalate directly into kaolinite, some can be introduced by displacement of a previously intercalated. Some compounds cannot intercalate into the basal spacing of kaolinite but are adsorbed on the edges of faces of the clay particles. Little systematic work appears to have been done to explain why kaolin minerals spontaneously expand in some polar organic compounds, but not in others.

In montmorillonite, organic compounds are adsorbed in the interlayer space of the clay. Desorption investigations indicated that the cation adsorbed on the edge of the kaolinite clay particles are not held as strong as those bound within the clay lattice

of the montmorillonite. The mechanism of organic adsorption on clay mineral is depicted according to the following exchange reaction [35].



#### 2.4.3.2 Anion exchange clays

A series of clay minerals of lesser importance, whose charge imbalance or layer substitution pattern has given them positively charge layers, are known. Such minerals as hydrotalcite ( $\text{Mg}_{4.5}\text{AlO}_{7.5}$ ) and xonotlite ( $\text{Ca}_6\text{Si}_6\text{O}_{17}(\text{OH})_2$ ) can be used as solid 'carriers' of hydroxy and *t*-butoxy anions, respectively.

#### 2.4.4 Acidity of Clays

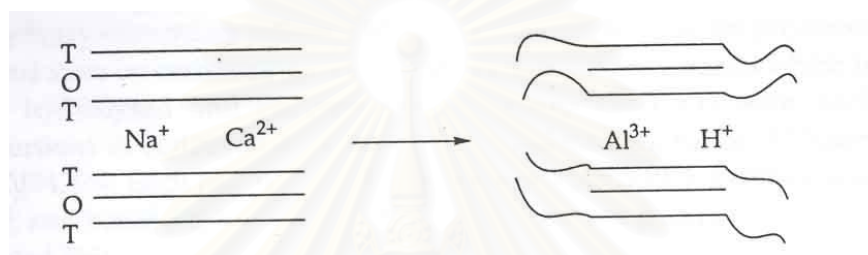
Several measurements of the acidity of clay surfaces have been carried out using surface conductivity, nuclear magnetic resonance and Hammett indicator methods. These experiments show that the effective pH of water at the mineral surface is usually between 1.5 and 3; which is 4 to 5 units more acidic than of bulk water.

Clay minerals show both Bronsted and Lewis acidity. Structural and environmental factors govern the degree to which is present and normally one type predominates for a given set conditions.

A further source of acidity is associated with the –OH groups of the octahedral layer which protrude into the interlayer region via the holes of the ring. The incidence of these protons may be increased by preparing a 'proton-exchanged' clay. This is achieved either a simply exchanging the clay with dilute acid, or less destructively to exchanging the clay with ammonium ions and calcining at 200-300 °C to expel ammonia. The exchanged proton can migrate into vacancies on the octahedral layer of dioctahedral clays where they protonate bridging oxygen.

### 2.4.5 Acid Activation of Clays

Smectite clays are often treated with strong mineral acid (acid activated) to give materials of very high surface area which have excellent activity as adsorbents and catalysts. The application of acid-activated clays as a developer for carbonless copying paper, requires a high brightness of the material. Acid activation improves the brightness mainly by removal of structural  $\text{Fe}^{3+}$  cations which cause the clay to be a grey or yellow colour.



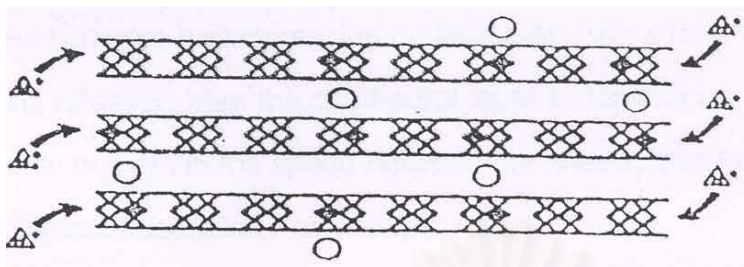
**Figure 2.12** Diagrammatic representation of the effects of acid activation.

The acid activation process is often quite severe ( $>5 \text{ mol dm}^{-3}$  hot mineral acid for several hours) and destroys much of the bentonite layer structure as it removes iron, aluminium and magnesium from the octahedral layer. Scanning electron microscopy shows that the edges of the clay particles become very disordered and consist mostly of ‘floppy’ silica sheets. The exchangeable cations are replaced mainly by  $\text{Al}^{3+}$  and  $\text{H}^{+}$  cations.

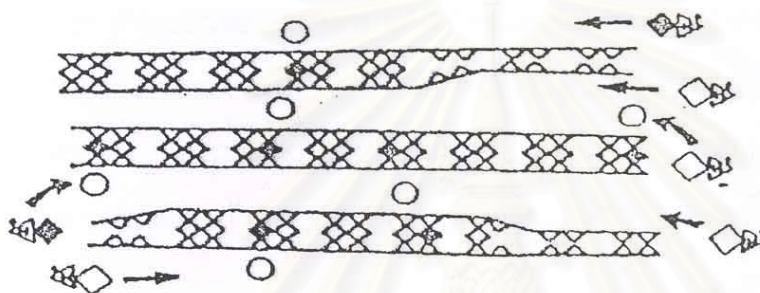
Acid activation enhances the natural ability of montmorillonites to adsorb polar molecules. The naturally occurring substitution of  $\text{Mg}^{2+}$  for  $\text{Al}^{3+}$  in certain clay deposits leaves a negative charge, which thus requires a positive charge go achieve electroneutrality. This positive charge is supplied by cations, such as  $\text{Na}^{+}$  or  $\text{Ca}^{2+}$ , which are located between the sheets.

Clay activation step by acid attack [36]

1. Acid attacks octahedral layer.

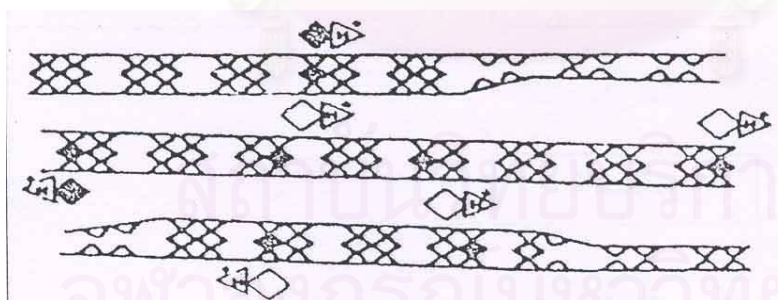


2. Structure disrupted surface area increases.



3. Acid cations released from structure exchanged for cations.

4. Acid activated clay with acid cations in exchange sites.

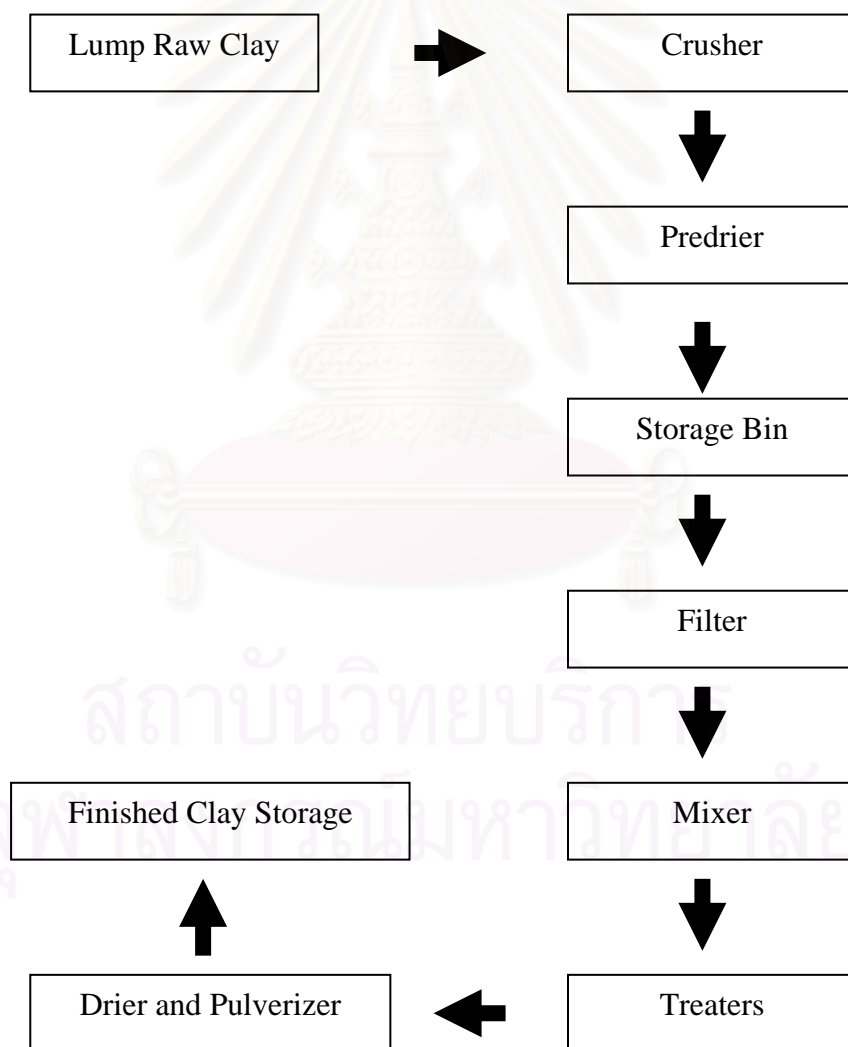


A well – controlled process has been developed to activate montmorillonites, exploiting the natural characteristics of its structure. The principle is to render soluble the structure cations in the octahedral layer, thus opening up the structure and increasing surface area. This allows more acidic (active) cations released from the octahedral layer to replace the less acidic cations originally present in the space

between the sheets, thus increasing its characteristic acidic adsorption and catalytic nature.

There is considerable evidence that acidic (active) cations serve as the active sites in the activated clay. When a pigment molecule (polar impurity) approaches one of the active sites, a carbonium ion (organic cation) forms and replaces, or associates with, one of the active, acidic inorganic interlayer cations. Now anchored in place by electrostatic force, the captive pigment molecule can be removed from the system along with the clay by simple filtration.

Figure 2.13 shows general stages of manufacturing of acid activated bentonite clay process. These clays are used as catalyst and adsorbent in the chemical, petroleum and petrochemical industries.



**Figure 2.13** Acid activated bentonite clay process



## 2.4.6 Some clays in Thailand

### 2.4.6.1 Ball clay

The name “ball clay” is derived from an early custom in the United Kingdom of excavating clay by cutting it into one-cubic foot blocks, which eventually became rounded to form balls. “Plastic clay” is a term used to describe fine-grained, highly plastic, principally kaolinitic sedimentary clays. However, the principal type of plastic clay, used in the whiteware industry because it fires to a white or near white colour, is called “ball clay” [37].

They are found from many resources in Thailand, scattering from northern, middle to southern parts of the country. Northern ball clays are Lampang ball clay from Ban jae Korn and brown ball clay from Ban Mae Than which is the largest clay production area. Ball clays in the middle and eastern parts of Thailand are in Pracheanburi and Chantaburi Provinces. Southern ball clays were found in the areas of Amphur Nasarn, Viensah, Kanchanadith, Surat Thani Province and in Nakoronsithammarat Province at Amphur Chwang, Pipoon, Lansaka, Ron Phibun and Sichon.

Ball clays of Thailand have high plasticity and fine grain size and often high content of organic material. Although the clay mineral is essentially kaolinite, it is much finer than that found in china clay, giving ball clays greater plasticity and dry-strength than china clays. They contain a greater quantity and variety of impurity than china clays. Iron and titanium impurities give the clays a fired colour which may vary from off white to dark brown depending on the amounts, of the clays. The advantages of ball clays are their high plasticity and dry-strength; their disadvantages are fired colour and low refractoriness.

Additionally, the clay mineral of the majority of ball clays is the disordered form of kaolinite and has a high cation exchange capacity (c.e.c.), of the order of 30-40 meq/ 100 g. Since, however, non-clay invariably present, ball clays as mined have a c.e.c. somewhat lower than this, the average being around 15 meq/ 100 g. organic matter makes a large contribution to the c.e.c. of ball clays [38].

High –quality ball clay can make up 30 percent of the body mix of wall tiles, vitreous china, sanitary ware, and insulator porcelain, and 5 to 25 percent of

earthenware and ordinary porcelain. Plastic clays of lower grade are used in heavy products such as pipe, brick, and tile. Ball clays are also used in refractories, as anti-caking agents in animal feedstuffs, and as fillers in rubber and plastics.

#### **2.4.6.2 China clay**

They are residual clays, and are essentially composed of the clay mineral kaolinite contaminated with silica, mica, feldspar and partly decomposed feldspar, all from the rock which the clay was formed. They are found in Northern parts; Lampang, Chiang Mai and southern parts: Ranong, Nakornsrihammarat.

China clay is much finer than the contaminating materials, and thus is relatively easily purified by sedimentation, giving a white clay which is also white burning due to its low content of colouring impurities, particularly iron and titanium compounds. Additionally, china clays have a comparatively low cation exchange capacity, ranging from about 2 to 10 meq/100g, the chief exchangeable ions being H, Ca, Mg, Na and K. Large quantities of china clay are used in paper manufacture as well as in the ceramics industry [39].

#### **2.4.6.3 Diatomite**

Diatomite (diatomaceous earth, kieselguhr) is a porous, lightweight sedimentary rock resulting from accumulation and compaction of diatom remains. The delicate shell or frustule of diatoms, which gives diatomite many of its useful properties, is composed of amorphous opaline silica ( $\text{SiO}_2 \cdot n\text{H}_2\text{O}$ ). While the specific gravity (density) of diatom frustules is nearly twice that of water, the perforations and open structure of the frustule renders diatomite a considerably lower effective density (between  $0.12\text{g/cm}^3$  and  $0.25\text{g/cm}^3$ ) and high porosity (from 75-85%), able to absorb and hold up to 3.5 times its own weight in liquid [40].

The purity of diatomites depends on the presence and amount of both classic particles (silt and clay) and of organic materials, which limit the utility of diatomite in industrial applications. Some high commercial-grade diatomite contains up to 90%  $\text{SiO}_2$  which can cause adsorption with polar molecules [41], with minor occurrence of

calcium carbonate, volcanic ash, terrigenous particles and other elements such as alumina, iron oxide, titanium oxide, phosphate, magnesium, sodium and potassium.

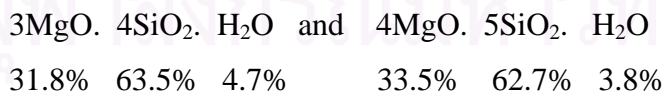
The physical properties of diatomite, it ranges from various shades of white to light gray or buff in colour, it can also be yellowish, light to dark brown, pink and green [42].

The characteristics that make diatomite most suitable for industrial uses are low density, high porosity(75-80%), low thermal conductivity, high melting point (1400°C to 1750°C depending on impurities), solubility only in strong alkaline solutions and hydrofluoric acid and being chemically inert. These properties have been applied in 300 to 500 commercial applications. Principal uses include filtration, thermal insulation, adsorption, catalyst carriers, fine abrasion, building materials, mineral fillers, as a pesticide, coatings, food additive and anti-caking agent. Diverse industries make use of diatomite:- food, beverage, pharmaceutical, chemical, agricultural, paint, plastics, paper, construction, dry cleansing, recreation, sewage treatment, among many others.

In Thailand there are several reserves of diatomite. Most of them are found in Lampang which is the second largest inland tertiary basin in the northern part. The main deposits were found at Amphoe Ko Kha Amphoe Muang and Amphoe Mae Tha. Diatomite in Lampang has a micro structure of diatoms, namely *Melosira granulata* which is a fresh-water diatom.

#### 2.4.6.4 Talcum

Talc is a different form of hydrated magnesium silicate which has a composition varying between the limits :



associated with impurities introducing alumina, iron, lime, alkalines and more water.

Talc is secondary rock formed by various interactions of water together with magnesium salts on the primary rocks. Although there are a large number of deposits, workable sources of a pure product are not nearly so frequent as those of clay. The mineral of clay is white to light green, extremely soft and has a greasy feel. Where the

mineral is laminated and very soft : it is termed talc ; impure varieties are known as soapstone. The more massive and relatively pure varieties are known as steatite [43].

The requirements for talc for steatite insulators (of which it is the major constituent) should be soft, substantially free from foliated, fibrous material and from gangue which makes dark spots when fired. The colour of the raw talc is of no importance, but should be white after heating to 1350°C in an oxidising or neutral atmosphere. The desirable chemical composition as compared with the theoretical is :

Theoretical :  $3\text{MgO} \cdot 4\text{SiO}_2 \cdot \text{H}_2\text{O}$  ( $\text{SiO}_2$  63.4%,  $\text{MgO}$  31.9%,  $\text{H}_2\text{O}$  4.7%)

Desired limits:  $\text{SiO}_2$  not less than 60% ,  $\text{MgO}$  not less than 30 %

$\text{Al}_2\text{O}_3$  not more than 2.5% ,  $\text{CaO}$  not more than 1.0 %

$\text{Fe}_2\text{O}_3$  not more than 1.5% ,  $\text{Na}_2\text{O}+\text{K}_2\text{O}$  not more than 0.4%

Loss on ignition not more than 6.0%

Acid soluble lime not more than 1.0%

The properties of talc are high melting point (1490°C) , specific gravity 2.6-2.8, thermal conductivity (2.3-2.8)/360 c.g.s., specific heat 0.2-0.3 c.g.s.

The main uses of talc are therefore those taking full advantage of the good electrical properties and thermal shock resistance, that is for electrical porcelain, saggars and kiln furniture for use up to 1250°C and use as electrical insulators.

## 2.5 Literature reviews

The invention related to the separation of carotenoids from palm oil by various methods, are the followings :

Desai *et al.* [5] studied the adsorption of carotenes from crude palm oil. They pointed out that acid-treated bentonite and alumina gel mixture are one of the adsorbents which can recover significant amounts of carotenoids without affecting its quality. Their experiments revealed that as the clay level increased, the amount of residual carotenoids in the palm oil decreased. The maximum adsorption of 79% carotenoids was possible at 4:1 ratio of bentonite and alumina gel.

Tan and Saleh [8] studied a process for concentrating carotene from palm oil which began with a transesterification step. The ester-rich phase obtained after settling is extracted with methanol and water to obtain a phase rich in carotene. Repeated extraction with methanol leaves a concentrated methanol/carotene mixture from which the alcohol is removed by evaporation in vacuo. In addition, the carotene-containing fatty acid methyl ester can be saponified after the transesterification step before it is extracted with petroleum ether. The disadvantage of this technique is the fatty acid methyl ester is contaminated with the extractant which prevents the ester from being further processed by hydrogenation to fatty alcohol. In general, the ester obtained in this process cannot be put to any further use and has to be disposed of.

The studied by Thanwongjathroengit [12] showed the outcome of the extraction of carotenes by adsorption techniques using different adsorbents. The activated carbon was used as adsorbent in stead of clay mineral under various conditions with two different systems; batch and column. The results revealed that the factors affected degradation of  $\beta$ -carotene extraction in palm oil were heat and antioxidant. They came up with the conclusions that the optimum condition for carotene adsorption was using activated carbon treated with antioxidant at a ratio of 4:1 by weight of palm oil to activated carbon at 80 °C adsorption temperature for 30 minutes and the appropriate condition for elution of adsorbed carotene from activated carbon was carried out at 25 °C by using toluene as an eluent which 30% yield. In column system, the recovery yield was 60% with 61% adsorption and 98% elution.

Baharin *et al.* [13] did the experiment by using synthetic polymer adsorbent (a styrene-divinyl benzene copolymer) for separation of palm carotene from crude palm oil. The studies showed that carotene was concentrated to about  $10^5$  ppm solution, which is about 160 times the original concentration in crude palm oil (CPO). Carotene recovery varied from 40 to 65 % depending upon chromatographic conditions. The fatty acid composition of the palm oil did not change during the

carotene recovery process, and the carotene composition was also almost the same as that in palm oil. Adsorption isotherms of the adsorbent differed from other adsorbents. This method for palm carotene may be suitable as an edible palm oil pretreatment process due to its efficient mass recovery of a valuable bioresources.

Khoo *et al.* [15] studied the adsorption using bleaching earth ; tonsil, fuller's earth , kaolin and silica as adsorbents. They came up with the ranking of effective adsorbents as follows : tonsil, fuller's earth, silica and kaolin. Experimental evidence indicated that silica is one of the example of physical adsorption process . The rest involved chemisorption which chemical reaction proceeded on the surface of clay minerals. Metallic ions, such as  $Fe^{3+}$ , are suggested to be the active sites for chemisorption and reaction. Oxygen was not essential for activity.

Nkpa *et al.* [16] introduced the method using local Nigerian clays as an adsorptive cleanser in the refining process of crude palm oil. The activated bentonite was used as the standard clay for comparison, and the performance of two local natural clays, leached with various concentrations of sulfuric acid, was evaluated for their use as alternative adsorptive cleansers in refining Nigerian crude palm oil. The degummed and alkali-neutralized palm oil was treated with clays leached with sulfuric acid at concentrations of 1M to 4M. Better results were obtained at acid concentrations of 2M and 3M. Bentonite proved better than both Nigerian clays. The yield of 62% is obtained from the experiment and the oxidative stability of the processed oil was reasonably good for an oil which was neither deodorized nor treated with antioxidants.

Nitsche *et al.* [44] reported in 1999 that carotene is recovered from a native fat or oil in five processing steps in an economical industrial process. The native fat or oil is catalytically reacted with an alkanol containing up to 4 carbon atoms to form fatty acid alkyl ester and glycerol. The ester phase of the reaction mixture is subjected to distillation to remove the fatty acid alkyl ester. The distillation residue obtained in the second process step is saponified, carotene is extracted from the product obtained

in the third process step and the extract phase is concentrated by evaporation. A yield of this process is about 80%. At the same time, a fatty acid alkyl ester suitable for further processing to fatty alcohol is possibly achieved.

Latip *et al.* [45] showed that palm carotene was successfully concentrated from CPO by adsorption process using synthetic adsorbents followed by solvent extraction. This process was a modified process for separation of palm carotene from CPO by adsorption chromatography with a synthetic polymer adsorbent. Carotene recovery varied from 30 to 62 % depending upon the process conditions. Different types of adsorbents, combinations of adsorbents, and adsorbent/CPO ratios were evaluated to determine the effect on the percentage of carotene extracted. An adsorbent /CPO ratio of 4 was most suitable for this process for optimal recovery and concentration of carotene.

Kumar *et al.* [46] evaluated the surface acidity and porosity of bentonite clay on treating with sulfuric acid. It was observed that clay treated with 4 N sulfuric acid showed the maximum surface acidity. Acid strength distribution as measured by Benesi's technique of nonaqueous titration show acidity range mainly between  $H_0 + 46$  and  $+3.3$ . Nitrogen adsorption-desorption hysteresis data indicated the transformation of pores from slit-shaped to spheroid inkbottle type as the acid concentration is increased from 1 to 8 N. The results from X-ray diffraction and FTIR showed the structural modifications of clay on treatment with acid.

Falaras *et al.* [47] evaluated the acid-activated montmorillonite to bleach cottonseed oil. A progressive decrease in cation exchange capacity (CEC) values was observed by treating Ca-montmorillonite with sulfuric acid clay. Elemental analysis showed that moderate activation occurred and only 25-30% of the octahedral cations was removed. X-ray and FTIR data confirmed that acid activation affects both the octahedral and the tetrahedral sheets. The oil acid value was not affected by the bleaching procedure but a light shift in the adsorption maximum of the bleached cottonseed oil was observed. Medium activation of the clay (treatment with 4M

H<sub>2</sub>SO<sub>4</sub>) was the most effective in bleaching the cottonseed oil, resulting in the best color index and the lowest peroxide value.

Diaz and Santos [48] studied on the acid activation of Brazilian smectitic clays used as bleaching earth for the industrial processing of vegetable, animal and mineral oils and waxes. The paper comments about the nomenclature used for these materials, the nature of the acid activation of smectitic clays (bentonites), activation laboratory procedures and presents a review of the acid activation bentonites from 20 deposits from several regions of Brazil. The activated clays were tested and showed good decolorizing power for soybean, castor, cottonseed, corn and sunflower oils.

Liew *et al.* [49] studied the adsorption of  $\beta$ -carotene on cation exchanged bleaching clays. Their experiment found that X-ray diffraction patterns of a number of commercial bleaching earths indicate that they consist mainly of montmorillonite mixed with smaller amount of kaolinites as well as mica and quartz. The active sites in these earths for the adsorption of  $\beta$ -carotene are identified through ion exchange and found to be the protonic sites and some metallic ions. The efficiencies of the various cations in imparting activities to the earths are found to be in the order  $Mg^{2+} > Fe^{3+} > H^+ > Ca^{2+} > Na^+$ . For  $Fe^{3+}$  and  $H^+$  exchanged clays, the activity was found to be linear function of the concentration of these ions. Metallic ions  $Fe^{3+}$ ,  $Mg^{2+}$  and  $Ca^{2+}$  impart considerable activity to clays for  $\beta$ -carotene adsorption. It is suggested that if the alkaline earth cations can interact with the  $\beta$ -carotene molecules, the activities of these cation exchanged clays may be expected to increase as their ionic radii decrease.



## CHAPTER III

### EXPERIMENTAL

In the present study, separation of carotenoids from palm oil by adsorption on clays was divided into:

1. Preparation and characterization of acid-activated clays and ion-exchanged clays
2. Separation of carotenoids from palm oil by adsorption on the prepared clays and desorped them from clays.
3. Quantitative analysis of carotenoid product

#### 3.1 Materials and chemicals

1. Thai clays were obtained from Cernic international Co.,Ltd. as followings:
  - 1.1 Bentonite clay (Chantaburi Province)
  - 1.2 Diatomite clay (Chiang Mai Province)
  - 1.3 Talcum clay (Utaradit Province)
  - 1.4 China clay (Lampang Province)
  - 1.5 China Clay (Ranong Province)
  - 1.6 Ball clay (Suratthani Province)
2. Crude palm oil was supplied from Olene Co., Ltd. The oil was used without further purification.
3.  $\beta$ -carotene  
Analytical grade; Fluka Chemie A.G., Switzerland
4. Sulphuric acid (96%)  
Analytical grade; Carlo Erba, Italy
5. Magnesium Chloride  
Analytical grade; Merck, Germany

6. Sodium Chloride  
Analytical grade; Merck, Germany
7. n-Hexane  
Analytical grade; Merck, Germany
8. Toluene  
Analytical grade; Carlo Erba, Italy
9. Ether  
Analytical grade; Carlo Erba, Italy
10. Tetrahydrofuran (THF)  
Analytical grade; UniLAB reagent, Australia
11. Butylated Hydroxytoluene (BHT)  
Analytical grade; Fluka Chemie A.G., Switzerland
12. Potassium bromide  
Analytical grade; Fluka Chemie A.G., Switzerland
13. Triethylamine  
Analytical grade; Merck, Germany
14. Acetonitrile  
HPLC grade; Merck, Germany
15. Dichloromethane  
HPLC grade; Carlo Erba, Italy
16. Methanol  
HPLC grade; Fluka Chemie A.G., Switzerland

### **3.2 Instruments and apparatus**

1. UV-visible Spectrophotometer  
UV-visible HP8453
2. Fourier Transform Infrared Spectrometer (FTIR)  
Nicolet FTIR-Impact 410
3. Scanning Electron Microscope (SEM)  
JEOL Japan JSM-541OLV SEM

4. X-ray Powder Diffractometer (XRD)  
Bruker AXS GmbH D8 Advance XRD
5. High Performance Liquid Chromatograph (HPLC)  
LC-3A Shimadzu Japan
6. Vacuum pump
7. Rotary evaporator
8. Magnetic stirrer

### **3.3 Experimental procedure**

#### **3.3.1 The acid activation of clay**

Fifty grams of each clay was refluxed with 250 ml of 4N H<sub>2</sub>SO<sub>4</sub> in a 500 ml round bottom flask at 90°C for 2 hours. The slurry was cooled in air and then filtered through a buchner funnel using a vacuum pump. The filter cake on filter paper was repeatedly washed with distilled water until the filtrate was almost neutral to pH indicator paper. The washed clay was dried at 120°C in an oven for 6 hours and ground to fine powder before use.

#### **3.3.2 The ion exchange of clay**

The acid activated clays were dried at 100°C overnight in an oven before ion exchange. Na<sup>+</sup> exchanged clays were prepared by the following procedure. A weighed sample of the clay was stirred with distilled water for about 30 minutes at room temperature. The slurry was filtered through a buchner funnel in a vacuum pump. The filter cake on filter paper was washed repeatedly with distilled water until a constant pH was obtained. 100g of the washed and dried clay was added 200ml of 1M NaCl solution and this mixture was stirred at room temperature for 3 hours before the slurry was filtered. The Na<sup>+</sup> exchanged clay was washed until free of residual sodium salt.

Preparation of  $Mg^{2+}$  exchanged clay was achieved as follows. Na-clay was equilibrated with solutions containing the appropriate cations at concentration 0.02 M at room temperature for about 1 hour. The slurry was filtered. The resulting clay was washed several times until it was free of any residual salt or acid. The exchanged clay was dried in an oven at 100°C overnight.

### 3.3.3 Determination of clay properties

#### 3.3.3.1 Structure of clays

The structure of clay was previously characterized by Scanning Electron Microscope, X-ray Powder Diffractometer and Fourier Transform Infrared Spectrometer.

#### 3.3.3.2 Acidity

Acidity of clays was determined by volumetric titration. In this method, 0.5 g of the clay, previously dried at 120°C for 6 hours, was taken in a conical flask to which 15 ml of 0.1N NaOH was added. After stirring the flask for 10 minutes, excess NaOH was titrated with 0.1N  $H_2SO_4$ . Acidity was determined as milliequivalents of NaOH used per 100g of clay.

$$\text{Acidity of clay (meq/100 g clay)} = \frac{(V_1 - V_2) \times [H_2SO_4] \times 100}{\text{amount of clay}}$$

Where:  $V_1$  is the volume of NaOH,  $V_2$  is the volume of  $H_2SO_4$ , and  $[H_2SO_4]$  is the  $H_2SO_4$  concentration. [36]

### 3.3.4 Adsorption of carotenoids from palm oil on the prepared clays

The adsorption was conducted in a 250-mL Erlenmeyer flask. Crude palm oil (CPO) was directly used without further purification. Adsorbents were dried in an oven for about 30 minutes before use. The adsorption process was initiated by adding

CPO (20 g) to the adsorbent (4 g). The mixture was heated to 70°C and stirred for 70 minutes. Then, the mixture was filtered through a buchner funnel which is connected to a vacuum pump. The oil which permeated filter paper was determined for the carotene contents by UV-visible Spectrophotometer. The adsorbent, separated from the mixture after the above treatment, was washed twice with purified hexane to remove residual oil. Then, the carotenes in adsorbent were removed by soaking the adsorbent in the eluting solvent at room temperature for 90 minutes and filtered them through filter paper. The excess solvent was removed in a rotary evaporator. The carotene contents were determined by UV-visible Spectrophotometer at  $\lambda_{\max}=463$  nm.

### 3.3.5 The influence of parameters for adsorption step

#### 3.3.5.1 Effect of processes for treatment of clay

Each clay was treated with these processes : acid activation (as in topic 3.3.1), non acid activation and ion-exchange (as in topic 3.3.2) before followed the adsorption experiment as in topic 3.3.4.

#### 3.3.5.2 Effect of type of adsorbents

Each adsorbent (4g) (as shown in Table 3.1) was used along with CPO (20 g) and followed the experiment as in topic 3.3.4.

**Table 3.1 Effect of type of adsorbents**

Adsorbent	Temperature (°C)	Time (mins)	Weight of clays (g)	Weight of palm oil (g)
Ball clay	70	70	4	20
China(Lampang)	70	70	4	20
China(Ranong)	70	70	4	20
Bentonite	70	70	4	20
Diatomite	70	70	4	20
Talcum	70	70	4	20

### 3.3.5.3 Effect of adsorption temperature

Each adsorbent (4g) was used along with CPO (20 g) at various temperature (as shown in Table 3.2) and followed the experiment as in topic 3.3.4.

**Table 3.2 Effect of adsorption temperature**

Adsorbent	Temperature (°C)	Time (mins)	Weight of clays (g)	Weight of palm oil (g)
Clay - 1	30	70	4	20
Clay - 2	70	70	4	20
Clay - 3	90	70	4	20
Clay - 4	110	70	4	20

### 3.3.5.4 Effect of adsorption time

Each adsorbent (4g) was used along with CPO (20 g) at various time (as shown in Table 3.3) and followed the experiment as in topic 3.3.4.

**Table 3.3 Effect of adsorption time**

Adsorbent	Temperature (°C)	Time (mins)	Weight of clays (g)	Weight of palm oil (g)
Clay - 1	70	50	4	20
Clay - 2	70	70	4	20
Clay - 3	70	90	4	20

### 3.3.5.5 Effect of ratios of clay to palm oil

Each adsorbent was used along with CPO at various ratios (as shown in Table 3.4) and followed the experiment as in topic 3.3.4.

**Table 3.4 Effect of ratios of clay to palm oil**

<b>Adsorbent</b>	<b>Temperature (°C)</b>	<b>Time (mins)</b>	<b>Weight of clays (g)</b>	<b>Weight of palm oil (g)</b>
Clay - 1	70	70	4	20
Clay - 2	70	70	4	28
Clay - 3	70	70	4	36

### **3.3.6 The influence of parameter for desorption step**

#### **3.3.6.1 Effect of type of eluting solvents**

Followed the experiment as in topic 3.3.4 and used hexane, toluene, ether and tetrahydrofuran as eluting solvents.

### **3.3.7 The influence of parameter for carotene degradation**

#### **3.3.7.1 Effect of antioxidant**

1% Butylated hydroxytoluene (BHT) in hexane 40 ml was used as antioxidant in this experiment. Triethylamine was added to this solution until it was alkalies (pH>7) to universal indicator pH. Then the adsorbent (4 g) was soaked in the above solution for 60 minutes. After that, the mixture was filtered through a buchner funnel and washed with purified hexane until the filtered solution was clear and colorless. The adsorbent after treatment with antioxidant was used to compare with the nontreated antioxidant adsorbent by following the experiment as in topic 3.3.4.

### 3.3.8 Quantitative analysis of carotene using UV-visible Spectrophotometer

The carotene contents in palm oil after adsorption and the extracted carotene were determined by diluting 1-mL aliquot of each supernatant with toluene to the appropriate dilution (25 mL) and measuring absorbance in a UV-visible HP8453 Spectrophotometer at  $\lambda_{\max}$  = 463 nm.

Standard  $\beta$ -carotene (>99%) was used to prepare the calibration curve.

### 3.3.9 Quantitative analysis of carotene using HPLC

The extracted carotene (topic 3.3.4) was diluted with dichloromethane to the appropriate dilution (10 mL) and injected onto the column under the chromatographic conditions as listed in Table 3.5.

The solution to be used as a mobile phase was acetonitrile : dichloromethane : methanol : water (79.9 : 10 : 10 : 0.1) which was degassed in an ultrasonic bath for least 30 minutes prior to use.

Standard  $\beta$ -carotene (>99%) was used to prepare the calibration curve.

**Table 3.5 HPLC chromatographic conditions for analysis of carotene**

HPLC Parameter	HPLC Condition
Analytical Column	Reverse phase C <sub>18</sub> 125x4 mm.
Mobile Phase	acetonitrile:dichloromethane:methanol: water (79.9 : 10 : 10 : 0.1)
Flow rate	1.5 mL/min
Column Temperature	25 °C
Detector	UV 463 nm
Pressure	120 kg/cm <sup>2</sup>
Sensitivity	0.2 AUFS
Attenuation	2 <sup>4</sup> $\mu$ Volt / full scale



## CHAPTER IV

### RESULTS AND DISCUSSION

Separation of carotenoids from palm oil was performed under various conditions of adsorption on various Thai clays.

The influence of parameters, i.e. different processes for treatment of clay, type of adsorbents, adsorption temperature, adsorption time, ratios of clay to palm oil and type of eluting solvents for adsorption on the prepared clays and desorption from clays were studied and the results from the experimental procedure mentioned in chapter III were presented in the following tables and figures. The results were also discussed as in the followings.

#### 4.1 Properties, structure and compositions of clays

##### 4.1.1 The chemical compositions of clays

The origin and chemical compositions of clays were presented in Table 4.1. (Informations were obtained from Cernic International Co.,Ltd.)

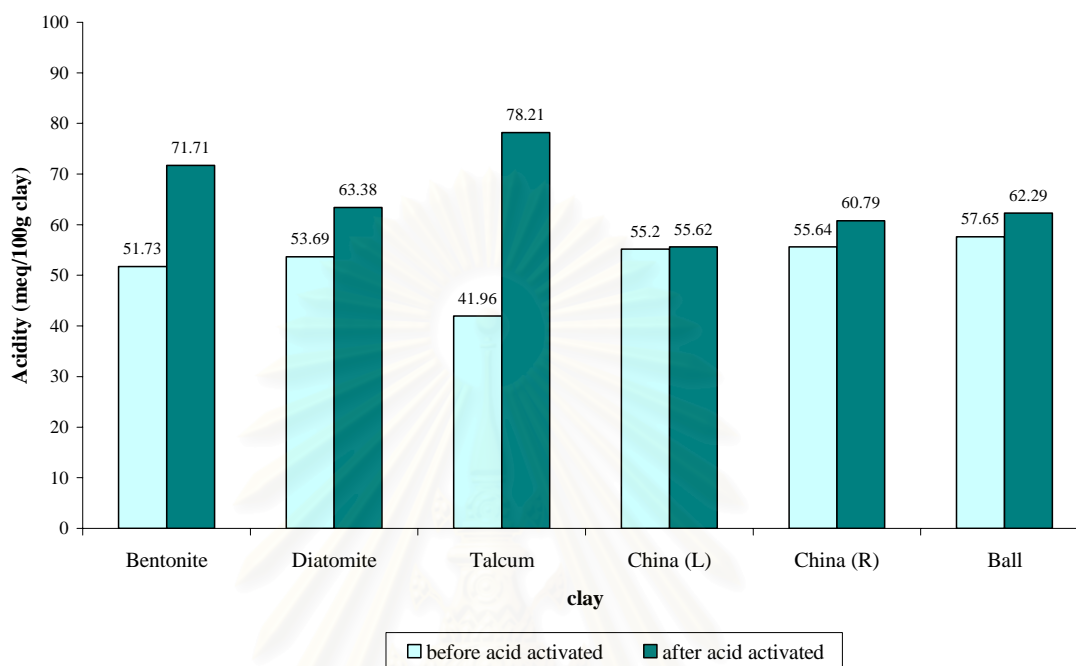
สถาบันวิทยบริการ  
จุฬาลงกรณ์มหาวิทยาลัย

**Table 4.1** Origin and chemical compositions of clays

Clay	Ball clay	China clay	China clay	Bentonite	Diatomite	Talcum
Origin	Suratthani	Ranong	Lampang	Chantaburi	Chiang Mai	Utaradit
SiO <sub>2</sub> (%)	51.50	44.85	66.05	63.60	74.35	61.54
Al <sub>2</sub> O <sub>3</sub> (%)	23.00	37.98	22.55	17.60	16.45	-
CaO(%)	0.37	0.06	0.08	3.00	0.44	0.35
MgO (%)	0.12	0.12	0.54	Trace	-	31.15
TiO <sub>2</sub> (%)	0.63	0.07	0.02	-	-	-
Fe <sub>2</sub> O <sub>3</sub> (%)	1.42	0.97	0.68	3.10	3.32	0.16
Na <sub>2</sub> O (%)	0.22	0.04	0.41	3.40	-	-
K <sub>2</sub> O(%)	0.92	1.23	4.19	0.50	-	-
H <sub>2</sub> O (%)	-	0.65	-	-	-	-
Loss on ignition (%)	19.60	13.94	4.65	5.80	-	-
Whiteness	-	-	-	-	-	94.30
Fineness	-	-	-	-	-	99.50

#### 4.1.2 Acidity characterization [36]

The total acidity of clay (determined by sodium hydroxide titrations and expressed in meq of NaOH used per 100 g of clay) was presented in Figure 4.1. From this Figure, talcum after acid activation exhibited the maximum acidity(78.21 meq/100 g) followed by bentonite, diatomite, ball clay, china (R) and china (L), respectively.



**Figure 4.1** Acidity values of clays before and after acid activation

**Table 4.2** Surface area and acidity of acid activated clays

Clay	Surface area (m <sup>2</sup> /g)	Acidity (meq/100 g)
Talcum <sup>a</sup>	324	78.21
Bentonite <sup>a</sup>	221	71.71
Diatomite <sup>a</sup>	187	63.38
Ball clay <sup>a</sup>	135	62.29
China (R) <sup>a</sup>	89	60.79
China (L) <sup>a</sup>	81	55.62

a) acid activated clay

### 4.1.3 Study of clay structure

The surfaces of clay samples were observed using Scanning Electron Microscope (SEM) as shown in Figure A1 in Appendix A. The surface of bentonite and talcum were found to have more porosity than the others. Thus, they should have more acid sites than the others. The IR spectroscopy is very sensitive to the structure changes, which occur in the clay upon acid treatment and the IR data recorded herein suggest that both the octahedral and tetrahedral sheets were susceptible to acid attack. Figures A2-A7 show IR spectra of acid activated clay samples and Table 4.4 summarizes the main vibrations observed. In Figures A2-A4, bentonite, diatomite and talcum, the band between 3800 and 3300  $\text{cm}^{-1}$  decreased in intensity from each parent clays. It showed that water was adsorbed on the free silica when acid treatment increases. Similar behavior was shown by the deformation band nearby 1638  $\text{cm}^{-1}$ , which may be used to indicate the amount of water in clay. The Si-O band, shift from 1034 to 1041  $\text{cm}^{-1}$  for bentonite, 1095 to 1098  $\text{cm}^{-1}$  for diatomite and 1018 to 1039  $\text{cm}^{-1}$  for talcum, indicated that the acid treatment had attacked the present clay structure because the tetrahedral sheet of the clay was collapsed, especially at higher acid concentration. Moreover, it is generally accepted that the rate of dissolution of tetrahedral cations is significantly lower than that of octahedral cations. The band at 915  $\text{cm}^{-1}$  corresponding to the AlAlOH bending deformation [47] decreased and became very weak for bentonite, diatomite and talcum, suggesting a significant depopulation of the octahedral sheet by acid treatment. [36]

**Table 4.3** Characteristic FTIR bands for acid activated clays

Wave number ( $\text{cm}^{-1}$ )						Band assignment
Bentonite		Diatomite		Talcum		
Parent	Acid <sup>a</sup>	Parent	Acid <sup>a</sup>	Parent	Acid <sup>a</sup>	
3432	3432 <sup>b</sup>	3443	3432	3432	3432 <sup>b</sup>	OH stretching
1635	1635 <sup>b</sup>	1626	1626 <sup>b</sup>	1658	1658 <sup>b</sup>	Hydration, HOH deformation
1034	1041	1095	1098	1018	1039	SiO stretching
912	912 <sup>b</sup>	917	917 <sup>b</sup>	912	912 <sup>b</sup>	OH deformation

a = acid activated clay

b = intensity decreased

On the other hand, Figures A5-A7, china (L), china (R) and ball clay indicated no change of IR spectra from the parent clays. Therefore, the acid activation of these clays did not change the structure.

Figure A8 shows a comparison of IR spectra of various acid activated Thai clays. The OH stretching band of bentonite has less intensity than the others. This means that OH groups in bentonite structure were attacked by acid. Thus, octahedral layers were collapsed and the number of acid sites increased [47]. This is a reason why bentonite has more acidity than the others.

Figures A9-A14 show IR spectra of ion-exchanged clay samples.

Figures A15-A20 show the XRD patterns of clay before and after acid activation. Gradual degradation of the layered structure was observed by the intensity of the (001) reflection. The untreated samples exhibited a well-defined and very intense 001 peak. When the samples were acid activated, the crystallinity of the samples decreased and the loss of intensity of 001 reflection. Without undergoing line broadening during the acid activation process, indicate that the removal of ions from the sheets occurs by proton attack at layer edge sites. The broad hump in the 20-30° (2 $\theta$ ) region has been attributed to amorphous silica [47].

## **4.2 Quantitative analysis of carotene using UV-visible Spectrophotometer**

### **4.2.1 Absorption maxima ( $\lambda_{\max}$ ) of carotene**

From the experiment,  $\lambda_{\max}$  of standard  $\beta$ -carotene by using toluene as a blank and measuring in a UV-visible HP8453 Spectrophotometer is 463 nm.

## 4.2.2 Calibration Curve

Standard  $\beta$ -carotene at various concentrations was used for measuring absorbance at  $\lambda_{\max} = 463$  nm to prepare calibration curve as shown in Figure B1 (Appendix B)

This calibration curve was used to calculate  $\beta$ -carotene content, % adsorption and % recovery of  $\beta$ -carotene.

Spectrum of  $\beta$ -carotene, CPO and the extracted carotene by using UV-visible spectroscopy was shown in Figure B2, B3 and B4, respectively.

## 4.3 Adsorption of carotenoids from palm oil on various clays.

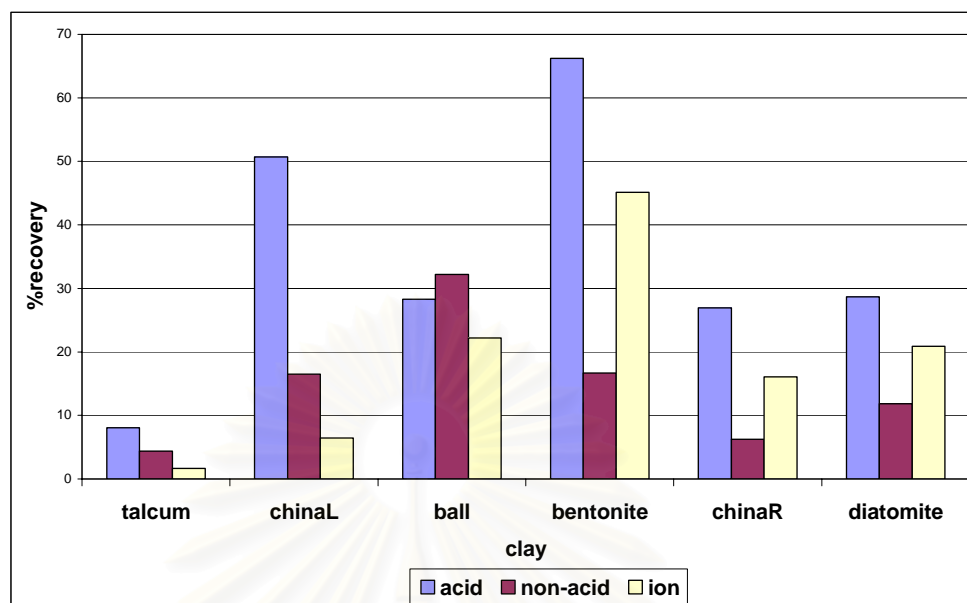
### 4.3.1 The influence of parameters for adsorption step

#### 4.3.1.1 Effect of processes for treatment of clay

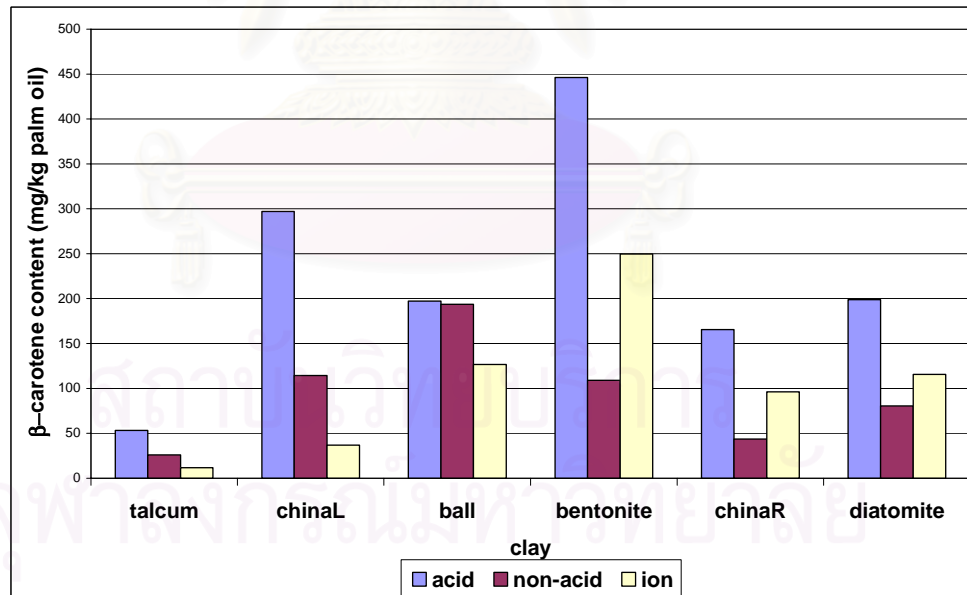
The  $\beta$ -carotene content, adsorption (%) and recovery (%) of  $\beta$ -carotene were determined by using different clay treatment processes which are acid activation, non acid activation and ion-exchange. Each adsorbent was treated with these processes before adsorption of carotene from palm oil.

Results are shown in Table C1, C2 (Appendix C) and Figure 4.2, 4.3. They showed that most of the acid activated clays show more  $\beta$ -carotene recovery (%) than non acid activated and ion-exchange. So this acid activation process was used to activate various clays to find the other optimum conditions.

From topic 4.1.3 and the results in this section, it was explained that during acid activation montmorillonite (e.g. bentonite, diatomite) undergoes considerable changes to its chemical composition and physical structure. A clay is formed of high surface area and the correct chemical composition at the surface to preferentially attract pigment molecules, e.g.  $\beta$ -carotene, of molecular weights lower than the triglyceride oil [50]. So the most acid activated clay adsorbs more pigments than the other clays [4].



**Figure 4.2** Effect of various processes for treatment of clay on the % recovery of  $\beta$ -carotene at  $70^\circ\text{C}$ , 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent.



**Figure 4.3** Effect of various processes for treatment of clay on the  $\beta$ -carotene content at  $70^\circ\text{C}$ , 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent.

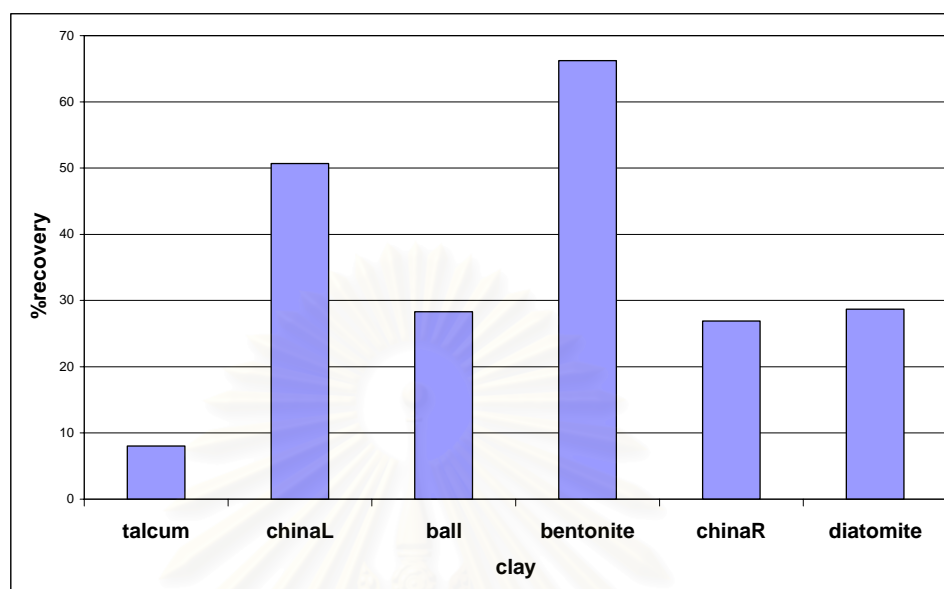
#### 4.3.1.2 Effect of type of adsorbents

Bentonite, diatomite, talcum, china (L), china (R) and ball clay, which were acid activated, were used for the evaluation of  $\beta$ -carotene extraction from CPO. Results are shown in Tables C3,C4 and Figures 4.4,4.5. All of the adsorbents tested were capable of adsorbing different amounts of  $\beta$ -carotene from CPO. The percentage of  $\beta$ -carotene adsorption ranged from 83 to 99 % and  $\beta$ -carotene recovery ranged from 8 to 66 %. The capability of the above-mentioned adsorbents to adsorb  $\beta$ -carotene from CPO is due to the similarity of the molecular structures of  $\beta$ -carotene and the adsorbents and also to hydrophobic interaction between the adsorbents and  $\beta$ -carotene [45].

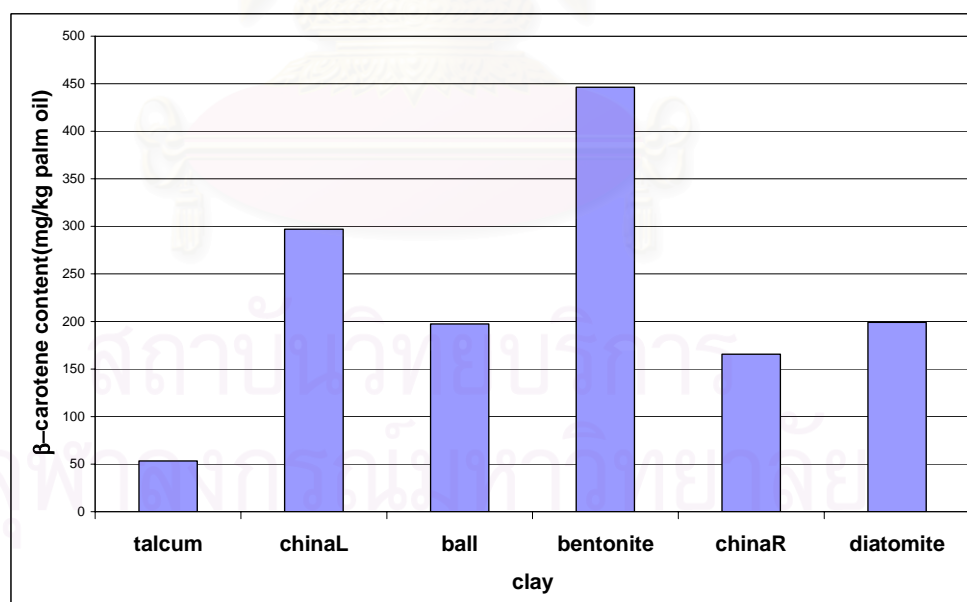
In addition, the acid treatment of montmorillonite clay, such as bentonite, diatomite, produces a specialty adsorbent from a naturally occurring mineral which displays limited adsorption properties and generally poorer in performance when compared to acid activated clays since these clays have a high surface area and are able to adsorb a variety of organic substances by means of van der Waals' force [45]. While clay minerals such as kaolinites (china clay and ball clay) do not have this potential to increase surface area on acid treatment.

From this experiment, it was concluded that acid activated bentonite gave better performance than the other clays because it gave the best carotene recovery (%) and the highest  $\beta$ -carotene content.





**Figure 4.4** Effect of different type of acid activated adsorbents on the % recovery of  $\beta$ -carotene at 70 °C, 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent



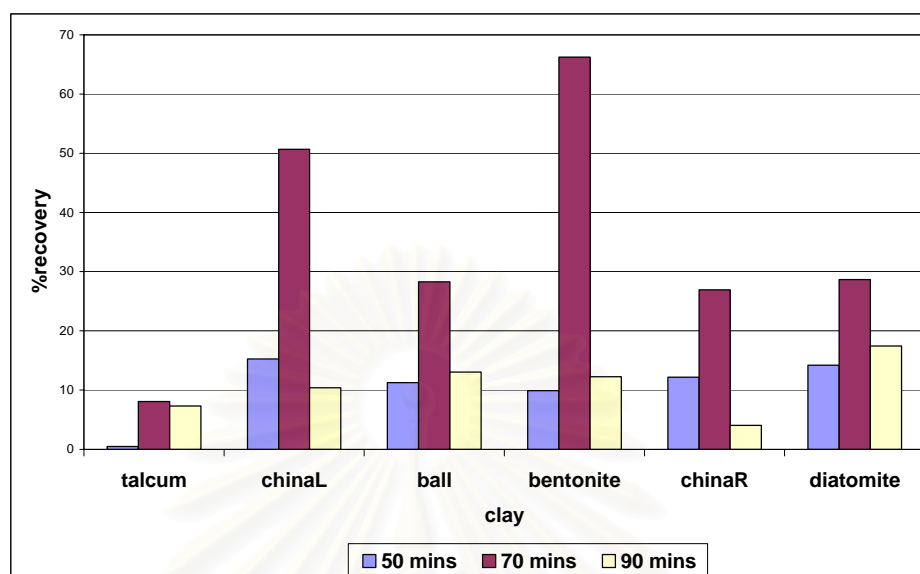
**Figure 4.5** Effect of different type of acid activated adsorbents on the desorbed  $\beta$ -carotene content at 70 °C, 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent

#### 4.3.1.3 Effect of adsorption time

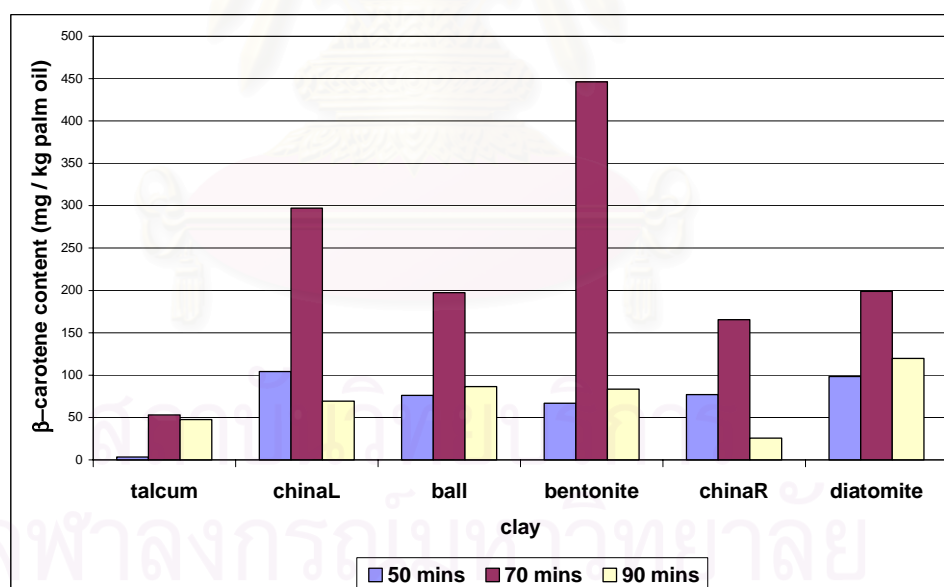
The effect of adsorption time has been studied. The results are shown in Table C5 and Figure 4.7 indicated that the  $\beta$ -carotene content at 70 minutes was higher than those at 50 and 90 minutes in every sample.

The  $\beta$ -carotene content can be converted to %  $\beta$ -carotene recovery as shown in Table C6 and Figure 4.6. Bentonite has the highest %  $\beta$ -carotene recovery, in comparison with other clays. Besides, diatomite showed nearly the same %  $\beta$ -carotene recovery with china (R) and ball clay at 70 minutes.

This experiment revealed that the suitable adsorption time for these 6 Thai clays is 70 minutes which is saturated point of adsorption of  $\beta$ -carotene on clays. When adsorption time was increased, resulted in the decreasing of %  $\beta$ -carotene recovery because some  $\beta$ -carotene was destroyed by heat during adsorption process. On the other hand, the less adsorption time, the less %  $\beta$ -carotene recovery due to unreachable saturated point.



**Figure 4.6** Effect of different adsorption times on the % recovery of  $\beta$ -carotene by using various acid activated clays as adsorbents at 70 °C, ratios of clay : palm oil = 1:5 and use THF as eluting solvent

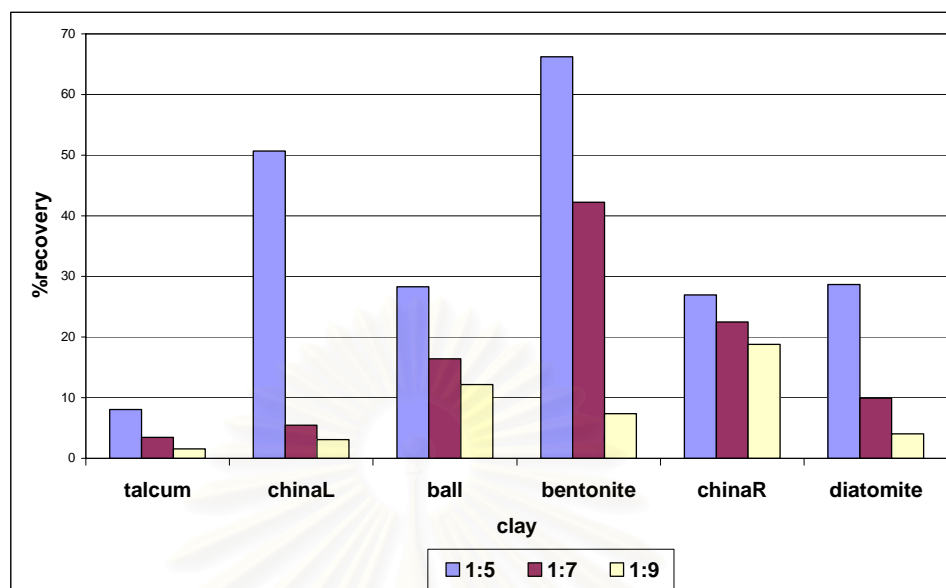


**Figure 4.7** Effect of different adsorption times on the desorbed  $\beta$ -carotene content by using various acid activated clays as adsorbent at 70 °C, ratios of clay : palm oil = 1:5 and use THF as eluting solvent

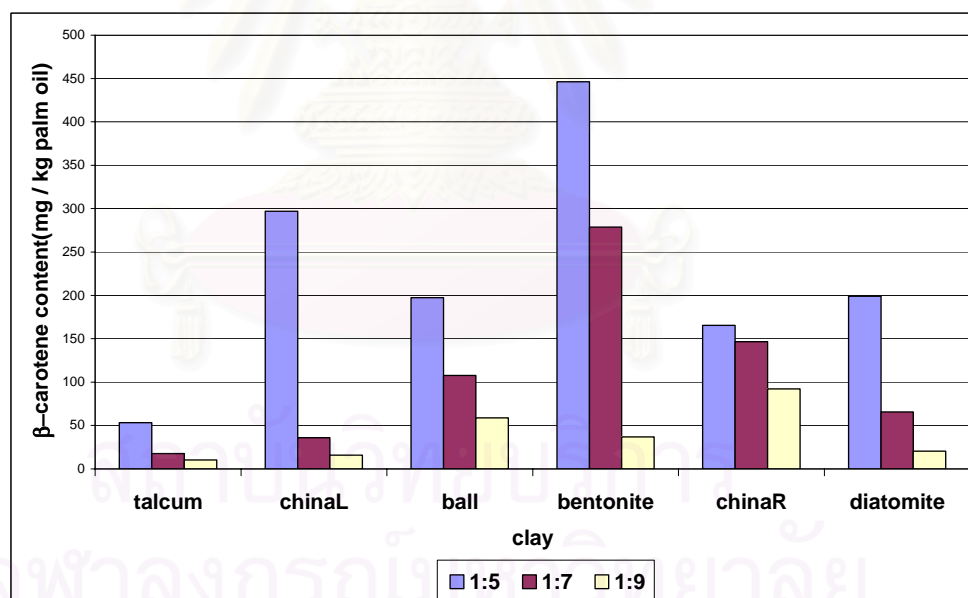
#### 4.3.1.4 Effect of ratios of clay to palm oil

Experiments were conducted to determine the optimal ratio of clay to palm oil for the highest yield of carotene extraction from CPO. In this section, the ratios of clay to palm oil were varied at different ratios : 1:5, 1:7 and 1:9. The results are shown in Tables C7,C8 and Figures 4.8, 4.9. They indicated that the percentage of  $\beta$ -carotene recovery increased with the increase of ratio of clay to palm oil as more carotene was adsorbed on the surface of the adsorbent . This is due to increased binding site availability. Clearly, the percentage of  $\beta$ -carotene recovery decreased with increasing amount of palm oil since competitive adsorption between the oil and carotene on clay surface [13]. Besides, in the chemical refining of palm oil, if the CPO feedstock is of bad quality, higher levels of the clay are required [20].

For these experiments, the ratio of clay to palm oil = 1:5 seemed to be the most suitable ratio since the percentage of  $\beta$ -carotene recovery was higher than the other ratios.



**Figure 4.8** Effect of different ratios of clay to palm oil on the % recovery of  $\beta$ -carotene by using various acid activated clays as adsorbents at 70 °C, 70 mins and use THF as eluting solvent



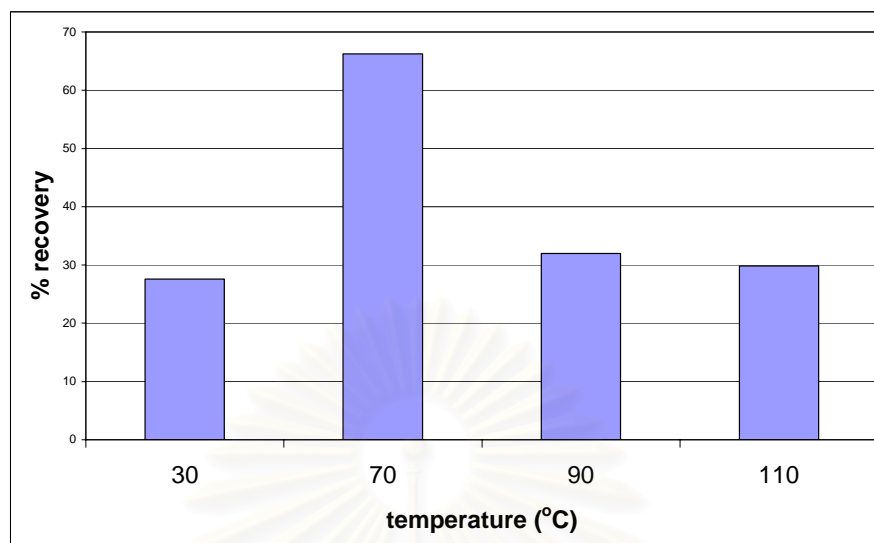
**Figure 4.9** Effect of different ratios of clay to palm oil on the desorbed  $\beta$ -carotene content by using various acid activated clays as adsorbents at 70 °C, 70 mins and use THF as eluting solvent

#### 4.3.1.5 Effect of adsorption temperature

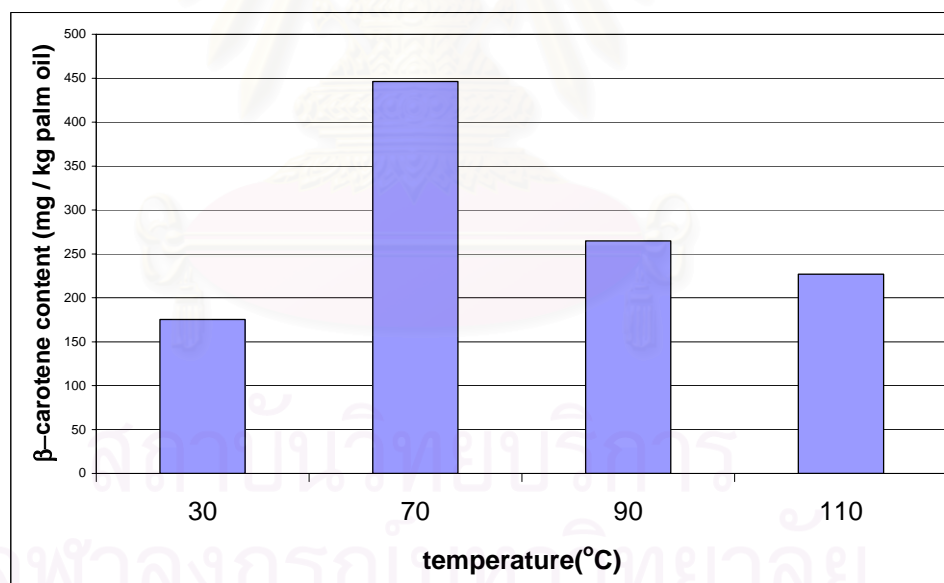
The effect of adsorption temperature has been studied. In this section, the temperature of the experiments was varied at different temperature of 30°C, 70°C, 90°C and 110°C for the acid activated bentonite. Since temperature in the bleaching process in the refining palm oil industry is about 80-130°C [20], therefore it is appropriate to use this range of temperatures in this experiment in order to minimize the difference from the industrial plant. The results are shown in Tables C9, C10 and Figures 4.10, 4.11. From this experiment, it was concluded that the suitable temperature is 70°C because degradation of carotene at elevated temperatures was possible.



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**Figure 4.10** Effect of different adsorption temperature on the % recovery of  $\beta$ -carotene by using acid activated bentonite as adsorbent for 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent



**Figure 4.11** Effect of different adsorption temperature on the desorbed  $\beta$ -carotene content by using acid activated bentonite as adsorbent for 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent

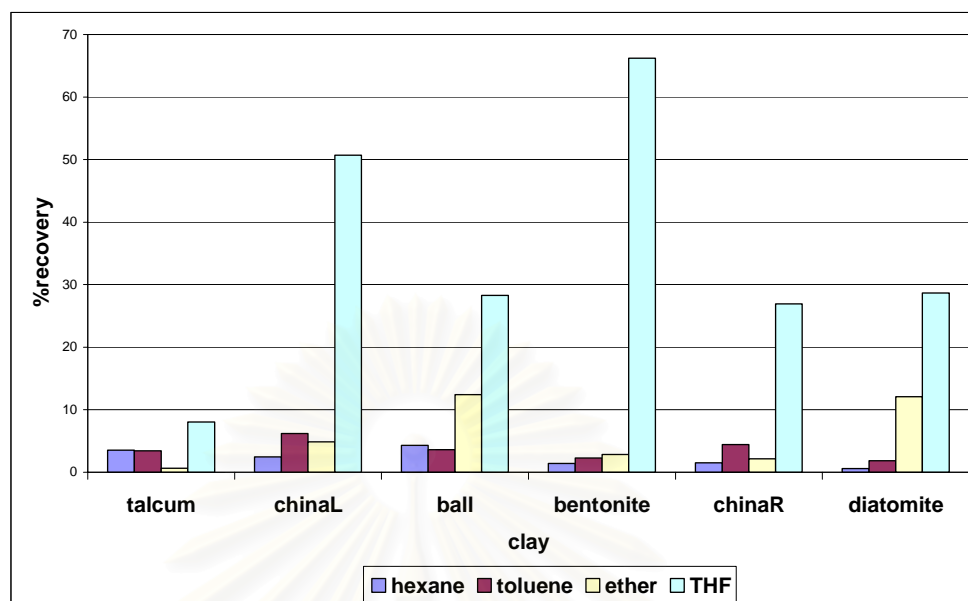
## 4.3.2 The influence of parameter for desorption step

### 4.3.2.1 Effect of type of eluting solvents

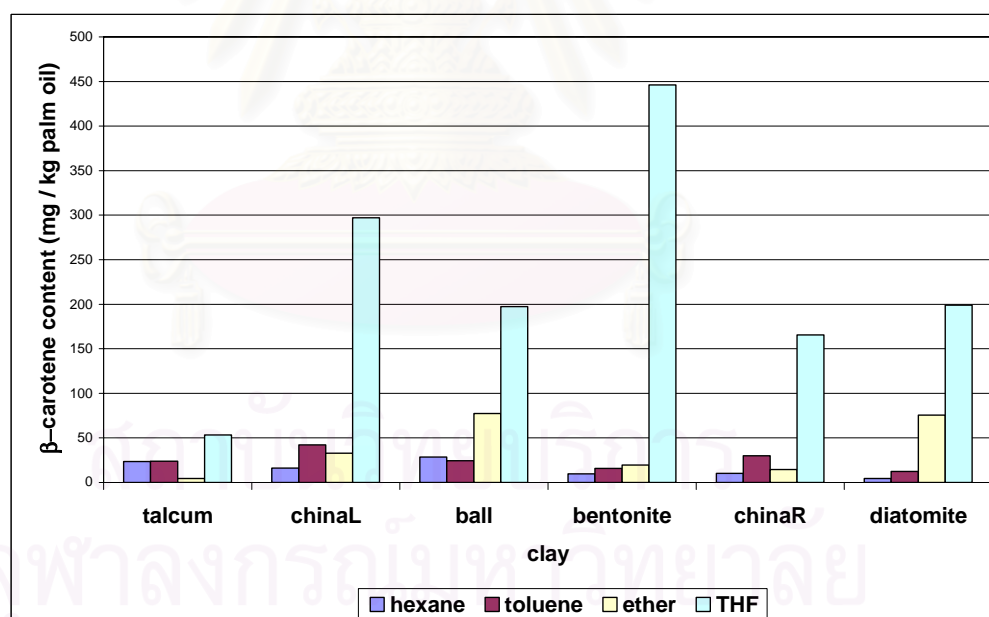
Eluting solvent was one of the important factor in desorption step. In this experiment, hexane, toluene, ether and THF were used as eluting solvents for the evaluation of  $\beta$ -carotene extraction from CPO. Results are shown in Tables C11,C12 and Figures 4.12, 4.13. For these experiments, the  $\beta$ -carotene content and %  $\beta$ -carotene recovery in hexane, toluene and ether was less than that from THF in every clay because desorption of adsorbed material is more difficult due to the greater adsorption forces between the adsorbents and carotene [13].

Besides solubility of  $\beta$ -carotene in the solvent, we must also consider about toxicity of solvent, cost and stability of  $\beta$ -carotene in the solvent. The study in stability of  $\beta$ -carotene in various solvents found that  $\beta$ -carotene has the most stability in THF with antioxidant (BHT).





**Figure 4.12** Effect of different type of eluting solvents on the % recovery of  $\beta$ -carotene by using various acid activated clays as adsorbents at 70 °C, 70 mins and ratios of clay : palm oil = 1:5.



**Figure 4.13** Effect of different type of eluting solvents on the desorbed  $\beta$ -carotene content by using various acid activated clays as adsorbents at 70 °C, 70 mins and ratios of clay : palm oil = 1:5.

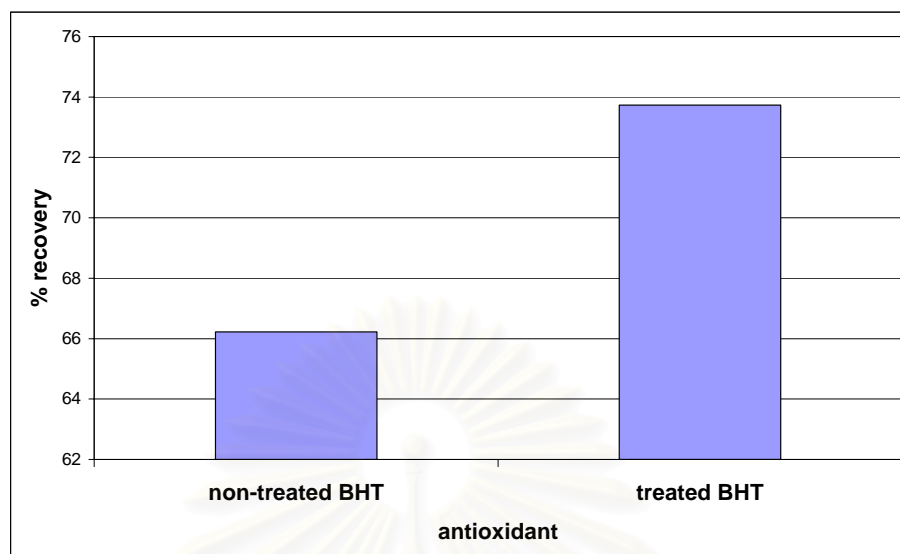
### 4.3.3 The influence of parameter for carotene degradation

#### 4.3.3.1 Effect of antioxidant

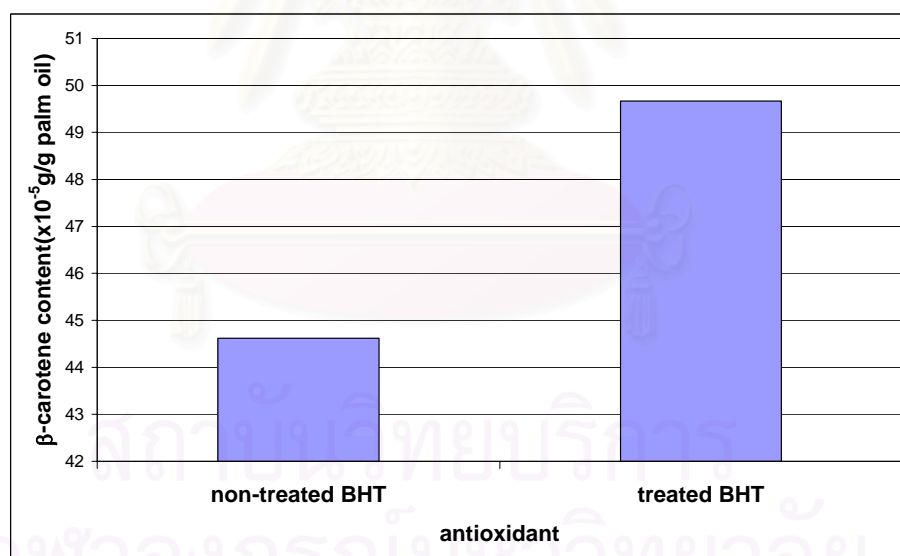
The effect of antioxidant has been studied. In this section, the antioxidant which used in this section is butylated hydroxytoluene : BHT. The acid activated bentonite treated with BHT was used in comparison with the nontreated BHT bentonite. Results are shown in Tables C13, C14 and Figures 4.14, 4.15. The acid activated bentonite treated with BHT gave higher  $\beta$ -carotene content and %  $\beta$ -carotene recovery than the nontreated BHT bentonite because BHT can protect oxidation which was one of the important cause of the degradation of the carotenoids.



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**Figure 4.14** Effect of antioxidant on the % recovery of  $\beta$ -carotene at 70 °C, 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent



**Figure 4.15** Effect of antioxidant on the desorbed  $\beta$ -carotene content at 70 °C, 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent

#### 4.4 Quantitative analysis of carotene using HPLC

Standard  $\beta$ -carotene at various concentrations was used to prepare calibration curve as shown in Figure B5 (Appendix B). This calibration curve was used to calculate  $\beta$ -carotene content in the extracted carotene.

The chromatograms of standard  $\beta$ -carotene, the extracted carotene and the spiking technique by using HPLC are shown in Figure B6.

The spiking technique was used to confirm that the extracted carotene matched with standard  $\beta$ -carotene which can be indicated that the peak area using the spiking technique was higher than the other peaks.

From the experiment under the chromatographic conditions (Table 3.5), the extracted carotene was separated from the column at 7.72 minutes, while the standard  $\beta$ -carotene was separated from the column at 8.39 minutes as shown in Figure B6. The calculation of  $\beta$ -carotene content, compared to calibration curve (Figure B5), indicated that  $\beta$ -carotene content in the extracted carotene was 241.53 mg/kg palm oil corresponding to 45.47 % by weight of carotenoids in crude palm oil.

#### 4.5 Carotenoids recovery

The optimum conditions for carotenoids recovery which use acid activated bentonite treated with BHT as adsorbent, 70°C adsorption temperature, 70 minutes adsorption time, 1:5 ratio of clay to palm oil and THF as eluting solvent are used in this section.

The initial crude palm oil (CPO) has carotenoids 675.01 mg/kg palm oil. The experiment use CPO 21.12 g, so the initial CPO has carotenoids 14.26 mg. After eluting solvent in carotenoids is evaporated, 1.45 g of the extracted carotenoids which has carotenoids content 10.49 mg is obtained. Therefore, overall carotenoids which can recovery from this experiment are 10.49 mg corresponding to 73.56% by weight of carotenoids in CPO.

## CHAPTER V

### CONCLUSION AND SUGGESTION

#### 5.1 Conclusion

In this research, separation of carotenoids from palm oil was carried out by adsorption on six types of Thai clays: bentonite, diatomite, talcum, china L, china R, and ball clay. The influence of various parameters for adsorption and desorption process was studied and the optimum conditions were investigated.

Results indicated that acid activation provided higher %  $\beta$ -carotene recovery than the non acid activation. The ion-exchange of various clays did not show better efficiency of %  $\beta$ -carotene recovery than the acid activated clay.

For type of adsorbents, acid activated bentonite showed higher %  $\beta$ -carotene recovery than the other clays. The commercial bleaching clays are usually acid activated bentonite. In this study, clay is treated with acid, the surface area of the clay is greatly enhanced. During this process, the physical structure and chemical composition are altered in a controlled way to maximize specific properties. Additionally, the acid activation process makes some of the aluminum ions dissolved from the lattice and mineral salts constituents was leached, which causes the surface and pore structures to be modified. The adsorption of carotene by bleaching clays is a chemical process and that the acid sites and metallic ions, such as  $\text{Fe}^{3+}$ , are active sites for adsorption. On the other hand, clay minerals such as kaolinites (china clay and ball clay) do not have this potential to increase surface area on acid treatment [50].

Therefore, the optimum conditions for  $\beta$ -carotene adsorption on adsorbent were carried out by using acid activated bentonite at a ratio of 1: 5 by weight of clay to

palm oil, at 70°C adsorption temperature for 70 minutes. Higher temperature was found to be one of the factors affecting degradation of  $\beta$ -carotene. Besides, the addition of antioxidant before adsorption process gave higher %  $\beta$ -carotene recovery.

The suitable condition for  $\beta$ -carotene desorption was performed at room temperature by using THF as an eluting solvent. These results suggested that carotene recovery depended mainly on two factors : (i) competitive adsorption between the oil and carotene on the clay surface, and (ii) the adsorption capacity of the clay for carotene in the presence of different solvents [13].

Under the above conditions, overall  $\beta$ -carotene recovery was 10.49 mg corresponding to 73.56 % by weight of carotenoids in crude palm oil which was 14.26 mg.

The results of this study point out that acid activated bentonite was suitable for using as adsorbent in the bleaching process and useful to recovery adsorbed pigment such as  $\beta$ -carotene for further uses. However, long term studies are necessary for commercial utilization of this process.

## **5.2 Suggestions for further work**

Further work is necessary for utilizing this method to be commercial and scale up for industry.

The choice of the correct bleaching earth to use in any specific application must take into consideration the removal of all undesirable impurities in addition to color. It is in this area where fully developed bleaching earths can greatly benefit the refiner.

To overcome technical problems in bleaching earth application and to improve their performance, other materials, especially Thai clays, will continue to be evaluated for possible application as bleaching earths such as in the edible oil industries.

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## APPENDICES

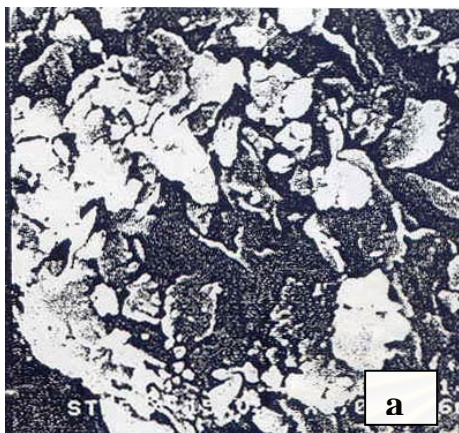
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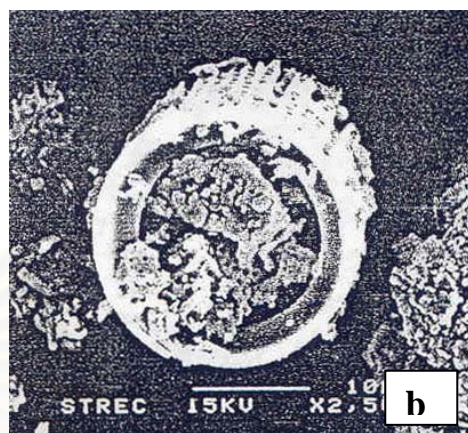
**APPENDIX A**

**The FTIR Spectrum and SEM of Clay Samples**

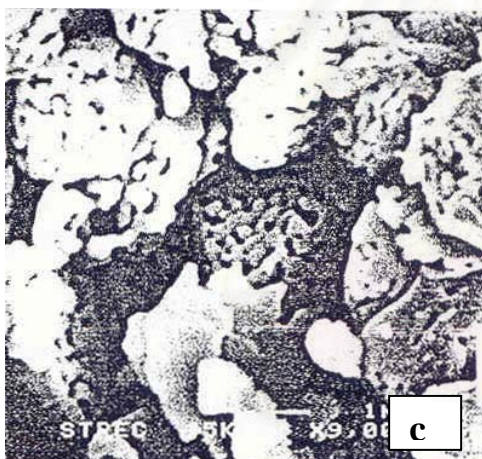
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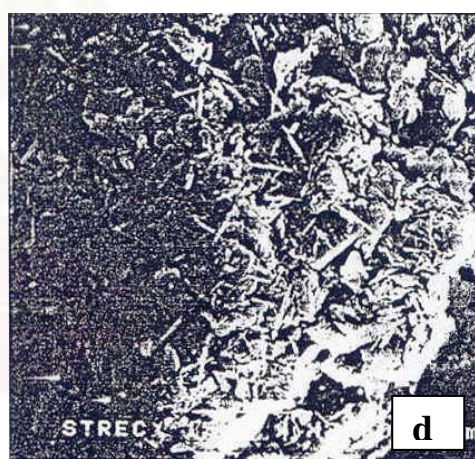
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Particle size 48.27-56.23  $\mu\text{m}$



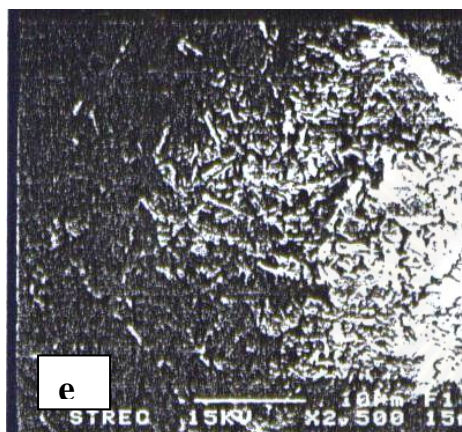
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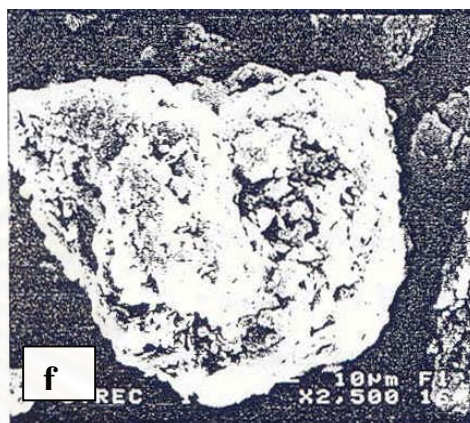
Particle size 26.20-30.53  $\mu\text{m}$

**Figure A1** Scanning electron photomicrographs of clay samples

(a) bentonite ; (b) diatomite ; (c) talcum ; (d) china L

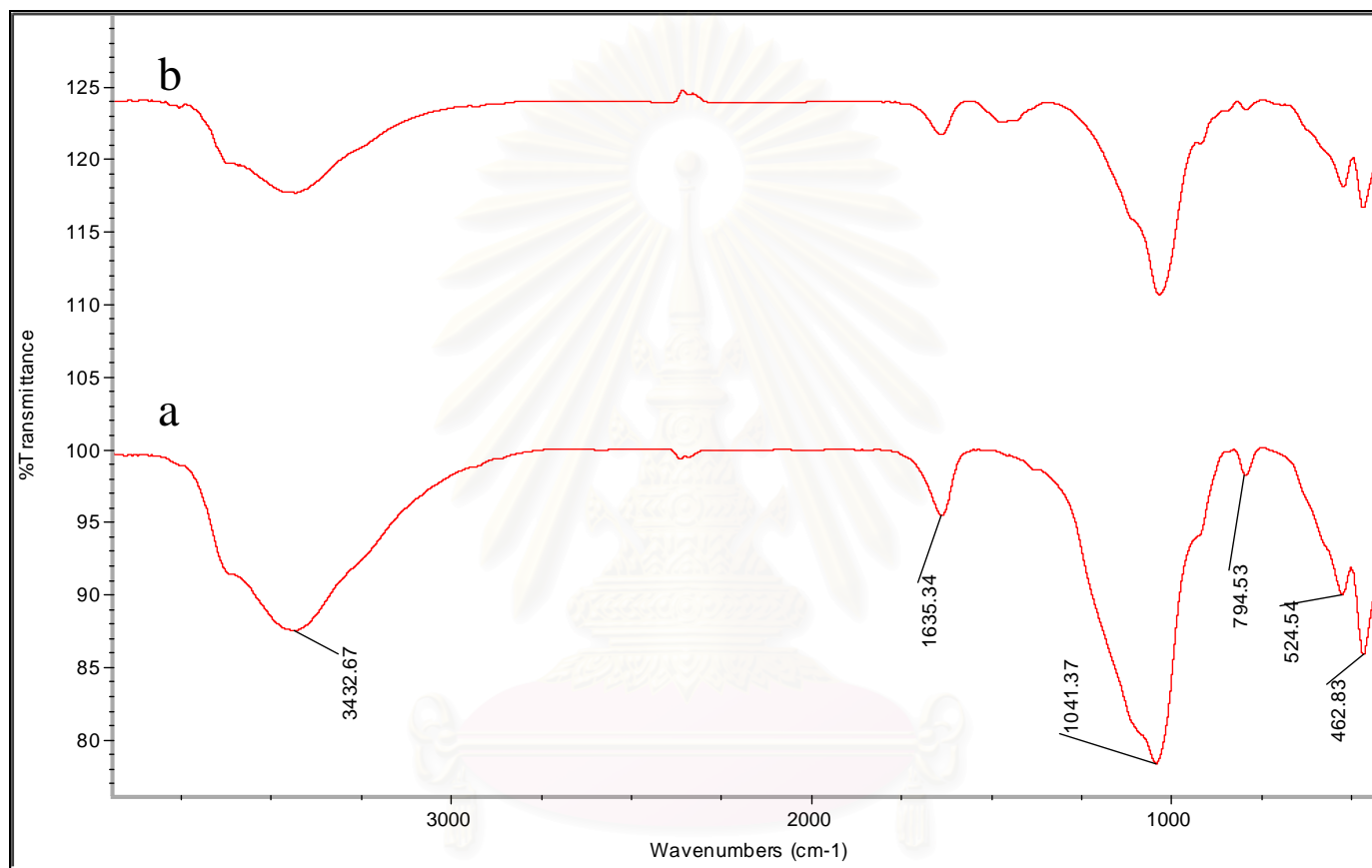


Particle size 26.20-30.53  $\mu\text{m}$

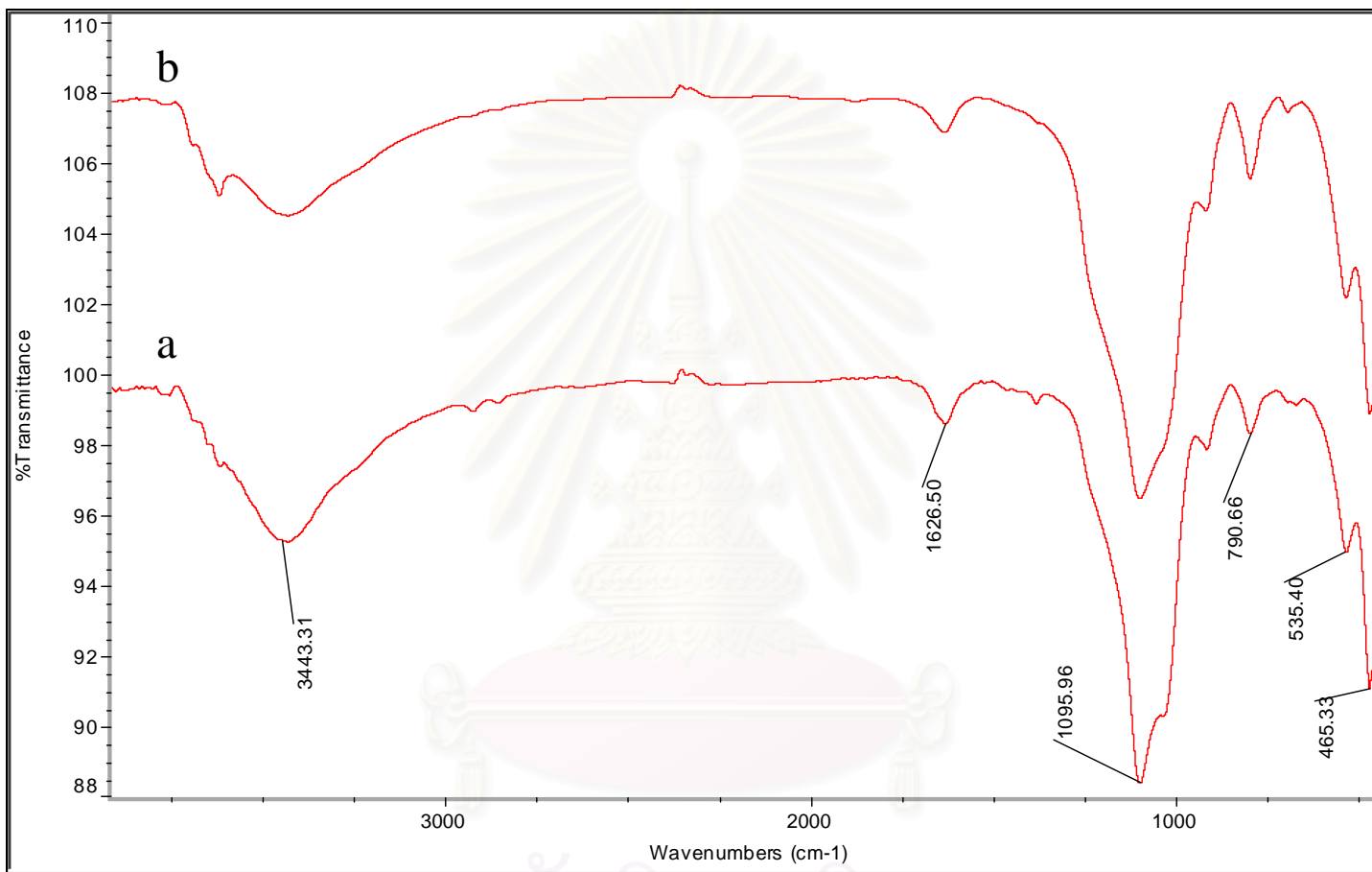


Particle size 22.49-26.20  $\mu\text{m}$

**Figure A1** (Continued) Scanning electron photomicrographs of clay samples  
(e) china L ; (f) ball clay



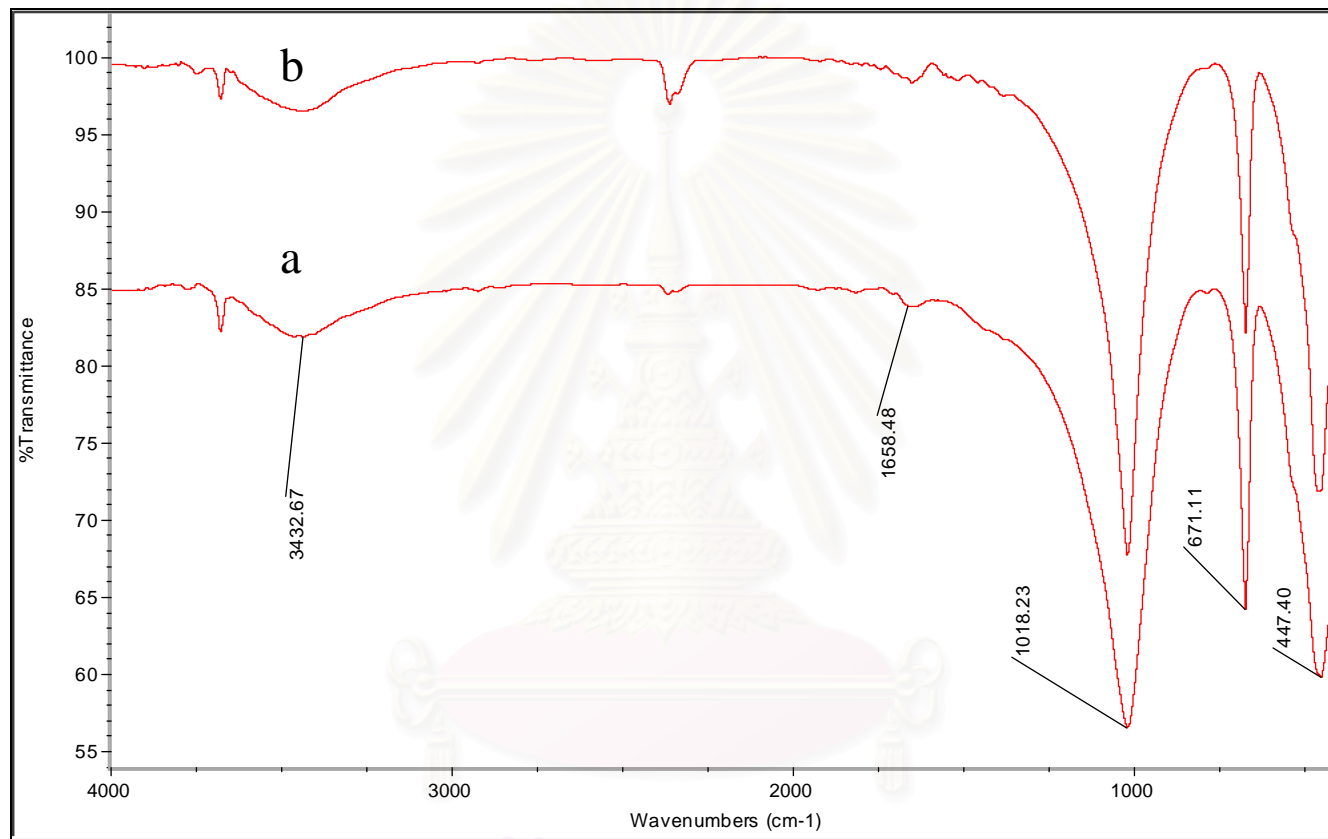
**Figure A2** FTIR spectra of bentonite (a) non-acid activated ; (b) acid activated with H<sub>2</sub>SO<sub>4</sub>



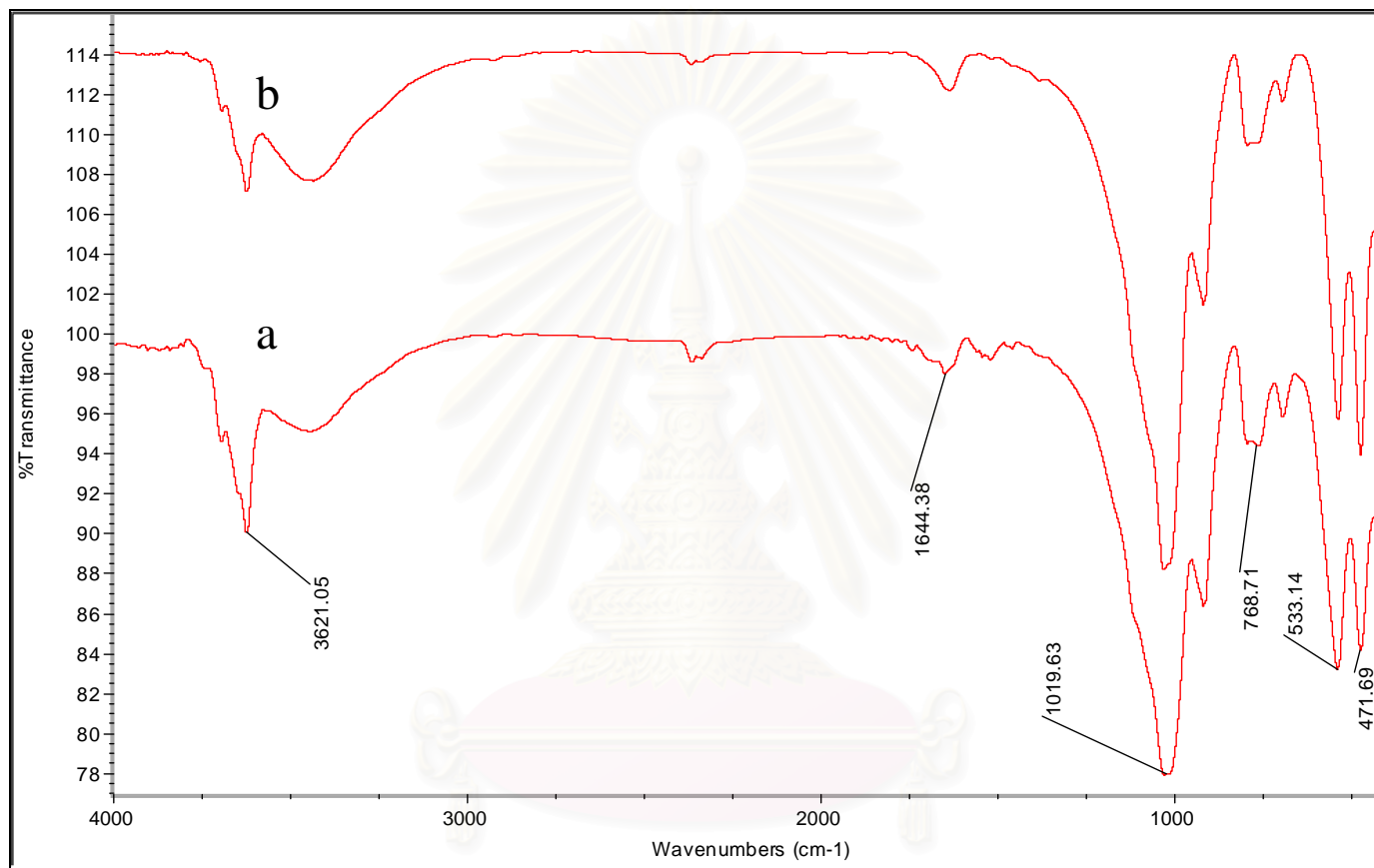
**Figure A3** FTIR spectra of diatomite (a) non-acid activated ; (b) acid activated with H<sub>2</sub>SO<sub>4</sub>

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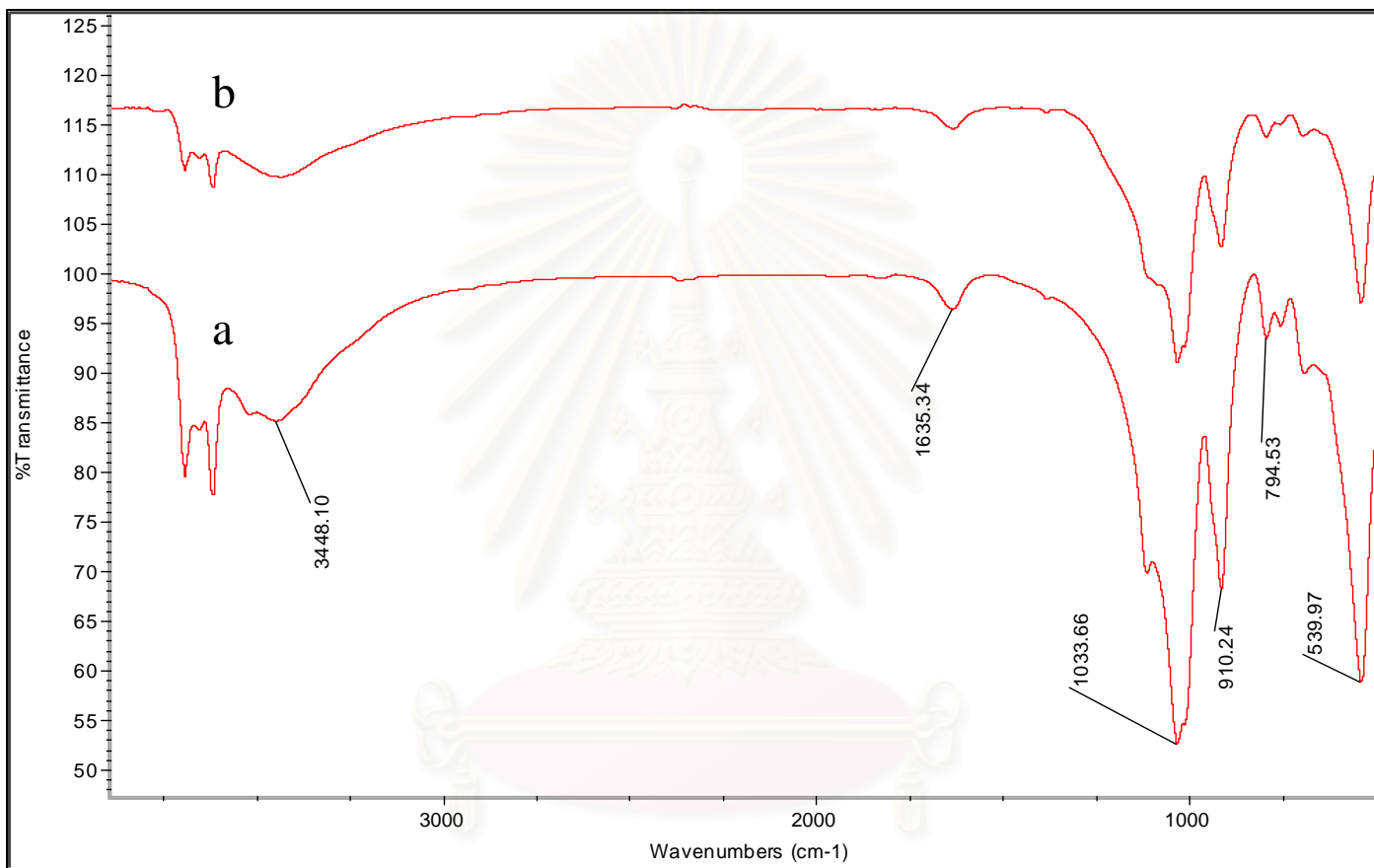


**Figure A4** FTIR spectra of talcum (a) non-acid activated ; (b) acid activated with H<sub>2</sub>SO<sub>4</sub>

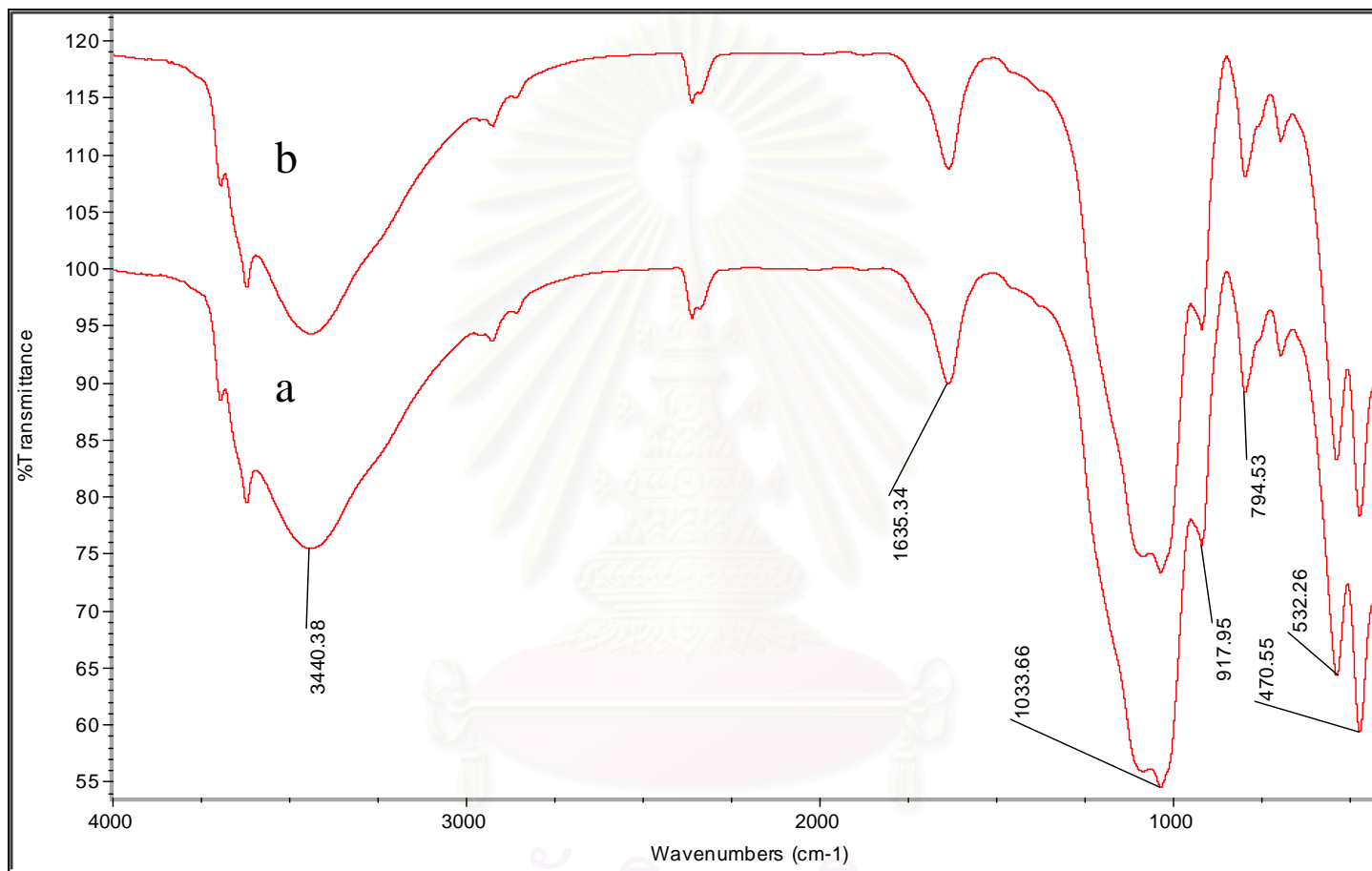


**Figure A5** FTIR spectra of chinaL (a) non-acid activated ; (b) acid activated with H<sub>2</sub>SO<sub>4</sub>

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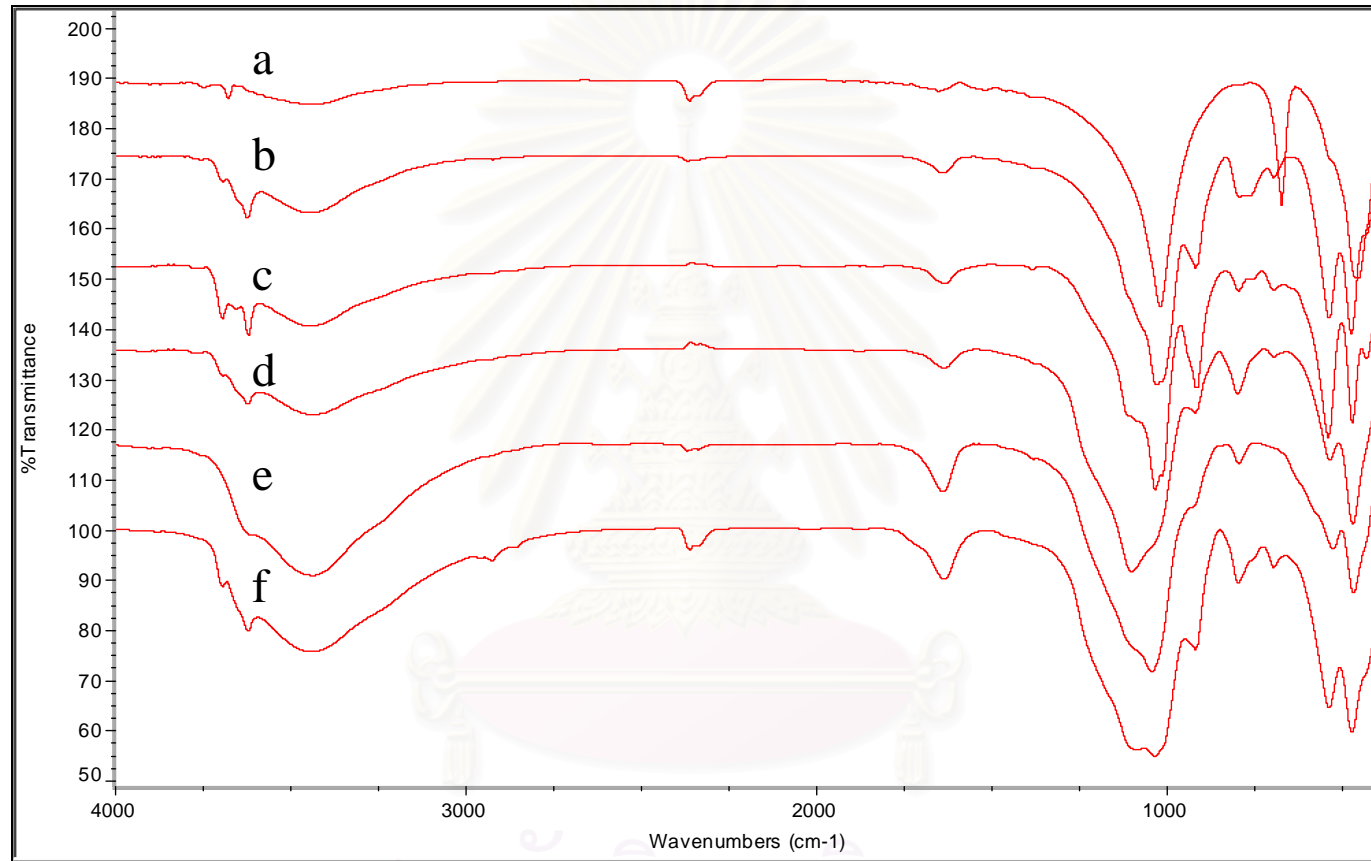


**Figure A6** FTIR spectra of china R (a) non-acid activated ; (b) acid activated with H<sub>2</sub>SO<sub>4</sub>

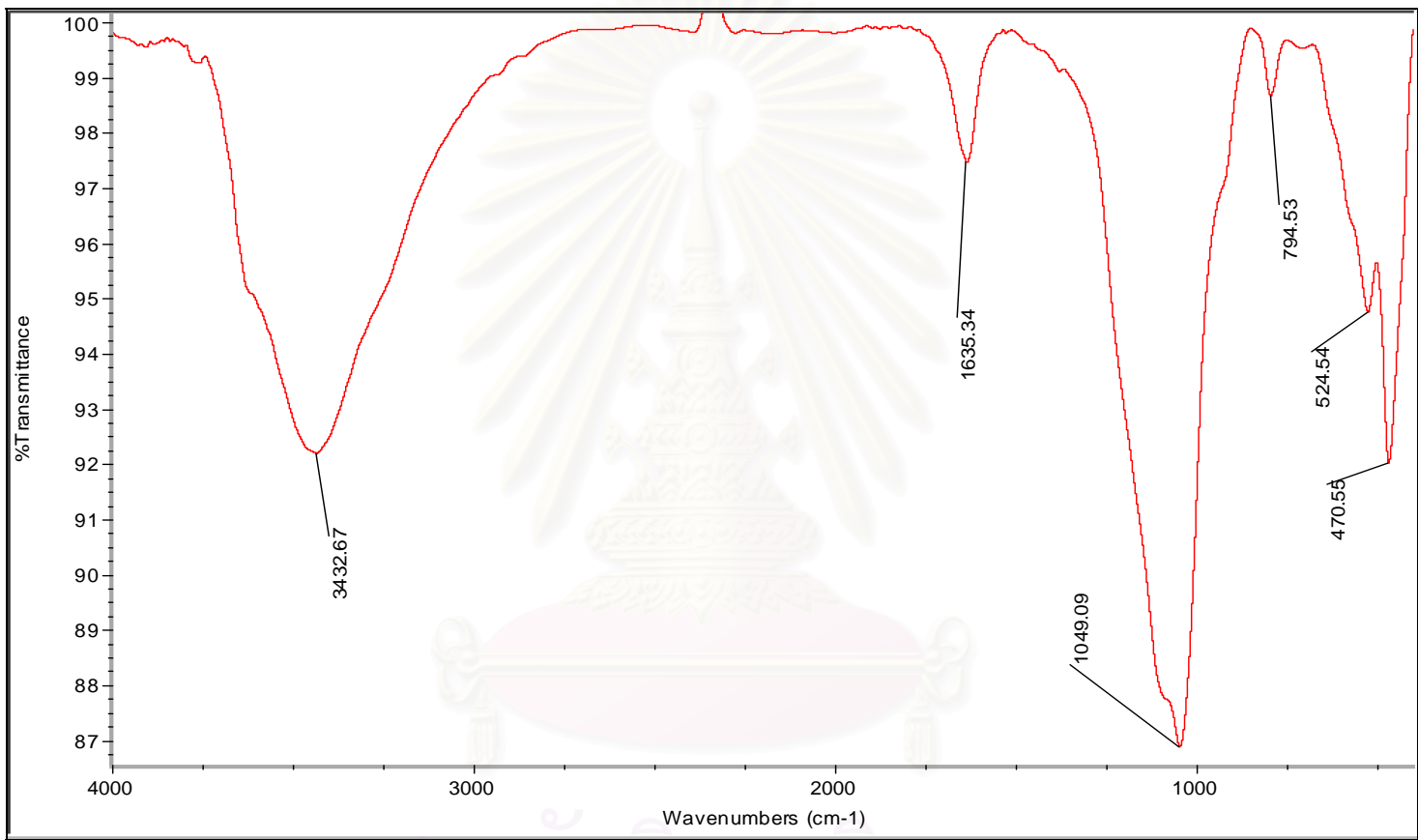


**Figure A7** FTIR spectra of ball clay (a) non-acid activated ; (b) acid activated with H<sub>2</sub>SO<sub>4</sub>

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**Figure A8** FTIR spectra of all acid activated clays (a) bentonite ;(b) talcum ;(c) diatomite;(d) ball clay;(e) china L;(f) china R



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**Figure A9** FTIR spectrum of ion-exchange bentonite

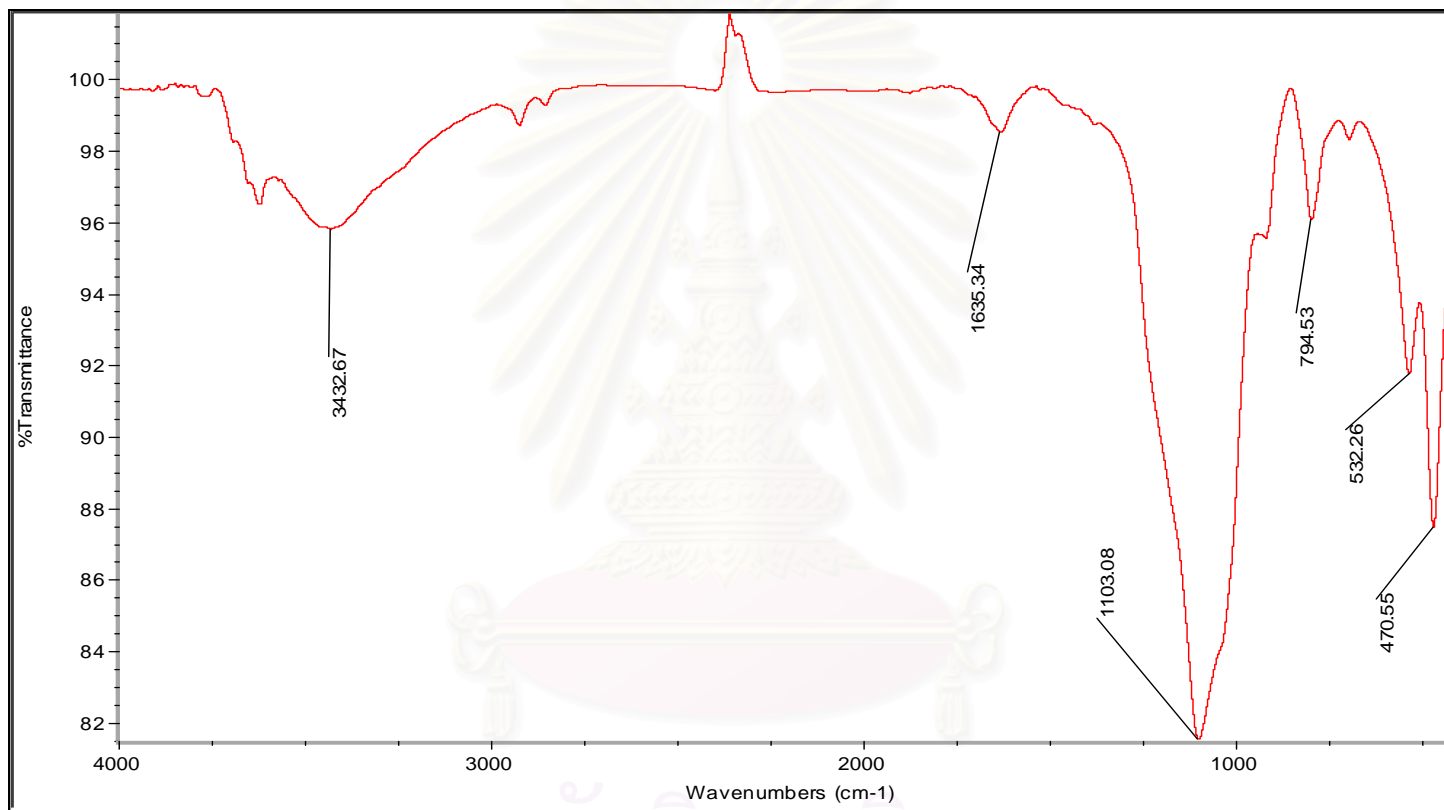
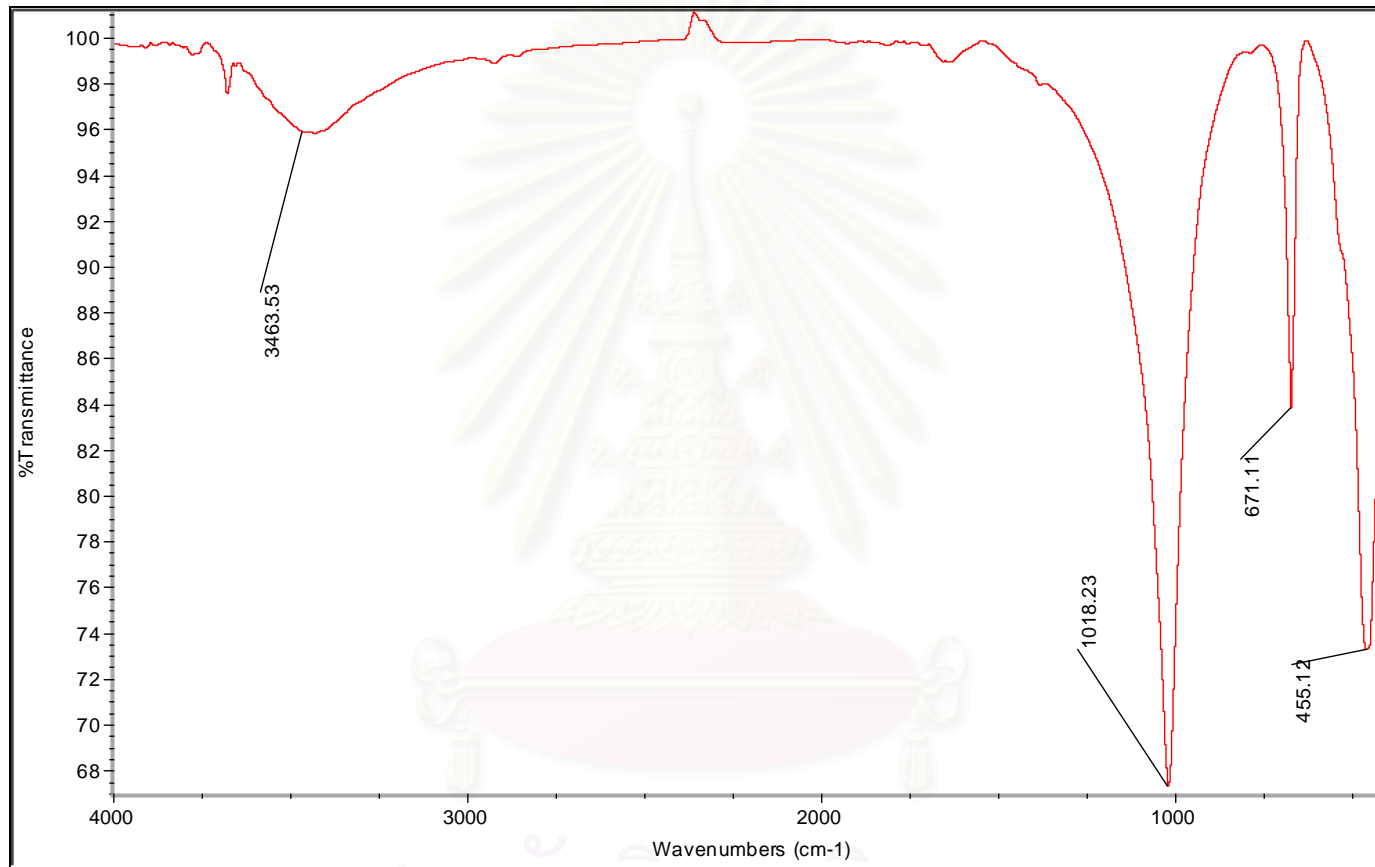


Figure A10 FTIR spectrum of ion-exchange diatomite



**Figure A11** FTIR spectrum of ion-exchange talcum



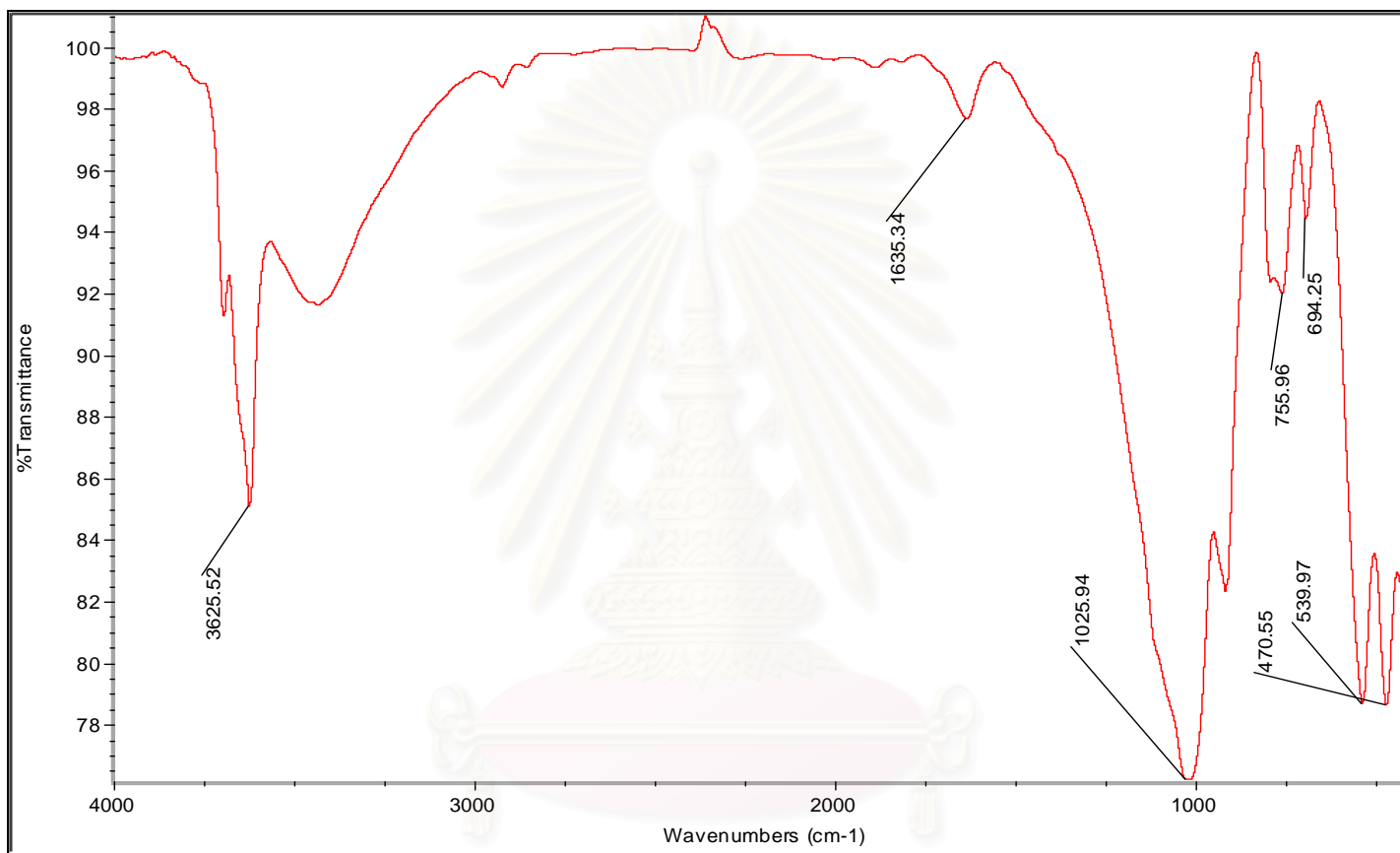


Figure A12 FTIR spectrum of ion-exchange china L

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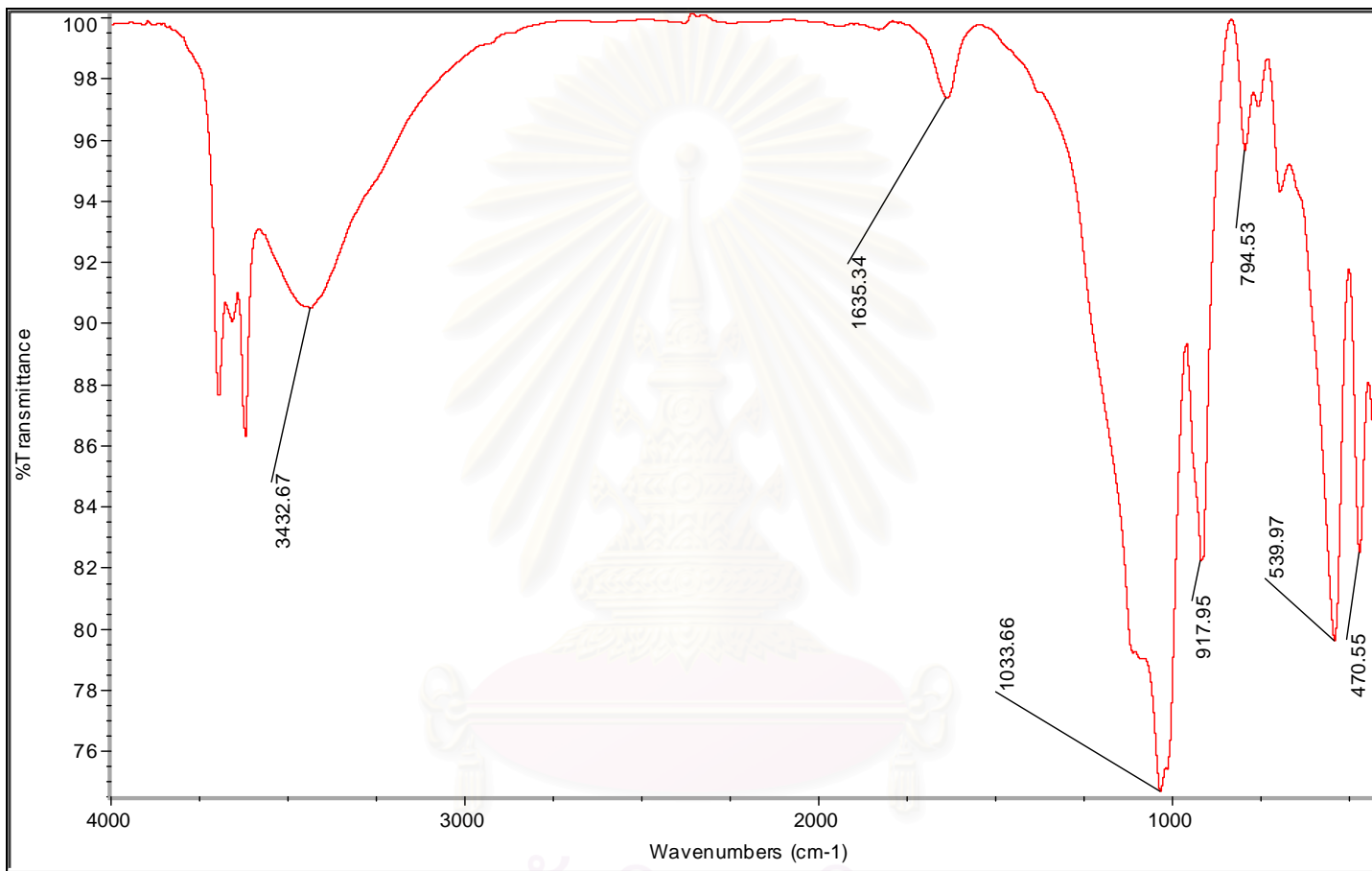


Figure A13 FTIR spectrum of ion-exchange china R

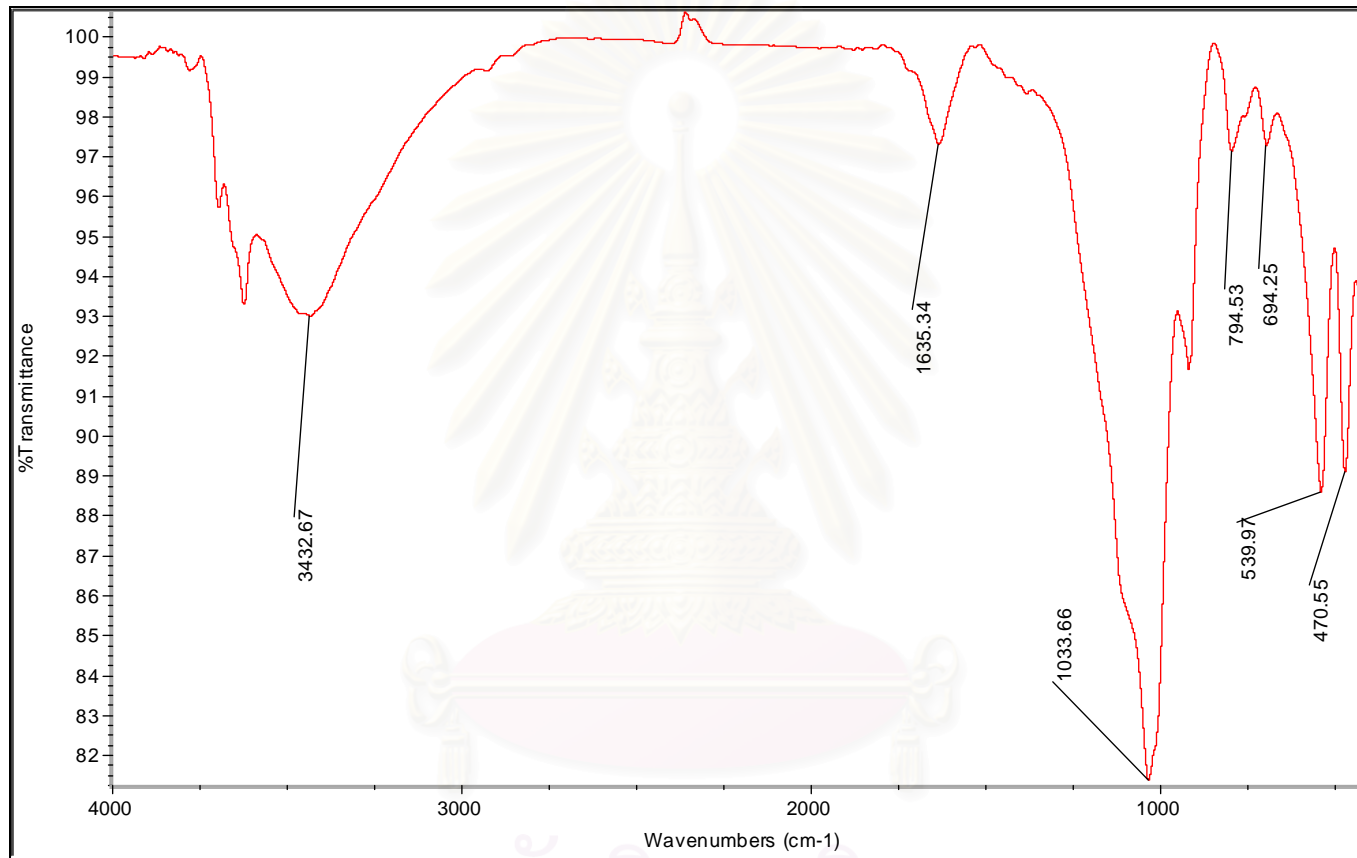
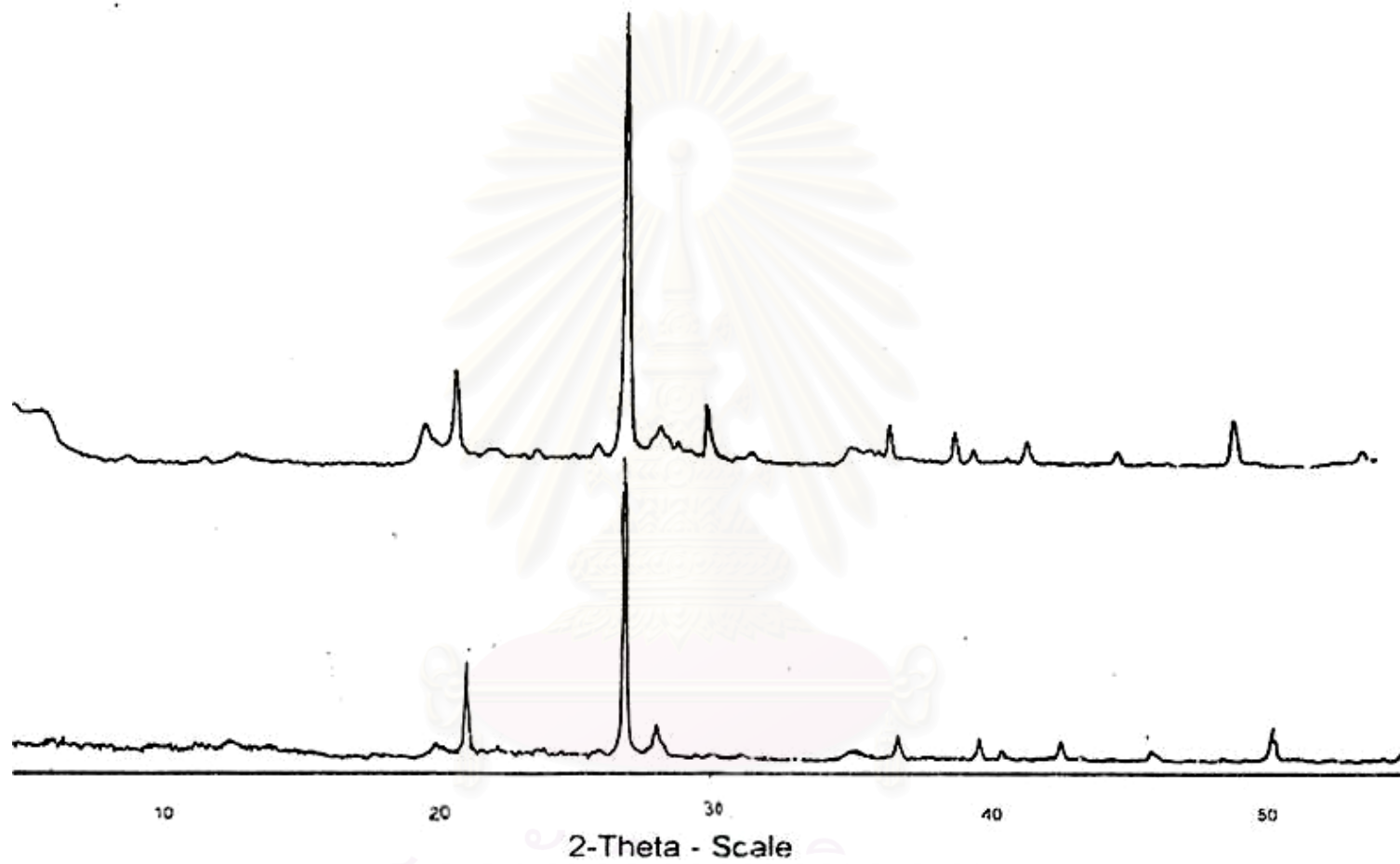
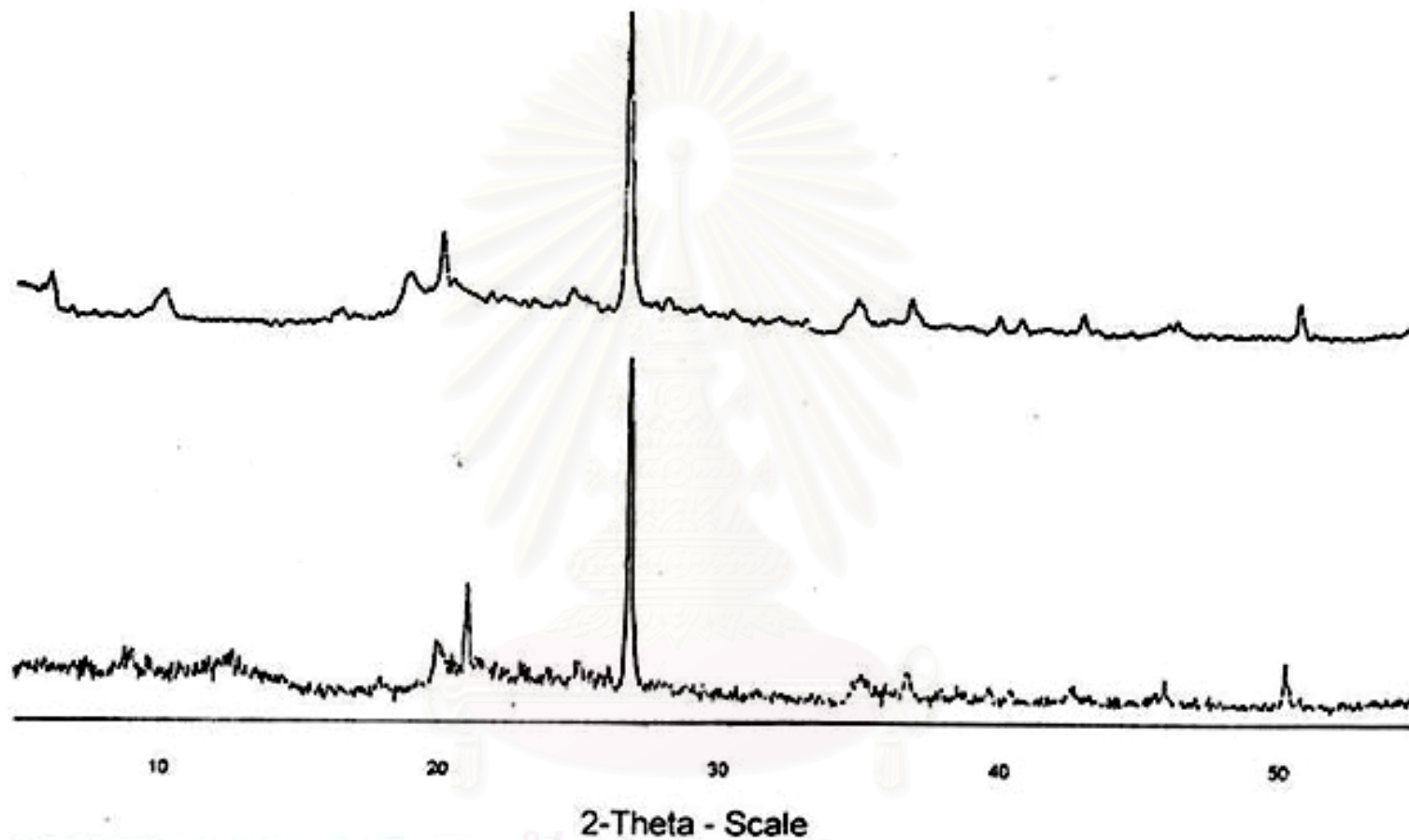


Figure A14 FTIR spectrum of ion-exchange ball clay

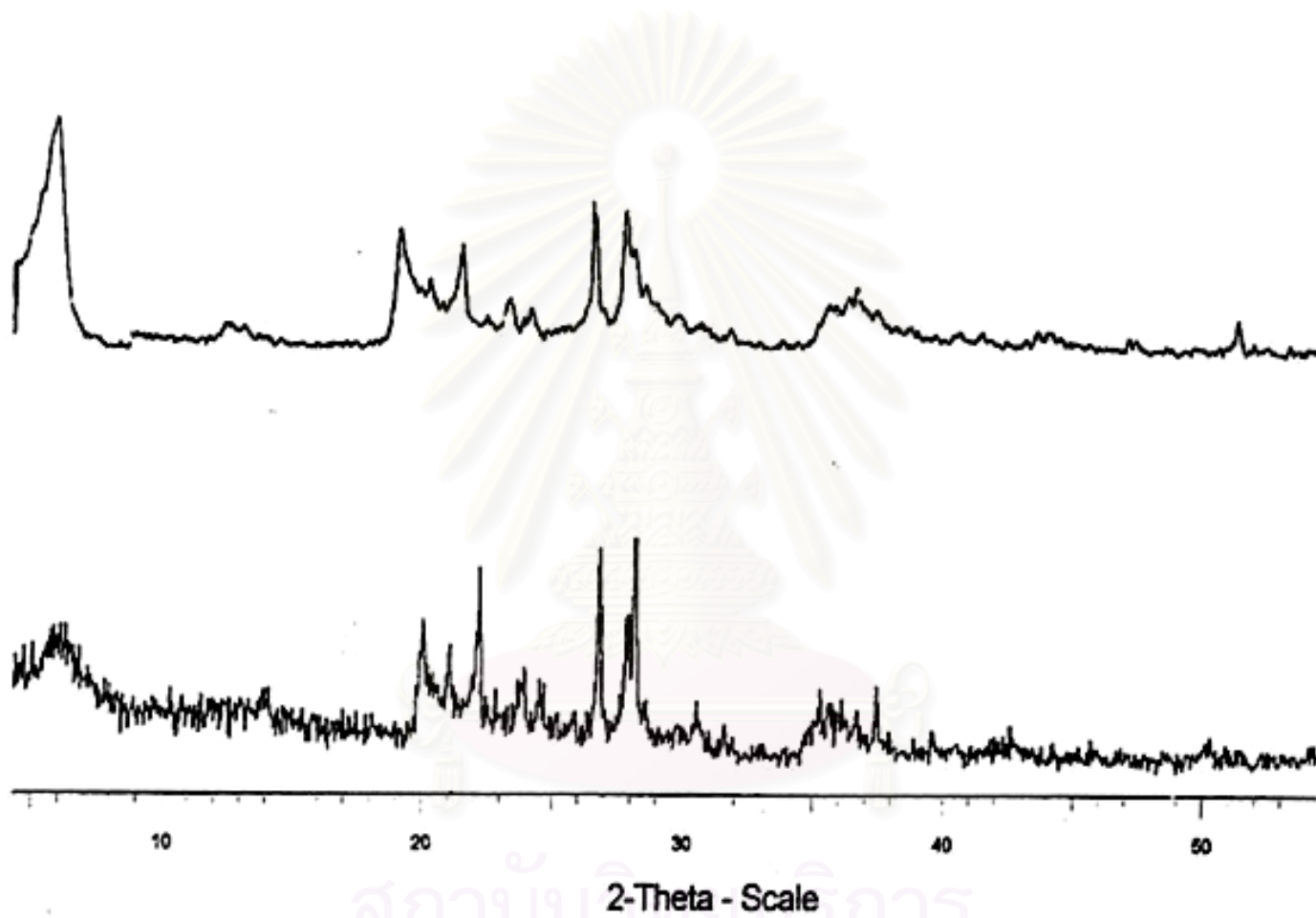
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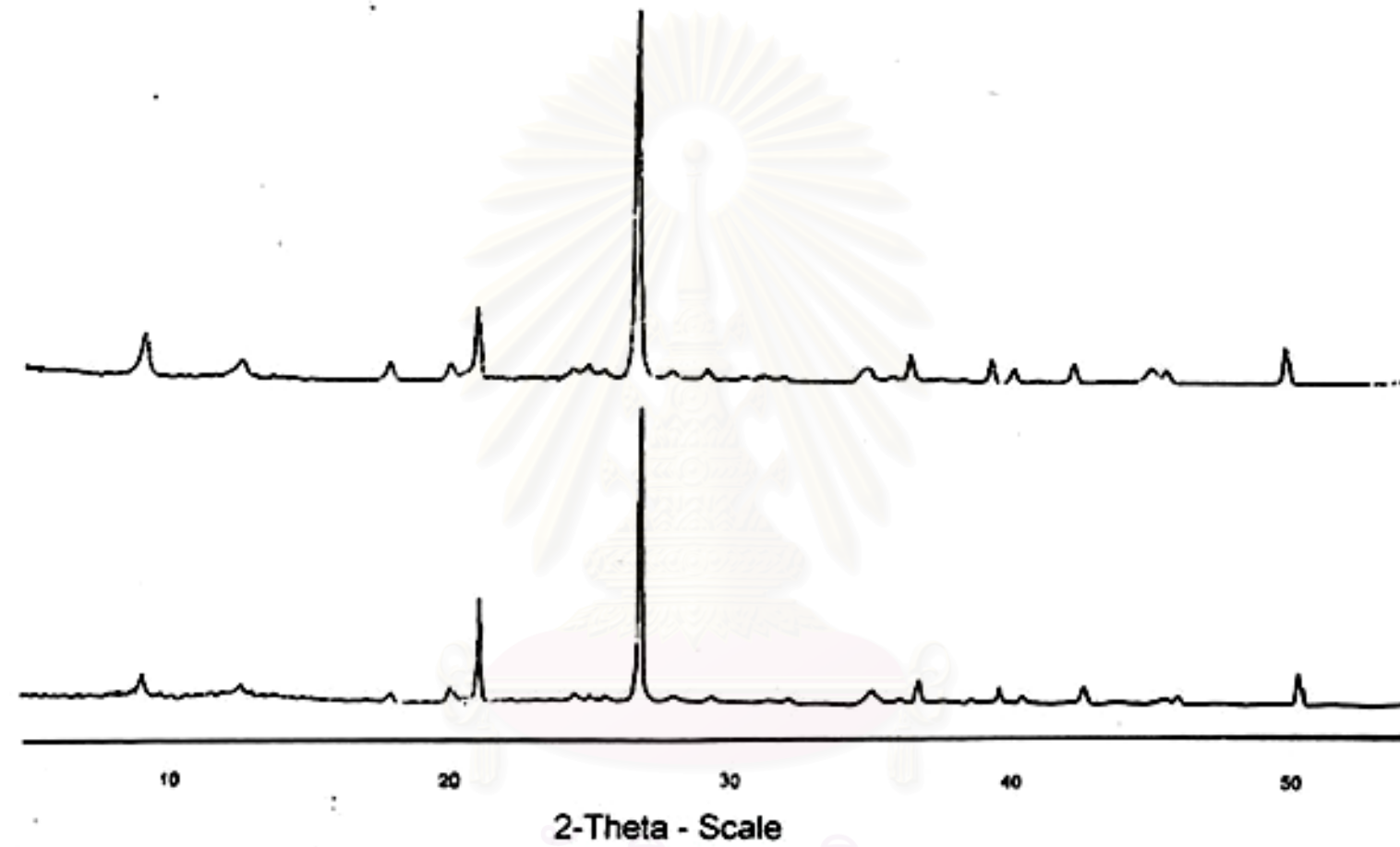
**Figure A15** XRD patterns of bentonite (a) non-acid activated; (b) acid activated with H<sub>2</sub>SO<sub>4</sub>



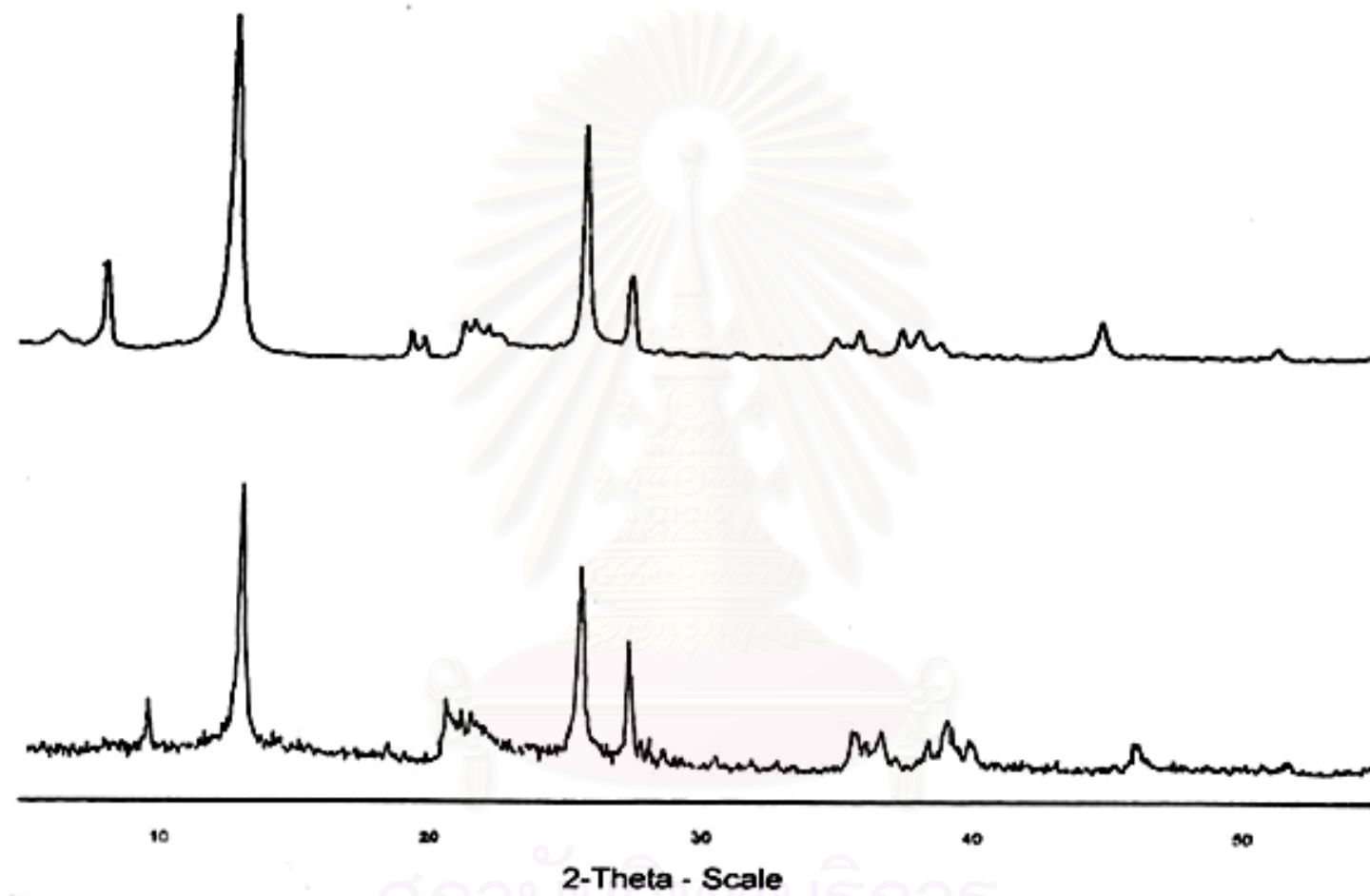
**Figure A16** XRD patterns of diatomite (a) non-acid activated; (b) acid activated with H<sub>2</sub>SO<sub>4</sub>



**Figure A17** XRD patterns of talcum (a) non-acid activated; (b) acid activated with  $H_2SO_4$

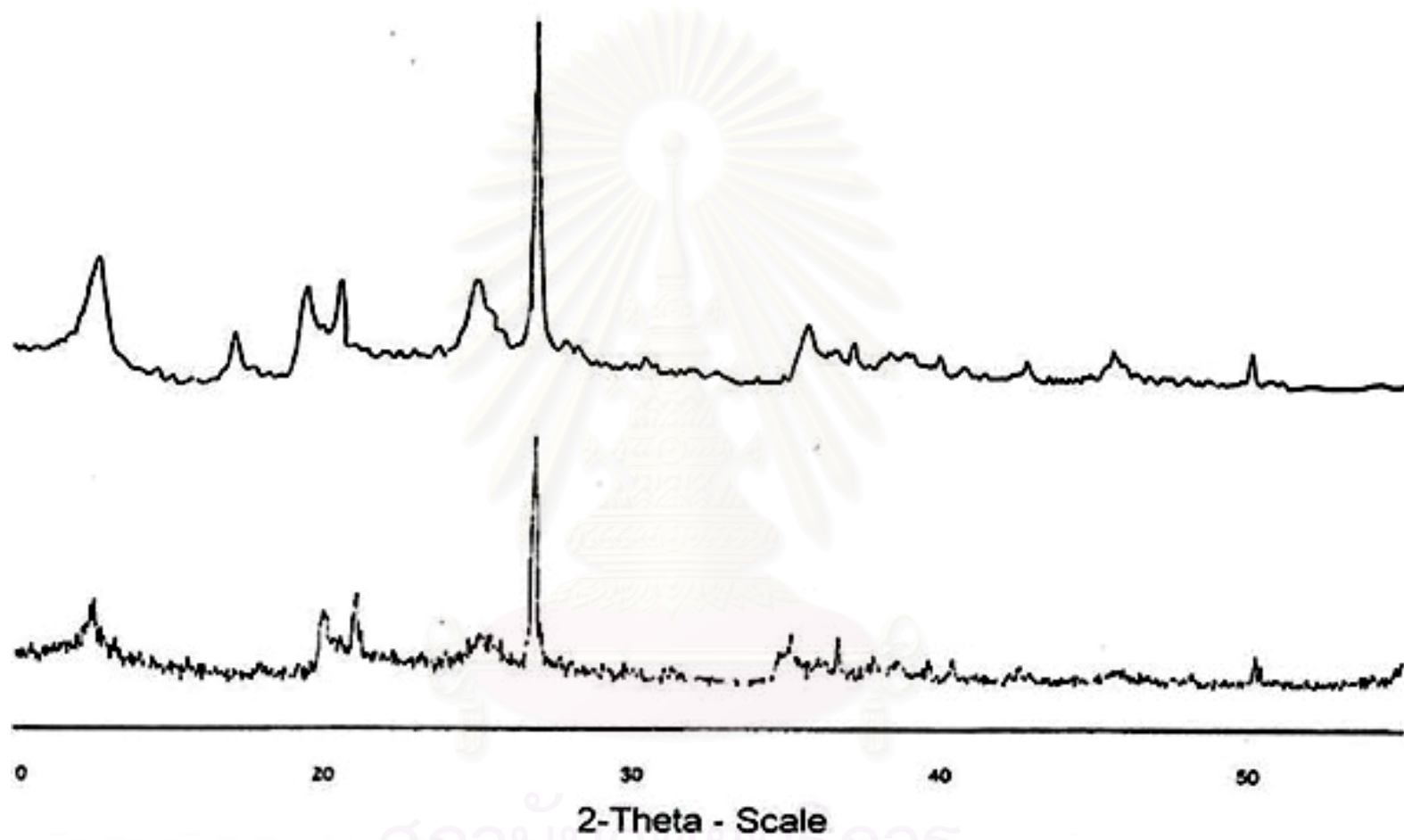


**Figure A18** XRD patterns of china L (a) non-acid activated; (b) acid activated with H<sub>2</sub>SO<sub>4</sub>



**Figure A19** XRD patterns of china R (a) non-acid activated; (b) acid activated with H<sub>2</sub>SO<sub>4</sub>





**Figure A20** XRD patterns of ball clay (a) non-acid activated; (b) acid activated with H<sub>2</sub>SO<sub>4</sub>



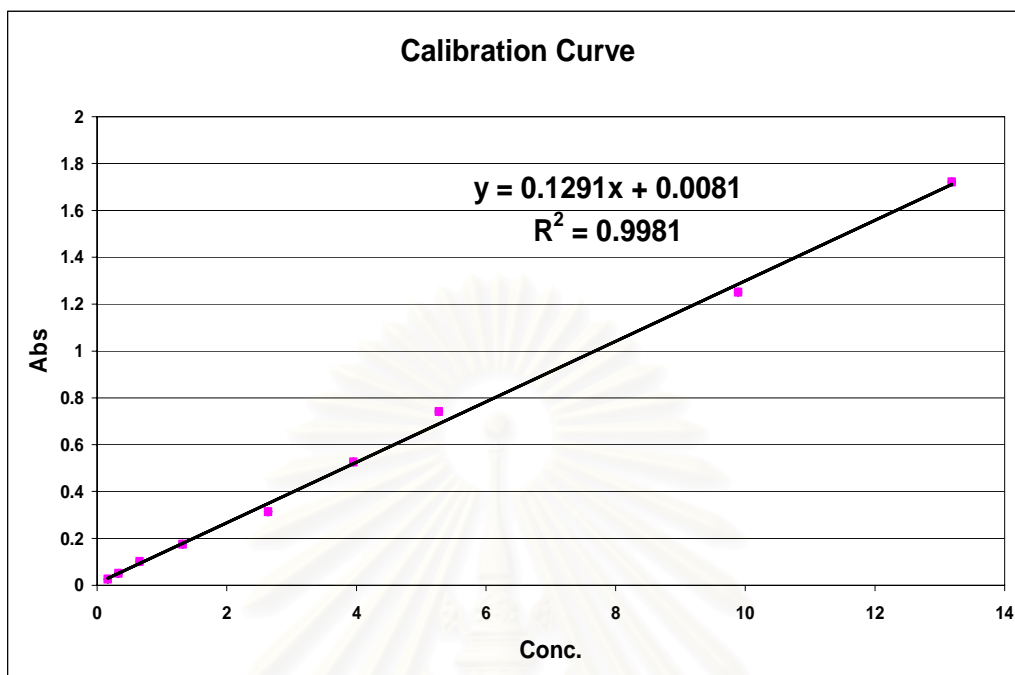
## **APPENDIX B**

**Calibration Curve of Standard  $\beta$  -carotene**

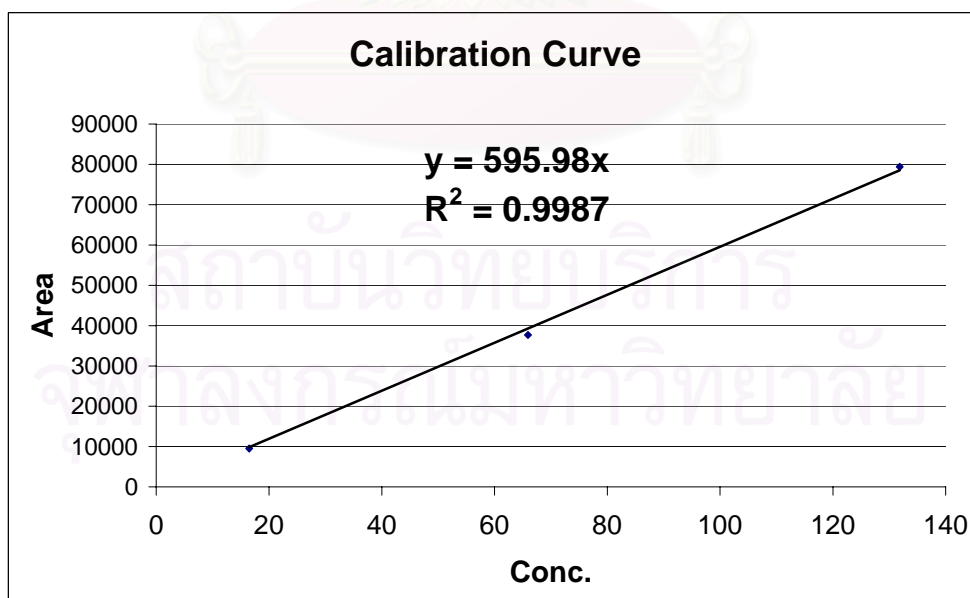
**UV-visible Spectrum of  $\beta$  -carotene and Palm oil**

**Chromatograms of  $\beta$  -carotene**

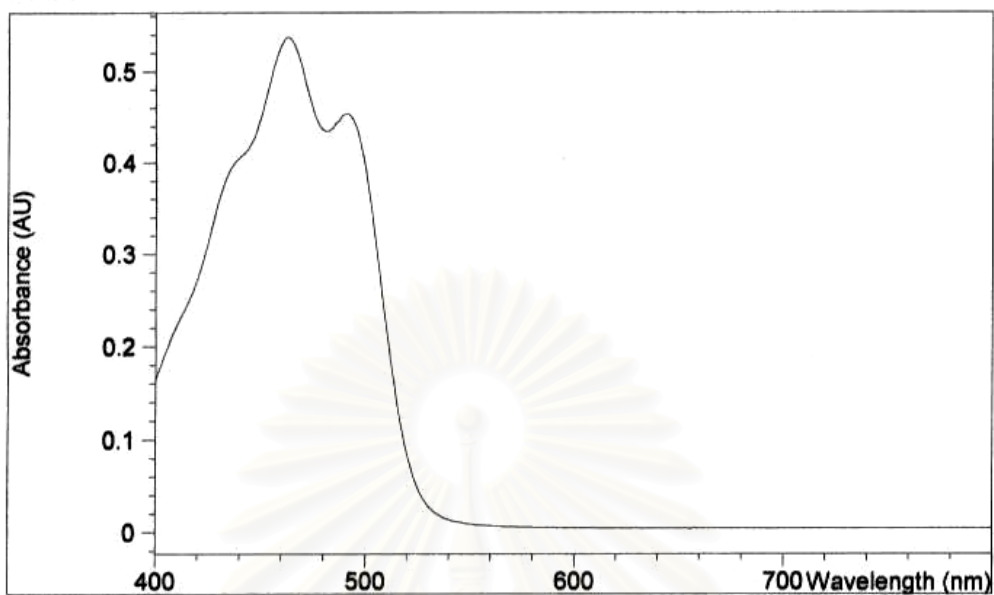
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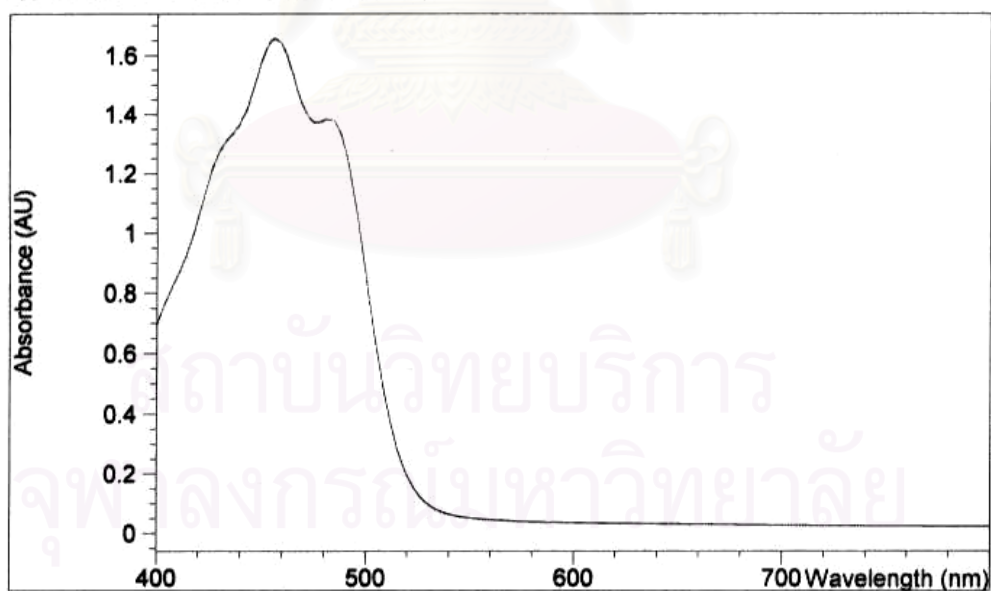
**Figure B1** Calibration Curve of standard  $\beta$ -carotene (UV-visible)



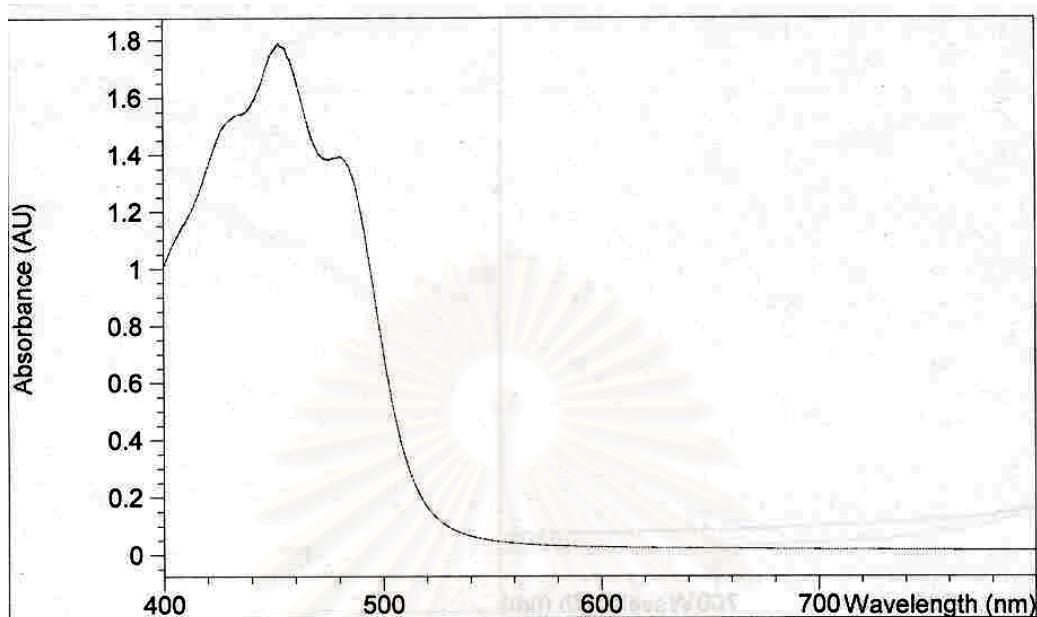
**Figure B2** Calibration Curve of standard  $\beta$ -carotene (HPLC)



**Figure B3** UV-visible spectrum of  $\beta$ -carotene

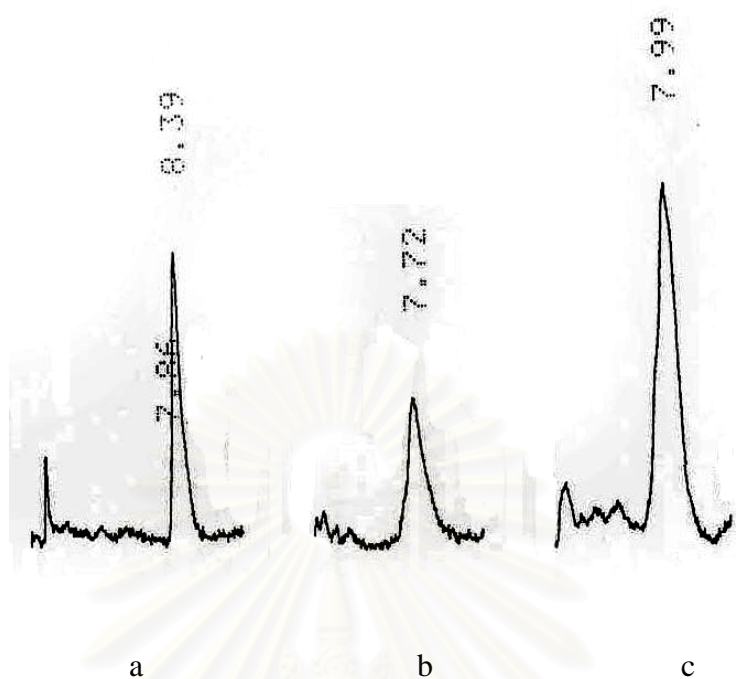


**Figure B4** UV-visible spectrum of Crude Palm Oil



**Figure B5** UV-visible spectrum of the extracted carotene

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**Figure B6** The Chromatograms of carotene using HPLC

(a) standard  $\beta$ -carotene; (b) the extracted carotene; (c) spiking technique

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**APPENDIX C**

**Experimental Data**

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**Table C1**  $\beta$ -carotene content of various processes for treatment of clay at 70 °C, 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent.

CLAY	Process	Initial palm oil content (mg/kg palm oil)	$\beta$ -carotene	
			Adsorbed content (mg/kg palm oil)	Desorbed content (mg/kg palm oil)
Talcum	Acid <sup>a</sup>	688.37	662.62	53.29
	Non-acid <sup>b</sup>	601.52	594.60	26.11
	Ion <sup>c</sup>	706.45	702.23	11.63
China L	Acid <sup>a</sup>	705.75	586.19	297.05
	Non-acid <sup>b</sup>	706.45	693.34	114.49
	Ion <sup>c</sup>	576.24	572.15	36.92
Ball clay	Acid <sup>a</sup>	702.27	697.40	197.39
	Non-acid <sup>b</sup>	605.36	601.89	193.66
	Ion <sup>c</sup>	596.49	570.80	126.67
Bentonite	Acid <sup>a</sup>	675.01	673.82	446.22
	Non-acid <sup>b</sup>	661.84	652.79	108.96
	Ion <sup>c</sup>	695.08	553.65	249.81
China R	Acid <sup>a</sup>	653.95	614.79	165.52
	Non-acid <sup>b</sup>	703.31	699.46	43.71
	Ion <sup>c</sup>	684.40	597.62	96.16
Diatomite	Acid <sup>a</sup>	703.31	693.34	198.95
	Non-acid <sup>b</sup>	701.58	679.99	80.49
	Ion <sup>c</sup>	559.94	553.61	115.56

a) Acid activation ; b) Non-acid activation ; c) Ion-exchange



**Table C2** % adsorption and % recovery of  $\beta$ -carotene of various processes for treatment of clay at 70 °C, 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent.

CLAY	Process	$\beta$ -carotene	
		%Adsorption	%Recovery
Talcum	Acid <sup>a</sup>	96.26	8.04
	Non-acid <sup>b</sup>	98.85	4.39
	Ion <sup>c</sup>	99.4	1.66
China L	Acid <sup>a</sup>	83.06	50.68
	Non-acid <sup>b</sup>	98.14	16.51
	Ion <sup>c</sup>	99.29	6.45
Ball clay	Acid <sup>a</sup>	99.31	28.3
	Non-acid <sup>b</sup>	99.43	32.18
	Ion <sup>c</sup>	95.69	22.19
Bentonite	Acid <sup>a</sup>	99.82	66.22
	Non-acid <sup>b</sup>	98.63	16.69
	Ion <sup>c</sup>	79.65	45.12
China R	Acid <sup>a</sup>	94.01	26.93
	Non-acid <sup>b</sup>	99.45	6.25
	Ion <sup>c</sup>	87.32	16.09
Diatomite	Acid <sup>a</sup>	99.58	28.69
	Non-acid <sup>b</sup>	96.92	11.84
	Ion <sup>c</sup>	98.87	20.87

a) Acid activation ; b) Non-acid activation ; c) Ion-exchange

**Table C3**  $\beta$ -carotene content of different type of acid activated adsorbents at 70 °C, 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent.

CLAY	Process	Initial palm oil content (mg/kg palm oil)	$\beta$ -carotene	
			Adsorbed content (mg/kg palm oil)	Desorbed content (mg/kg palm oil)
Talcum	Acid <sup>a</sup>	688.37	662.62	53.29
China L	Acid <sup>a</sup>	705.75	586.19	297.05
Ball clay	Acid <sup>a</sup>	702.27	697.40	197.39
Bentonite	Acid <sup>a</sup>	675.01	673.83	446.22
China R	Acid <sup>a</sup>	653.95	614.79	165.52
Diatomite	Acid <sup>a</sup>	703.31	693.34	198.95

a) Acid activation

**Table C4** % adsorption and % recovery of  $\beta$ -carotene of different type of acid activated adsorbents at 70 °C, 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent.

CLAY	Process	$\beta$ -carotene	
		%Adsorption	%Recovery
Talcum	Acid <sup>a</sup>	96.26	8.04
China L	Acid <sup>a</sup>	83.06	50.68
Ball clay	Acid <sup>a</sup>	99.31	28.3
Bentonite	Acid <sup>a</sup>	99.82	66.22
China R	Acid <sup>a</sup>	94.01	26.93
Diatomite	Acid <sup>a</sup>	99.58	28.69

a) Acid activation

**Table C5**  $\beta$ -carotene content of different adsorption times by using various acid activated clays as adsorbents at 70 °C, ratios of clay : palm oil = 1:5 and use THF as eluting solvent.

CLAY	Time (mins)	Initial palm oil content (mg/kg palm oil)	$\beta$ -carotene	
			Adsorbed content (mg/kg palm oil)	Desorbed content (mg/kg palm oil)
Talcum	50	701.24	698.94	3.25
	70	688.37	662.62	53.29
	90	696.78	649.23	47.66
China L	50	698.83	683.59	104.25
	70	705.75	586.19	297.05
	90	680.16	666.55	69.34
Ball clay	50	697.80	677.15	76.25
	70	702.27	697.40	197.39
	90	688.04	664.02	86.48
Bentonite	50	688.70	676.56	66.95
	70	675.01	673.83	446.22
	90	695.42	681.15	83.64
China R	50	683.42	632.28	77.04
	70	653.95	614.79	165.52
	90	649.19	637.98	25.61
Diatomite	50	695.76	693.25	98.37
	70	703.31	693.34	198.95
	90	691.71	685.04	119.66

**Table C6** % adsorption and % recovery of  $\beta$ -carotene of different adsorption times by using various acid activated clays as adsorbents at 70 °C, ratios of clay : palm oil = 1:5 and use THF as eluting solvent.

CLAY	Time (mins)	$\beta$ -carotene	
		%Adsorption	%Recovery
Talcum	50	99.67	0.46
	70	96.26	8.04
	90	93.18	7.34
China L	50	97.82	15.25
	70	83.06	50.68
	90	97.99	10.4
Ball clay	50	97.04	11.26
	70	99.31	28.3
	90	96.51	13.02
Bentonite	50	98.24	9.9
	70	99.82	66.22
	90	97.95	12.28
China R	50	92.52	12.18
	70	94.01	26.93
	90	98.27	4.01
Diatomite	50	99.64	14.19
	70	99.58	28.69
	90	99.04	17.47

**Table C7**  $\beta$ -carotene content of different ratios of clay to palm oil by using various acid activated clays as adsorbents at 70 °C, 70 mins and use THF as eluting solvent.

CLAY	Ratio clay/CPO	Initial palm oil content (mg/kg palm oil)	$\beta$ -carotene	
			Adsorbed content (mg/kg palm oil)	Desorbed content (mg/kg palm oil)
Talcum	1:5	688.37	662.62	53.29
	1:7	509.33	507.78	17.64
	1:9	661.85	657.46	10.28
China L	1:5	705.75	586.19	297.05
	1:7	667.42	656.07	35.85
	1:9	521.25	513.60	15.80
Ball clay	1:5	702.27	697.40	197.39
	1:7	663.69	656.67	107.76
	1:9	488.22	483.89	58.86
Bentonite	1:5	675.01	673.83	446.22
	1:7	665.24	659.59	278.68
	1:9	515.97	499.00	36.76
China R	1:5	653.95	614.79	165.52
	1:7	671.83	652.65	146.63
	1:9	520.49	490.19	92.17
Diatomite	1:5	703.31	693.34	198.95
	1:7	669.30	662.58	65.73
	1:9	515.22	508.10	20.54

**Table C8** % adsorption and % recovery of  $\beta$ -carotene of different ratios of clay to palm oil by using various acid activated clays as adsorbents at 70 °C, 70 mins and use THF as eluting solvent.

CLAY	Ratio clay/CPO	$\beta$ -carotene	
		%Adsorption	%Recovery
Talcum	1:5	96.26	8.04
	1:7	99.7	3.47
	1:9	99.33	1.56
China L	1:5	83.06	50.68
	1:7	98.3	5.47
	1:9	98.53	3.08
Ball clay	1:5	99.31	28.3
	1:7	98.94	16.41
	1:9	99.11	12.16
Bentonite	1:5	99.82	66.22
	1:7	99.15	42.25
	1:9	96.71	7.37
China R	1:5	94.01	26.93
	1:7	97.15	22.47
	1:9	94.18	18.8
Diatomite	1:5	99.58	28.69
	1:7	98.99	9.92
	1:9	98.62	4.04

**Table C9**  $\beta$ -carotene content of different adsorption temperature by using acid activated bentonite as adsorbent for 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent.

CLAY	Temperature (°C)	Initial palm oil content (mg/kg palm oil)	$\beta$ -carotene	
			Adsorbed content (mg/kg palm oil)	Desorbed content (mg/kg palm oil)
Bentonite	30	689.70	635.46	175.26
	70	675.01	673.83	446.22
	90	850.60	828.80	264.81
	110	856.74	761.06	226.97

**Table C10** % adsorption and % recovery of  $\beta$ -carotene of different adsorption temperature by using acid activated bentonite as adsorbent for 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent.

CLAY	Temperature(°C)	$\beta$ -carotene	
		%Adsorption	%Recovery
Bentonite	30	92.14	27.58
	70	99.82	66.22
	90	97.44	31.95
	110	88.83	29.82

**Table C11**  $\beta$ -carotene content of different type of eluting solvents by using various acid activated clays as adsorbents at 70 °C , 70 mins and ratios of clay : palm oil = 1:5.

CLAY	Eluting solvent	Initial palm oil content (mg/kg palm oil)	$\beta$ -carotene	
			Adsorbed content (mg/kg palm oil)	Desorbed content (mg/kg palm oil)
Talcum	Hexane	693.73	672.07	23.56
	Toluene	704.01	693.83	23.92
	Ether	676.93	672.13	4.40
	THF	688.37	662.62	53.29
China L	Hexane	680.48	667.45	16.19
	Toluene	695.08	676.90	41.94
	Ether	697.12	671.01	32.71
	THF	705.75	586.19	297.05
Ball clay	Hexane	694.07	661.73	28.53
	Toluene	681.79	676.47	24.47
	Ether	680.81	622.62	77.47
	THF	702.27	697.40	197.39
Bentonite	Hexane	688.70	675.84	9.46
	Toluene	700.55	693.30	15.68
	Ether	694.07	688.40	19.61
	THF	675.01	673.83	446.22
China R	Hexane	692.38	682.95	10.21
	Toluene	684.73	677.82	29.96
	Ether	691.71	668.20	14.45
	THF	653.95	614.79	165.52
Diatomite	Hexane	694.40	690.28	4.27
	Toluene	684.08	673.30	12.37
	Ether	695.08	624.63	75.37
	THF	703.31	693.34	198.95



**Table C12** % adsorption and % recovery of  $\beta$ -carotene of different type of eluting solvents by using various acid activated clays as adsorbents at 70°C , 70 mins and ratios of clay : palm oil = 1:5.

CLAY	Eluting solvent	$\beta$ -carotene	
		%Adsorption	%Recovery
Talcum	Hexane	96.88	3.51
	Toluene	98.56	3.45
	Ether	99.29	0.65
	THF	96.26	8.04
China L	Hexane	98.09	2.43
	Toluene	97.38	6.2
	Ether	96.25	4.87
	THF	83.06	50.68
Ball clay	Hexane	95.34	4.31
	Toluene	99.22	3.62
	Ether	91.45	12.44
	THF	99.31	28.3
Bentonite	Hexane	98.13	1.4
	Toluene	98.97	2.26
	Ether	99.18	2.85
	THF	99.82	66.22
China R	Hexane	98.64	1.5
	Toluene	98.99	4.42
	Ether	96.6	2.16
	THF	94.01	26.93
Diatomite	Hexane	99.41	0.62
	Toluene	98.43	1.84
	Ether	89.86	12.07
	THF	99.58	28.69

**Table C13**  $\beta$ -carotene content of acid activated bentonite before and after treated with antioxidant at 70 °C, 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent.

CLAY	Process	Initial palm oil content (mg/kg palm oil)	$\beta$ -carotene	
			Adsorbed content (mg/kg palm oil)	Desorbed content (mg/kg palm oil)
Bentonite	non treated with BHT	675.01	673.83	446.22
Bentonite	treated with BHT	675.01	673.54	496.71

**Table C14** % adsorption and % recovery of  $\beta$ -carotene of acid activated bentonite before and after treated with antioxidant at 70 °C, 70 mins, ratios of clay : palm oil = 1:5 and use THF as eluting solvent.

CLAY	Process	$\beta$ -carotene	
		%Adsorption	%Recovery
Bentonite	non treated with BHT	99.82	66.22
Bentonite	treated with BHT	99.78	73.73



**Figure C15** an experimental photo of the oil after adsorption of carotenoids on the following clays (1) bentonite; (2) talcum; (3) china L; (4) china R; (5) diatomite; (6) ball clay and (7) the initial palm oil

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## Calculation

### 1. $\beta$ -carotene content

Calibration curve (UV-visible),  $Abs. = 0.1291X + 0.0081$  ( $X = \text{conc.}$ )

Example The experiment which use acid activated bentonite as adsorbent,  $70^{\circ}\text{C}$  adsorption temperature, 70 minutes adsorption time,

1:5 ratio of clay to palm oil, THF as eluting solvent,  $Abs. = 0.0339$

$$Abs = 0.1291X + 0.0081$$

$$0.0339 = 0.1291X + 0.0081$$

$$X = 0.1998 \text{ ppm} = 0.1998 \text{ mg/L}$$

$$(1 \text{ ppm} = 1 \text{ mg/L})$$

In solution 1000 ml has  $\beta$ -carotene  $0.1998 \times 10^{-3} \text{ g}$

In solution 25 ml has  $\beta$ -carotene  $4.9919 \times 10^{-6} \text{ g}$

$$\begin{aligned} \text{Due to dilution 5 times, so } \beta\text{-carotene} &= 4.9919 \times 10^{-6} \times 5 \\ &= 2.4959 \times 10^{-5} \text{ g} \end{aligned}$$

In CPO 21.12 g has  $\beta$ -carotene  $2.4959 \times 10^{-5} \text{ g}$

$$\begin{aligned} \text{So, in CPO 1 g has } \beta\text{-carotene} &= 1.1818 \times 10^{-6} \text{ g} \\ &= 1.1818 \times 10^{-6} \text{ g/g palm oil} \\ &= 1.1818 \text{ mg/kg palm oil} \end{aligned}$$

Therefore,  $\beta$ -carotene in the oil after adsorption (not adsorption)

$$= 1.1818 \text{ mg/kg palm oil} \longrightarrow \boxed{\text{B}}$$

Therefore,

Initial palm oil has  $\beta$ -carotene = 675.0063 mg/kg palm oil

$$\longrightarrow \boxed{\text{A}}$$

The extracted  $\beta$ -carotene from adsorbent

$$= 446.22 \text{ mg/kg palm oil}$$

$$\longrightarrow \boxed{\text{D}}$$

So, the adsorbed  $\beta$ -carotene on adsorbent

$$= 675.0063 - 1.1818 = 673.8245 \text{ mg/kg palm oil}$$

$$\longrightarrow \boxed{\text{C}}$$

**2. %  $\beta$ -carotene adsorption and %  $\beta$ -carotene recovery**

$$\begin{aligned}\% \beta\text{-carotene adsorption} &= \frac{\boxed{\text{C}}}{\boxed{\text{A}}} \times 100 \\ &= \frac{673.8245 \times 100}{675.0063} = 99.82\%\end{aligned}$$

$$\begin{aligned}\% \beta\text{-carotene recovery} &= \frac{\boxed{\text{D}}}{\boxed{\text{C}}} \times 100 \\ &= \frac{446.22 \times 100}{673.8245} = 66.22\%\end{aligned}$$

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## VITA

Miss Winunta Poopunpanich was born on December 30, 1978 in Bangkok, Thailand. She received her Bachelor's Degree of Science in Chemistry, Faculty of Science, Chulalongkorn University in 2000. She attended the Master's Degree Program of Petrochemical and Polymer Science, Faculty of Science, Chulalongkorn University in 2000 and completed her study in 2004.



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