การแยกแคโรทีนอยด์จากน้ำมันปาล์มโดยใช้เคลย์ที่ปรับสภาพด้วยเงิน

นางสาวพนินทร เดชฟุ้ง

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรมหาบัณฑิต
สาขาวิชาปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์
คณะวิทยาศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย
ปีการศึกษา 2548

ISBN 974-53-2993-2

SEPARATION OF CAROTENOIDS FROM PALM OIL USING Ag-TREATED CLAYS

Miss Panintorn Dechfoong

A Thesis Submitted in Partial Fulfillment of the Requirements
for the Degree of Master of Science Program in Petrochemistry and Polymer Science
Faculty of Science
Chulalongkorn University
Academic Year 2005

ISBN 974-53-2993-2

Thesis Title SEPARATION OF CAROTENOIDS FROM PALM OIL USING Ag-TREATED CLAYS
By Miss Panintorn Dechfoong
Field of Study Petrochemistry and Polymer Science
Thesis Advisor Associate Professor Amorn Petsom, Ph.D.
Thesis Co-advisor Assistant Professor Polkit Sangvanich, Ph.D.

Accepted by the Faculty of Science, Chulalongkorn University in Partial Fulfillment of the Requirements for the Master's Degree

.... Dean of the Faculty of Science
(Professor Piamsak Menasveta, Ph.D.)

THESIS COMMITTEE

.... Chairman
(Professor Pattarapan Prasassarakich, Ph.D.)

.... Thesis Advisor
(Associate Professor Amorn Petsom, Ph.D.)

..................Polkit Sangvanich.................. Thesis Co-advisor
(Assistant Professor Polkit Sangvanich, Ph.D.)

.... Member
(Professor Sophon Roengsumran, Ph.D.)

..........Warinthorn Chavasiri.......... Member
(Assistant Professor Warinthorn Chavasiri, Ph.D.)

พนินทร เดชฟุ้ง : การแยกแคโรทีนอยด์จากน้ำมันปาล์มโดยใช้เคลย์ที่ปรับสภาพด้วยเงิน. (SEPARATION OF CAROTENOIDS FROM PALM OIL USING Ag-TREATED CLAYS) อ. ที่ปรึกษา: รศ. ดร. อมร เพชรสม, อ. ที่ปรึกษาร่วม: ผศ. ดร. พลกฤษณ์ แสงวณิช 127 หน้า. ISBN 974-53-2993-2.

งานวิจัยนี้มีจุดประสงค์เพื่อ หาภาวะที่เหมาะสมสำหรับการแยกสารแคโรทีนอยด์จากน้ำมันปาล์มโดยการดูดซับด้วยเคลย์ที่ปรับสภาพด้วยเงินในระบบแบทช์ เคลย์ที่นำมาใช้ในงานวิจัยนี้คือ เคลย์ชนิดเบนโทไนท์ซึ่งมีแหล่งที่มาจากประเทศจีนและประเทศญี่ปุ่น โดยจะนำเบนโทไนท์ทั้งสองชนิดมาปรับสภาพด้วยเงินโดยวิธีการแลกเปลี่ยนไอออน และการรีดักชันด้วยเอทิลีนไดเอมีนเตตระอะซิติก แอซิด ดินเหนียวที่ปรับสภาพด้วยเงินที่เตรียมได้นั้นจะมีเงินอยู่ในรูปแบบ ไอออนเงิน ออกไซด์ของเงิน เงินคลอไรด์ และโลหะเงิน และปริมาณที่แตกต่างกัน จากนั้นจึงนำเคลย์ดังกล่าวไปทดลองศึกษาตัวแปรที่มีผลต่อการแยกแคโรทีนออกจากน้ำมันปาล์มดังต่อไปนี้ ชนิดของเคลย์ที่ปรับสภาพด้วยเงิน เวลาในการดูดซับ อัตราส่วนโดยน้ำหนักระหว่างเคลย์และน้ำมันปาล์ม อุณหภูมิในการดูดซับ ชนิดของตัวทำละลายที่ใช้ชะแคโรทีนออกจากตัวดูดซับ และอุณหภูมิในการชะ เพื่อหาสภาวะที่เหมาะสมที่จะให้เปอร์เซ็นต์คืนกลับของแคโรทีนจากน้ำมันปาล์มสูงที่สุด และศึกษาการบำบัดตัวดูดซับด้วยสารป้องกันการเกิดออกซิเดชัน บิวทิเลเทดไฮดรอกซีโทลูอีน ที่มีผลต่อการสลายตัวของเบตา-แคโรทีนระหว่างกระบวนการดูดซับ นอกจากนี้งานวิจัยนี้ยังศึกษาวิธีการนำตัวดูดซับที่ใช้แล้วนำกลับมาใช้ใหม่เพื่อเป็นการรักษาสิ่งแวดล้อม จากผลการทดลองพบว่าสภาวะที่เหมาะสมที่สุดคือ การใช้ 0.5 % ซิลเวอร์เบนโทไนท์จากประเทศจีนในรูปคลอไรด์ และผ่านการบำบัดด้วยสารป้องกันการเกิดออกซิเดชันเป็นตัวดูดซับ โดยใช้อัตราส่วนระหว่างดินเหนียวกับน้ำมันปาล์ม 1:5 โดยน้ำหนัก ที่อุณหภูมิ 60 องศาเซลเซียส เป็นเวลา 60 นาที โดยสามารถดูดซับแคโรทีนจากน้ำมันปาล์มได้ถึง 86.82 เปอร์เซนต์ จากนั้นทำการชะสารแคโรทีนออกจากตัวดูดซับโดยการแช่ตัวดูดซับในเตตระไฮโดรฟิวแรน ที่อุณหภูมิห้อง เป็นเวลา 90 นาที ภายใต้สภาวะดังกล่าวสามารถชะแคโรทีนออกมาได้ 75.76 เปอร์เซ็นต์ และได้ผลิตผลคืนกลับของแคโรทีนจากน้ำมันปาล์มถึง 65.77 เปอร์เซนต์โดยน้ำหนัก

สาขาวิชา ปิโตรเคมีและวิทยาศาสตร์พอลิเมอร์ ลายมือชื่อนิสิต พนินทร เดชฟุ้ง
ปีการศึกษา 2548 ลายมือชื่ออาจารย์ที่ปรึกษา
ลายมือชื่ออาจารย์ที่ปรึกษาร่วม

4572399923 : MAJOR PETROCHEMISTRY AND POLYMER SCIENCE
KEY WORD: CAROTENOID / PALM OIL / CLAY / ADSORPTION

PANINTORN DECHFOONG: SEPARATION OF CAROTENOIDS FROM PALM OIL USING Ag-TREATED CLAYS. THESIS ADVISOR: ASSOC. PROF. AMORN PETSOM, Ph.D., THESIS COADVISOR: ASST. PROF. POLKIT SANGVANICH, Ph.D.,127 pp. ISBN: 974-53-2993-2.

The purpose of this study was to determine the separation condition of carotenoids from palm oil using Ag-treated clay as adsorbents in batch system. Clays used in this experiment were bentonites originated from China and Japan. The Ag-treated clays at different Ag types and content in various forms (Ag^+ form, AgO form, AgCl form and Ag^0 form) were prepared. The factors affecting the adsorption step such as type of Ag-treated clays, adsorption time, ratio of clay to palm oil and adsorption temperature were studied. Then, factors affecting the desorption step such as type of eluting solvents and desorption temperature were investigated to determine the optimum condition. The study of factors affecting degradation of β-carotene extraction with antioxidant (BHT) were studied for improving recovery of carotene from palm oil. Moreover, regeneration of used clay was studied as a mean of environment protection. Results show that the optimum condition for carotene adsorption on 0.5% AgCl-treated bentonite from China (2-steps) which was treated with antioxidant (BHT) at a ratio of 1:5 by weight of clay to palm oil, at 60 ^{0}C for 60 minutes could adsorb 86.82 % carotene. The suitable condition of carotene desorption from clay was carried out at room temperature by using tetrahydrofuran as an eluting solvent resulted in 75.76 % carotene desorption. From the above conditions, overall carotene recovery from crude palm oil corresponding to 65.77 % by weight of carotene in crude palm oil.

Field of study: Petrochemistry and Polymer Science Student's signature Panintorn Dechfoong
Academic year: 2005 Advisor's signature
Co-advisor's signature Polkit Sangvanich

ACKNOWLEDGEMENTS

I wish to express my deepest gratitude to my advisor, Associate Professor Dr. Amorn Petsom and co-advisor, Assistant Professor Dr. Polkit Sangvanich, for their guidance, advice and kindness throughout the course of this research. In addition, I am also grateful to Professor Dr. Pattarapan Prasassarakich, Professor Dr. Sophon Roengsumran and Assistant Professor Dr. Warinthorn Chavasiri for their discussion and comments.

I would like to express special thanks to Cernic International Company Limited for clay supports. I am gratefully thanks to the Olene Company Limited for their kindness in donating the crude palm oil used as raw material in this research. I also thank staff of the Scientific and Technological Research Equipment Center and Metallurgy and Materials Science Research Institute, Chulalongkorn University for clay characterization experiments. Moreover, appreciation is also extended to Program of Petrochemistry and Polymer Science, Faculty of Science and the Graduate School of Chulalongkorn University for financial supports to fulfill this study and provision of experimental facilities.

Finally, I am grateful to my family and friends for their morale, understanding and encouragement throughout the course of this study.

CONTENTS

Page

CHAPTER

Page

CHAPTER

Page

CHAPTER

LIST OF FIGURES

Figure **Page**

Figure **Page**

LIST OF TABLES

Table **Page**

LIST OF ABBREVIATIONS

λ_{max}	Adsorption maxima
^oC	Degree celsius
cm^{-1}	Unit of wave number
XRF	X-ray Fluorescence Spectrometer
SEM	Scanning Electron Microscope
BET	Surface Area Analyzer
UV	Ultraviolet
Meq	Millimolequivalent
ml	Millilitre (s)
mg	Milligram (s)
nm	Nanometre
ppm	parts per million
% wt	percent by weight
THF	Tetrahydrofuran
BHT	Butylated hydroxytoluene
EDTA	Ethylenediaminetetra-acetic acid
$AgNO_3$	Silver nitrate
CPO	Crude palm oil
Benotnite^C	Bentonite from China
Bentonite^J	Bentonite from Japan

CHAPTER I

INTRODUCTION

1.1 Statement of problems

Palm oil is a fruit flesh fat derived form the species *Elaeis guineensis* and owes its distinctive orange-red colour to its relatively high content of nature carotenoids [1]. Crude palm oil (CPO) is the world's richest source of natural plant carotenoid in term of retinol (provitamin A) equivalent. Crude palm oil contains about 15 to 300 times more retinal equivalent than carrots, green leaf vegetables and tomatoes. The carotene content in palm oil varies between 500-700 ppm. The major carotenes include α-carotene and β-carotene that constitute to 90 % of the total carotene. The β-carotene is important as the precursor of vitamin A. The main use of carotenoids is for the direct coloration of food. Moreover, carotene play an important role in prevent of cancer, cataracts and degenerative diseases such as heart disease [2].

Most of the carotenoids in palm oil are destroyed and discarded in the present refining process to produce the light color to colorless oil, as per the demand from major consumers. This represents a loss of a potential source of natural carotenoids [3, 4, 5]. Since the Thai production of refined palm oil amounts to about 500,000 tons/year, every year 281-393 tons of carotenoids are destroyed [6]. If recovered, the value of carotene concentrated would be close to that of the oil itself, excluding the processing cost. The importance of carotenoids is well documented, and various methods of carotenoid recover from palm oil have been developed. These include extraction with suitable solvent [7], extraction by saponification or tranesterification, followed by both phase separation and distillation of the ester [8, 9, 10, 11], gel permeation chromatoghaphy [12], and crystallization [13]. The known processes as above have been found that the yield of carotene is too low as a result of thermal and chemical decomposition. After the process has been carried out the palm oil and the palm oil derivative is no longer suitable for the production of fatty alcohol. Most of methods are difficult to perform, inefficient or costly. Therefore, carotenoids extraction by adsorption without 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 synthetic polymer [14, 15], silica [16], kaolin-carbon [17], activated carbon [18], regenerated clay [19], and activated clays [20, 21, 22, 23, 24]. However, these above adsorbents are alternatives to conventional bleaching clays such as fuller's earth and bentonite. Unfortunately, β-carotene is sensitive to acid, heat and light and it decomposes during adsorption.

In the present study, separation of carotenoids from palm oil is obtained by adsorption on Ag-treated clay in easier procedure. The adsorbents are Ag-treated bentonites (China and Japan) which were prepared with cation exchange and reduction with EDTA. These Ag-treated clays were developed for improving recovery of carotene from palm oil with decrease carotene decomposition. The regeneration of used clay was studied as a mean of environment protection. Moreover, this work also evaluates 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 The objective of the research

1. To study the preparation of the appropriate Ag-treated clays in the adsorption of carotenoids from palm oil.

2. To determine the optimum condition for separation of β-carotene from palm oil and extraction of adsorbed carotene from adsorbent.

1.3 The scope of this research

1. Preparing various Ag-treated clays at different Ag content in various forms (Ag^+ form, AgO form, AgCl form and Ag^0 form) with cation exchange and reduction with EDTA and then characterize them by :

- X-ray Fluorescence Spectrometer (XRF)
- Scanning Electron Microscope (SEM)
- Surface area analyzer (BET)
- Acidity characterization
- Determination of adsorption isotherm

2. Separating carotenoids from palm oil by adsorption on prepared clays.
3. Studying the effect of these following parameters for adsorption step:
    - Types of Ag-treated clay at different Ag content in various forms
    - Adsorption time
    - Ratio of clay to palm oil
    - Adsorption temperature
4. Studying the effect of these following parameters for desorption step:
    - Types of eluting solvent
    - Desorption temperature
5. Studying the effect of antioxidant to carotene degradation.
6. Determination of the optimum condition for adsorption and desorption.
7. Studying the regeneration of used clay.
8. Summarizing the results.

CHAPTER II

THEORETICAL AND LITERATURE REVIEWS

2.1 Palm oil

2.1.1 Introduction

The oil palm known as *Elaeis guineensis* originated from West Africa. Each palm tree produced approximately one fruit bunch, containing as many as 3000 fruits, per month. In addition, each palm tree continues producing fruit economically for up to 25 years. The current planting material in Malaysia is a cross of Dura and Pisifera variety known as Tenera. Palm oil is derived from the mesocarp of oil palm fruit and palm kernel oil is obtained from the kernel inside the nut. The palm is the most prolific oil producing plant and the national average yield in Malaysia is 4.0 tons per hectare per year with 11% of that quantity as palm kernel oil, a co-product. The production of palm oil in the world for 1992 was 12.86 million tons of which Malaysia (53.2 %) and Indonesia (24.1%) are two largest producers. In 1992, the market share of palm oil production in the world was 14.2%. In 2000, palm oil is as important as soyabene oil, contributing 21.1% of the world's total oils and fats [20, 21]. In Thailand, The growth habitats of oil palm are Krabi, Surat Thani, Chumphon, Trang, Satun and others. The production of palm oil in Thailand was 500,000 tons per year.

Naturally, palm oil is characterized as stabilized oil due to its chemical composition. As such, it can be used in most food applications without hydrogenation. Palm oil is available in a variety forms: crude palm oil, palm olein, palm sterin, RBD palm oil, fractionated palm olein and pal mid-fraction. The palm oil is wildly used in food industries and non-food industries. Approximately 90% of palm oil are used in food products such as edible oils, margarine, bakery shortenings, ice-cream, and cheese leaving about 10 % for non-food such as detergents, soaps and candles [19, 28].

2.1.2 The Composition of Palm Oil [27]

Palm oil like all natural fats and oils comprises mainly triglycerides, diglycerides and monoglycerides. Palm oil also contains other minor constituents such as free fatty acids and non-glyceride components.The fatty acid composition of crude Malaysia palm oil is giving in table 2.1. About 50% of the fatty acids are saturated, 40% mono-unsaturated, and 10% polysaturated. In its content of monounsaturated 18:1 acid, palm oil is similar to olive oil, which is as effective as the more polyunsaturated oils in reduction blood cholesterol and the risk of coronary heart disease.

Table 2.1 Fatty acid composition of Malaysian palm oil [27]

Fatty acid in palm oil	**Fatty acid composition (%)**
12 : 0	0.1-1.0
14 : 0	0.9-1.5
16 : 0	41.8-46.8
16 : 1	0.1-0.3
18 : 0	4.2-5.1
18 : 1	37.3-40.8
18 : 2	9.1-11.0
18 : 3	0.0-0.6
20 : 0	0.2-0.7

Crude palm oil contains approximately 1% of minor components including carotenoids, tocopherols, sterols, triterpene alcohol, phospholipids, aliphatic hydrocarbons and other trace impurities. Carotene 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 constituents determine the quality characteristics of palm oil. The minor components composition of crude palm oil is shown in table 2.2.

Table 2.2 Minor components compostion of crude palm oil [27]

Minor Components fo crude palm oil	Concentration (ppm)
Carotenoids	500-700
Tocopherols and Tocotrienols	600 -1000
Sterols	326-527
Phospholipids	5-130
Triterpene alcohol	40-80
Methylsterols	40-80
Squalene	200-500
Aliphatic alcohols	100-200
Aliphatic hydrocarbons	50

2.1.3 Refining of Palm Oil [28]

Palm oil consist mainly of glycerides, like other oils in their crude form, small and variable portions of non-glyceride components as well. In order to render the oils to an edible form, some of these non-glycerides need to be either removed or reduced to acceptalble levels. The insoluble impurities consisting of fruit fibres, nut shells and free moisture, are readily removed. The oil soluble non-glycerides which include free fatty acids, phospholipids, trace metals, carotenoids, tocopherols or tocotrienols, oxidation products and sterols are more difficult to remove and thus, the oil needs to undergo various stages of refining. Not all of the above non-glyceride components are undesirable. The tocopherols and tocotrienols not only help to protect the oil from oxidation, which is detrimental to flavour and keep ability of the finished oil, but also have nutritional attributes, α-carotene and β-carotene, the major constituents of carotenoids, are precursors of vitamin A. The other impurities generally are detrimental to the oil’s flavour, odour, colour and keep ability and thus influence the oil’s usefulness.

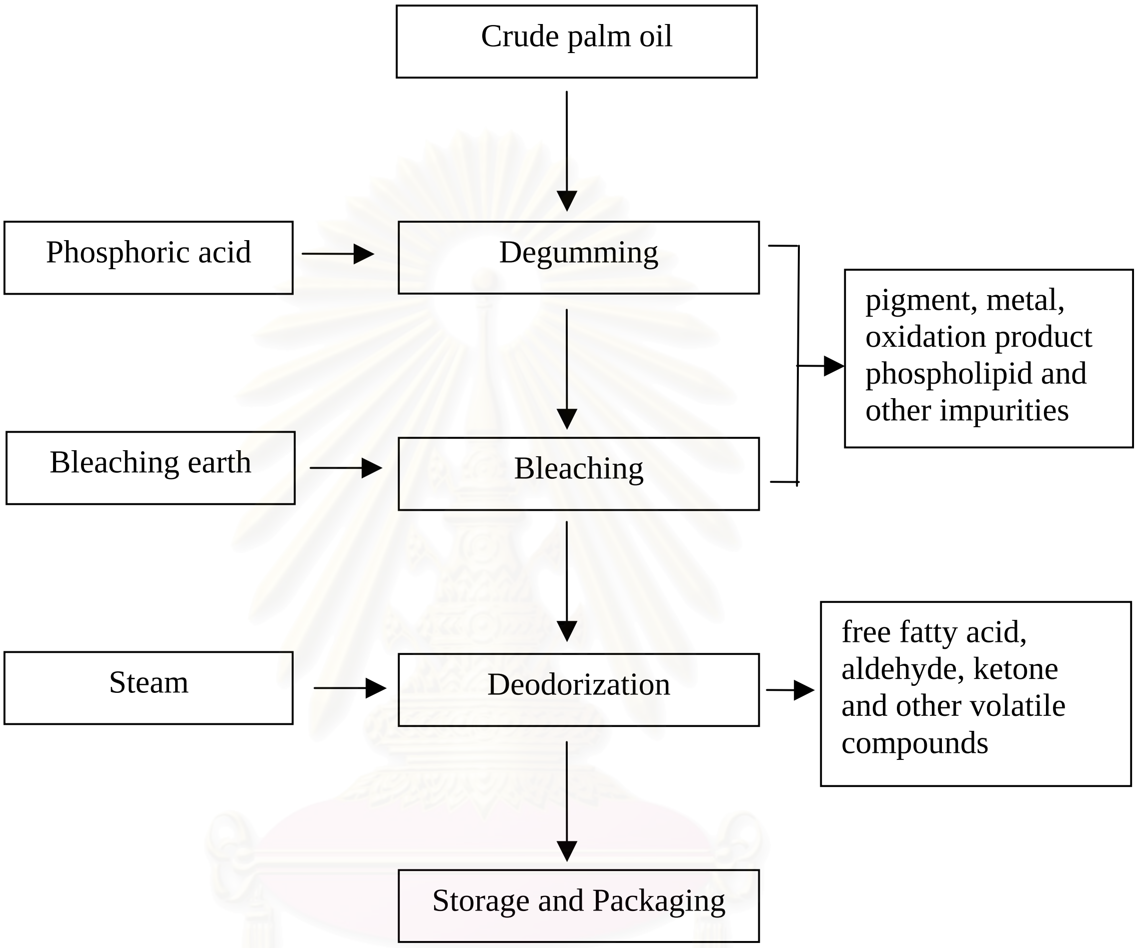
The aim of refining is therefore to convert the crude to quality edible oil by removing objectionable impurities to the desired levels in the most efficient manner. This also means that, where possible, losses in the desirable component are kept minimal.The refining route of palm oil is quite indentical. There are two routes are taken to process crude oil into refined oil; which are chemical refining and physical refining.

2.1.3.1. Physical refining

For this process, firstly the crude feedstock is subjected to pretreatment. The pretreatment comprises a degumming step and an earth bleaching step which together remove certain nonvolatile impurities. Crude palm oil is first mixed with about 0.1% of concentrated of phosphoric acid for precipitation up the gums. After holding, about 1 to 2% activated bleaching earth is added under vacuum and at a temperature of 80 - 130 ^{0}C. After a suitable contact time the earth is removed by filtration. The practice of bleaching involves the addition of bleaching earth to remove any undesirable impurities (all pigments, trace metals, oxidation products) from crude palm oil and improves the final flavor, colour and oxidative stability of product. It also helps to overcome problems in subsequent processing by adsorption of soap traces, pro-oxidant metal ion, decomposes peroxides and other impurities.

After the pretreatment, crude palm oil is subjected to deacidification and deodorization. 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. The 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 ^{0}C. Careful cooling and a polishing filter then yield the refined, bleached and deodorized product (RDB palm oil). 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 do not arise. The physical refining process is shown in figure 2.1.

Figure 2.1 Physical refining



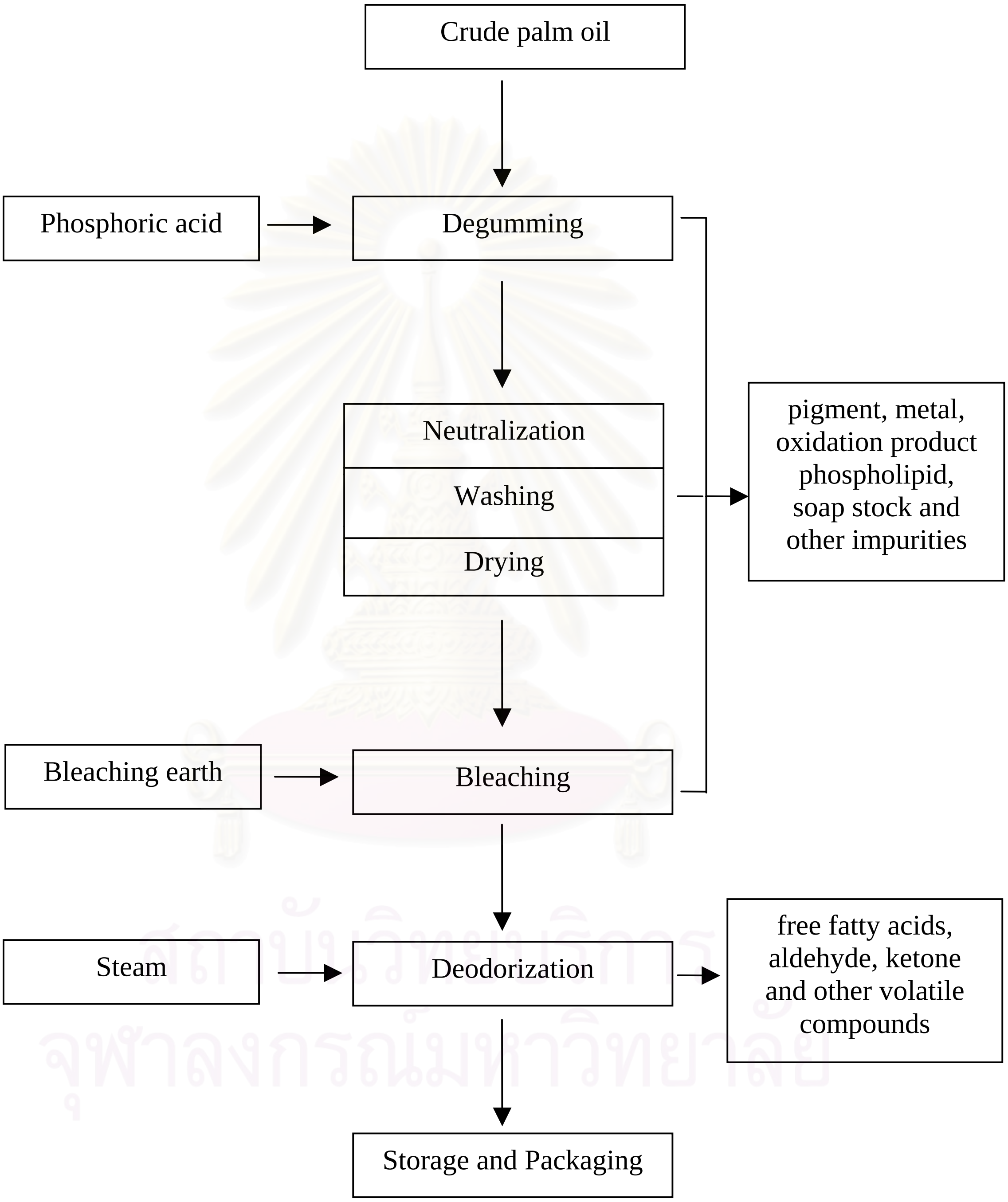
2.1.3.2. Chemical refining

For this process, crude palm oil is subjected to neutralization with alkali after degumming. Caustic soda is used in this procedure to remove free fatty acids. It is vigorously mixed with heated fat at relatively high temperature (60-80 ^{0}C) and left to stand for the aqueous phase to settle. The aqueous solution remains is known as foots or soapstock and, after separation, maybe used for producing soap. Residual foots are eliminated from the neutral oil by washing it with hot water, along with settling or centrifugation. Even though the main purpose of the alkali treatment is the removal of free fatty acid.

Nomally, 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. After the pretreatment, crude palm oil is subjected to deodorization. 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. The degummed and bleached oil is first deaerated before steam stripping under a vauum of 2 to 6 mm Hg at a temperature between 240 and 270 ^{0}C. Careful cooling and a polishing filter then yield the refined, bleached and deodorized product (RDB palm oil).

With the pretreatment process physic refining of palm oil is not only much more economical than chemical refining in connection with stripping steam deodorization, but also causes much less pollution by waste water and exhaust air. The chemical refining process is shown in figure 2.2

Figure 2.2 Chemical refining



2.2 Carotenoids

2.2.1 Introduction

Carotenoids are a large group of yellow, orange and orange red fat-soluble C_{40} plant pigments that synthesized by photosynthetic microorganisms and plants. Fruit and vegetable of green, orange and red color are the most important sources of carotenoids in the human diet. Over 600 carotenoids have been identified and are classified into hydrocarbon carotenoids, with β-carotene and lycopene being the most prominent members, and oxycarotenoids (xanthophylls), to which belong β-cryptoxanthin, lutein, zeaxanthin, canthaxanthin, and astaxanthin. The carotenoids pigment found largly in plants with chlorophyll are polyenes belonging to four principal groups given below [29]

1. The carotene, $C_{40}H_{56}$, which include α-carotene, β-carotene, γ-carotene and lycopenes.

2. The xanthophylls and oxo and hydorxy derivatieves of the carotenes which include among others, cryptoxanthin ($C_{40}H_{55}OH$), and lutein [$C_{40}H_{54}(OH)$]

3. The xanthophylls esters, which are esters of xanthophylls with fatty acid.

4. The carotenoid acids, which are carboxyl derivatives of the carotenes.

Carotenoids are of physiological interest in human nutrition because β-carotene and other carotenoids containing an unsubstitued β-ring as provitamin A. These carotenoids are cleaved enzymically in the intestine to yield retinal and are the main source of vitamin A. β-Carotene has also been used successfully for the treatment of erythropoietic protoporphyria, a condition in which the patients are extremely sensitive to light because of an abnormality in haem synthesis, which leads to the accumulation of free porphyrins in the skin. The free porphyrin molecule act as photosensitizers, in the same way as chlorophyll in plants, and cause the production, in the skin, of $^1O_2$ which causes tissue damage and inflammation. β-Carotene can afford substantial protection against this and alleviate the unpleasant symptoms.

In recent years there have been many reports to suggest that β-carotene may also be important in affording protection against cancer, heart disease and other conditions, and that it may have anti-ageing benefits. Several studies since then have concluded that individuals whose dietary intake and serum level of β-carotene are low have higher risk of developing some cancer, for example of the lung, skin and bladder. Similar correlations have been found for low carotene levels and increased risk of coronary heart disease.

It is usually considered that the beneficial protection action of β-carotene are due to its antioxidant properties. Under appropriate condition, β-carotene can function as an effective antioxidant, not only against $^1O_2$ but also against lipid peroxidation and the highly destructive hydroxyl radical $HO^\cdot$ that is implicated in many diseases. It is by no means sure, however, that carotenoids can act as antioxidants at the low concentration found *in vivo* [30].

2.2.2 Carotenoids in palm oil [26]

Crude palm oil is the world's richest natural plant source of carotenes in terms of retinol (provitamin A) equivalent. The carotenoid content of crude palm oils varies between 500 and 700 ppm, the compounds contained within this portion of saponifiables contain α-carotene, β-carotene, γ-carotene, xanthophylls and lycopene. α-Carotene and β-carotene constitue ca. 91% of the fraction while the other components are represented more or less equally (see table 2.3).

Table 2.3 Carotenoids composition in palm oil

Compound	Quantity
β-carotene	62%
α-carotene	29%
γ-carotene	4%
xanthopylls	3%
lycopene	2%

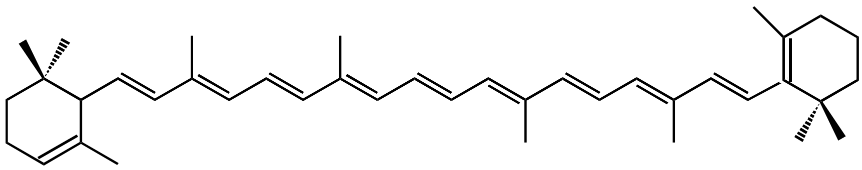
Carotenes are the precursors of vitamin A, with β-carotene having the high provitamin A activity. Palm oil has the highest carotene-derived vitamin A activity. This is primarily due to β-carotene and secondarily, to α-carotene. For example, red palm oil has 15 times more vitamin A activity than carrots and 300 times more than tomatoes. Retinol equivalent of red palm oil compared with other foods, are illustrated in table 2.4 Unfortunately, all this carotene is destroyed by present refining processes which traditionally produce only light colored to colorless oils for major consumers.

Table 2.4 Retinol equivalents (RE) of red palm oil compared with other foods [27]

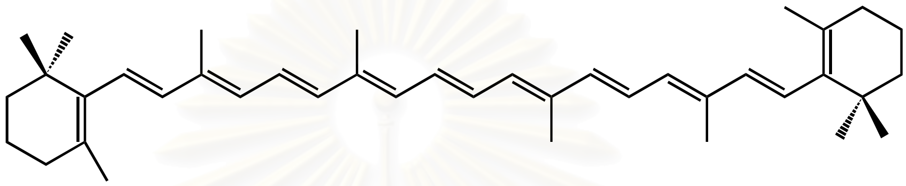
Fruits and vegetables	**Retinol equivalents (RE)**	**Relative quality**	
		> RPO	**< RPO**
red palm oil (RPO)	30,000		
carrots	2,000		15
leafy vegetables	685		44
apricots	250		120
tomatoes	100		300
bananas	30		1,000
orange juice	8		3,750

2.2.3 Structure and nomenclature [30]

Carotenoid hydrocarbons collectively are called *carotenes*. Derivative that contain oxygen functions (most commonly hydroxyl, keto, epoxy, methoxy or carboxylic acid groups) are called *xanthophylls*.The polyene backbone of the carotenoids is characteristically conneted to any two of the seven 9-carbon recognized end-groups. Some carotenoids are acyclic (e.g. lycopene) but more common are those that contain a six-membered (or occasionally five-membered) ring at one end or both ends of the molecule. The structure and numbering of the parent acyclic and dicyclic carotenoids, lycopene and β-carotene, are illustrated in figure 2.3



α-carotene



β-carotene

Figure 2.3 Structures of α-carotene and β-carotene

Traditionally, natural carotenoids have been given trivial names that are usually derived from the name of the biological source from which they were first isolated. In recent years, however, a semi-systematic nomenclature that conveys structural information has been devised. According to this figure, the carotenoid molecule is considered in two halves. Each individual compound is then named as a derivative of the patent carotene, as specified by the Greek letters that describe its two end groups, with conventional prefixes and suffixes being used to indicate change in hydrogenation level and the presence of substituent groups. The seven end groups that have been recognized are illustrated in figure 2.4.

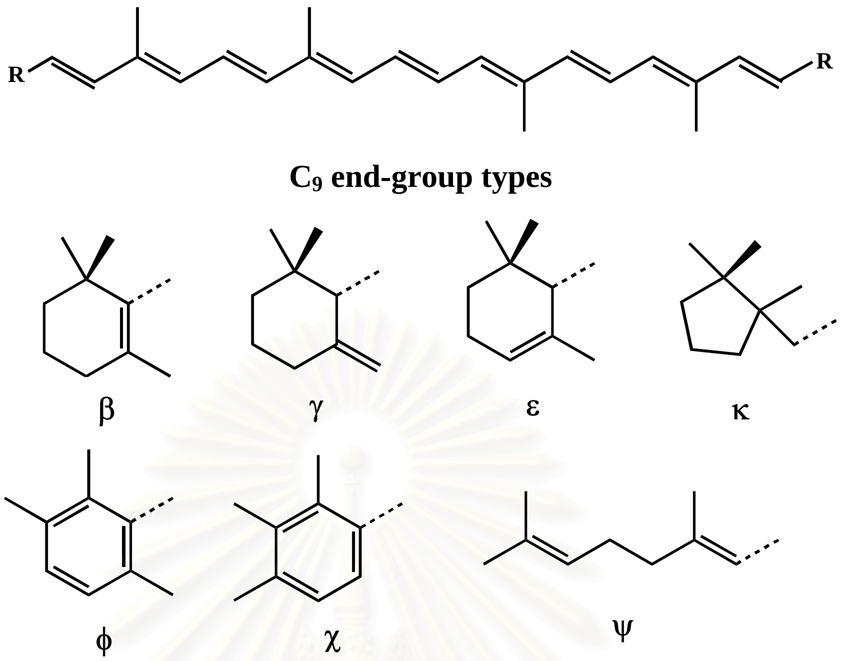


Figure 2.4 Structure of the seven carotenoid end-group types.

Compounds from which an end group has been removed from the normal C_{40} structure are known as *apocarotenoids*; some of these are important food colorants. In keeping with current practice, trivial names will be used in this article for common carotnoids, but the semi-systematic names of these are given in table 2.5.

Table 2.5 Semi-systematic names of carotenoids

Trivial name	Semi-systematic name
α-carotene	β,ε-carotene
β-carotene	β,β-carotene
γ-carotene	β,ψ-carotene
δ-carotene	ε,ψ-carotene
ε-carotene	ε,ε-carotene
lycopene	ψ,ψ-carotene

Because of the extensive double-bond system in the molecule, any carotenoid can, theoretically, exist in many geometrical isomeric forms (Z-E isomers). Most carotenoids occur naturally in the all-E (all-*trans*) form, but Z-isomers (*cis*-isomers) are frequently present in small amounts, and are easily formed as artifacts from the all-E isomer.

2.2.4 Solubility of β-carotene [31]

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

β-Carotene could be dissolved in nonpolar organic solvents. Solubility in many organic solvents is shown in table 2.6.

Table 2.6 Solubility of β-carotene in organic solvents

Type of Solvents	**Solubility (mg/l)**
acetone	200
acetonitrile	10
benzene	4,000
chloroform	2,000
dichloromethane	6,000
ethyl acetate	500
ethyl ether	1,000
methanol	10
tetrahydrofuran	10,000
toluene	4,000

Besides solubility of β-carotene in the solvent, we must also consider about toxicity of solvent, cost and stability of β-carotene in the solvent. The study in stability of β-carotene in various solvents found that β-carotene has the most stability in THF which has antioxidant (butylated hydroxytoluene : BHT). Degradation of β-carotene in various solvents is shown in table 2.7

Table 2.7 Degradation of β-carotene in various solvents

Type of Solvents	**% Adsorption of β-carotene**			
	Period of time (days)			
	1	**3**	**6**	**10**
acetonitrile	98	96	96	92
methanol	97	92	89	88
ethanol	98	94	92	91
ethyl ether	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 carotenoids degradation [32]

The carotenoids are sensitive to light, acid, oxygen and heating, which leads to degradation and isomerisation of carotenoids in vegetables oils. The degradation and isomerisation of carotenoids are illustrated in figure 2.5.

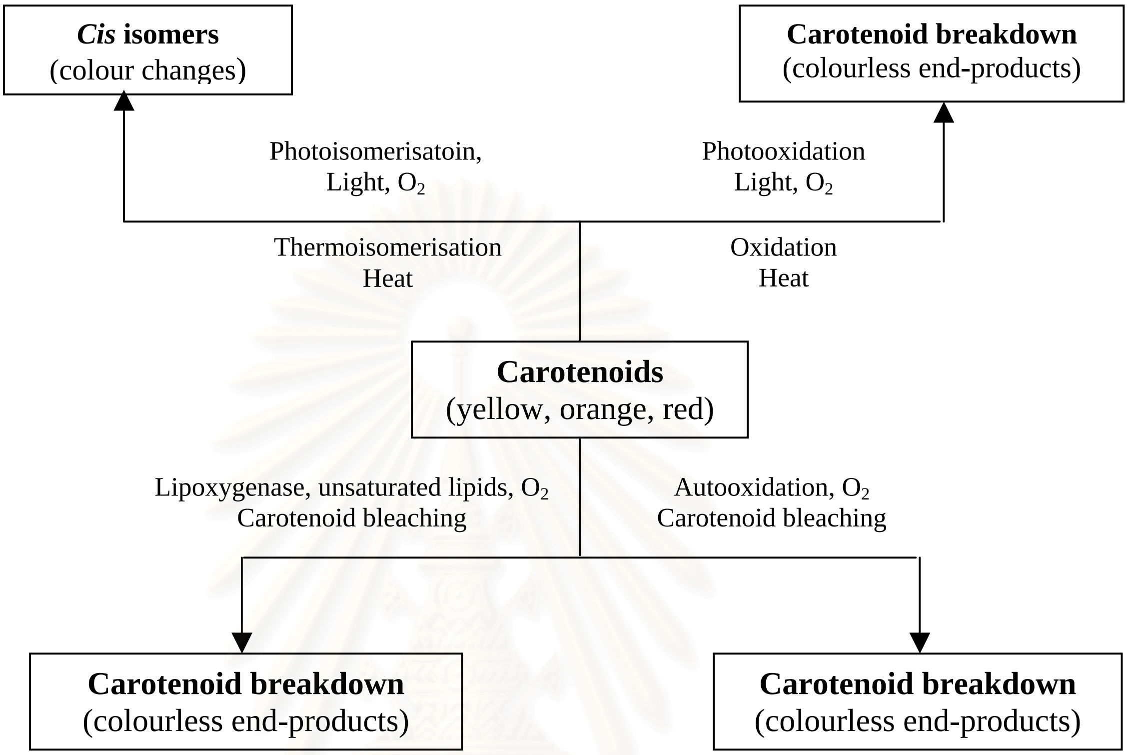


Figure 2.5 The degradation of carotenoids

2.2.5.1 Oxidation

The major cause of carotenoids losses in vegetables is due to oxidation of the highly unsaturated carotenoid structrure. 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 and (c) coupled oxidation in lipid containing systems. The oxidation may result in carotene bleaching, which is a consequence of formation of colourless end-products. Color bleaching leads to changes or loss of the colour. The severity of oxidation depends on the structure of the carotenoids and the environmental conditions, and the compounds being form depend on the oxidation process and the carotenoid structure.

- *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 β-carotene were identified as a mixture of products with epoxy, hydroxyl and carbonyl groups.

The rate of autooxidation between carotenoids and free oxygen radicals depends for example on structure of carotenoids, available oxygen, temperature, light, water activity, pH and the presence of prooxidants and antioxidants. If one or more β-ionone ring are present in the carotenoid structure, the rate of autooxidation seems to depend on their polarity. The most apolar carotenoids, e.g., β-carotene, are more suspectible to autooxidation than the polar xanthophylls, e.g., zeaxanthin, containing two hydroxyl groups. Enhanced temperature increase the β-carotene oxidation. At temperature above 170 ^{0}C, β-carotene may be fragmented to polar short-chain hydrocarbons and/or longer-chain aromatic hydrocarbon, e.g. ionene. Volatile compounds, which compounds, which predominately were formed by thermal degradation of β-carotene at 190-220 ^{0}C in the presence of air, were β-ionone, dihydroactinidiolide and 5,6-epoxy-β-ionone. The presence of prooxidants and antioxidants affects the rate of autooxidation. Addition of unsaturated lipids result in increased β-carotene degradation. The pro-oxidative effect of the lipids increases with the degree of unsaturation.

- *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 of sensitizers and the intensity of illumination by fluorescent light. In the photosensitized oxidation, an excited sensitiser produced singlet oxygen ($^1O_2$), which can be quenched by the carotenoids. In general, photooxidation of β-carotene results in formation of *cis*-isomers of β-carotene and its 5,6- and 5,8-epoxides.

- *Coupled oxidation*

The carotenoids may undergo coupled oxidation in a lipid system, which also leads to carotenoid bleaching. Coupled oxidation of β-carotene is associated with the linoleate-lipoxygenase system in raw vegetables that have been expose to stress. The peroxides being formed oxidize the carotenoids by a secondary or a coupled reaction, resulting in decolouration of the carotenoids. Other factors that affect the rate of coupled oxidation of the carotenoids in lipid systems are available oxygen and the presence of antioxidants, e.g., phenolics. The products obtained by oxidatoni of β-carotene in a linolate-lipoxygenase system are identified as *cis* isomers of β-carotene, β-carotene monoepoxide, aurochrome, and several conjugated ketones. Volatile products being formed during bleaching with soybean lipoxygenase are β-ionone, β-ionone epoxide and dihydroactinidiolide.

2.2.5.2 *cis-trans*-isomerisation

As described above, different *cis*-somers are formed during oxidation. The carotenoid structure makes a *cis*-*trans*-isomerisation possible around the double bonds. In vegetables, the carotenoids are predominatly the all-*trans* isomers, which may be converted to the *cis*-isomers. *Cis*-*trans* isomerisation results in colour changes in vegetable products as the spectra properties of the *cis*-carotenoids are different from the corresponding *trans*-carotenoids. Insertion of one or more *cis*-double bonds in an all-*trans*- conjugated system results in a hypsochromic shift of 2 to 5 nm. A subsidiary peak is also present in the near-ultraviolet adsorption spectrum, if a *cis*-isomer is present. These different spectral properties of *cis*-isomers result in a lighter hue of lower intensity compared to the corresponding *trans*-isomers.

Acid, light and heating promote isomerisation of all-*trans*-carotenoids to *cis*-forms. In general, the rate of *cis*-isomerisation increase with enhanced temperatures. Heating of tomatoes at 100 ^{0}C for 30 minutes resulted in conversion of all-*trans*-β-carotene and all-*trans*-lutein to the *cis* configurations. However, the isomerisation depends on the structure of the carotenoids. Especially the epoxycarotenoids are sensitive to heat treatment, whereas lutein and carotenes, e.g., lycopene, α-carotene and β-carotene survive the heat treatment. The concentration of all-*trans*-α-carotene and all-*trans*-β-carotene was only slightly affected at 50 ^{0}C or 100 ^{0}C for 30 minutes. At 150^{0}C all-*trans*-β-carotene was isomerised, 9-*cis*-β-carotene and 13-*cis*-β-carotene were the major isomer being formed. Isomerisation of all-*trans*-α-carotene resulted in formation of 13-*cis*-β-carotene in the highest concentration.

Illumination promotes isomerisation of carotenoids. During iodine-catalysed photoisomerisation of all-*trans*-α-carotene and all-*trans*-β-carotene the major isomer was 13,15-di- *cis*-carotene.

2.2.6 Uses of carotenoids [30]

2.2.6.1 Direct use in food coloration

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. β-Carotene and 8’-apo-β-carotene-8’-al are the main carotenoids used in oil suspensions and solutions, and can provide yellow-orange and orange-red colours, respectively, depending on concentration. The solubility of canthaxanthin in triglyceride oils is too low for practical application. Although lycopene shows good coloration properties, its poor solubility and stability present serious difficulties for its practical application. The oil-based preparations, especially of β-carotene, are widely used for colouring butter, margarine, cheese, cooking fats, industrial egg products, bakery products, pasta, salad dressing, dairy product substitutes, pop-corn, potato products and many others. Water-dispersible forms of β-carotene, 8’-apo-β-carotene-8’-al canthaxanthin, and the water-soluble (norbixin) forms of annatto, are used extensively for colouring soft drinks (especially ‘orange juice’), ice-cream, desserts, sweets, soups and meat products.

2.2.6.2 Animal feeds Feeds for cattle, birds and fish will be considered

- *Cattle*

Natural pasture is very rich in β-carotene and provides sufficient of this compound to full fill the vitamin A requirement of the animals and also to give a desirable yellow color to the fat and rich cream colour to cream and butter. Artificial diets must be supplemented with β-carotene to ensure adequate vitamin A levels and to maintain the required yellow colour or dairy products and fat.

- *Bird*

Many ornamental birds owe their exotic yellow and red colours to the presence of carotenoids in the feathers. Adequate dietary supplieds of carotenoid therefore need to be maintained in captive birds. A well-known example is the flamingo, which needs to be provided with substantial quantities of the oxocarotenoids that the wild birds obtain from their diet of crustaceans, otherwise the characteristic deep pink colour is lost. Of far greater commercial importance is the poultry industry. Chicken adsorb and accumulate xanthophylls rather than carotenes and need substantial supples of xanthopylls rather than carotenes and need substantial supplies of xanthophylls to ensure the required golden-yellow colour of the egg yolk and also the yellow skin colour demaned in many countries. The apocarotenoids and canthaxanthin can be used for yolk coloration, but the most natural colours of yolk and skin are achieved with zeaxanthin. Marigolds, as a source of lutein, are included in the diet in some countries.

- *Fish*

With the recent rapid development of farming methods for salmon and trout, it is essential that the products should have the same desired pink flesh coloration as the wild fish. This is now attained by the inclusion of astaxanthin (salmon) or canthaxanthin (trout) in the diet for several weeks before harvesting.

2.2.6.3. Medical and health products

The beneficial role of β-carotene as provitamin A has long been known. β-Carotene is also now used successfully to alleviate the symptoms of light-sensitivity disease, especially erythropoietic protoporphyria, that are characterized by extreme irritation of the skin when exposed to strong light, because of the formation of singlet oxygen sensitized by the free porphyrins that accumulate in this condition. β-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.

β-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 β-carotene preparations on the health market, including crystalline carotene, extracts of natural sources such as carrots and algae, carotene drinks and even dried algal cells (*Dunaliella*). There seems little doubt that the demand for β-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, capsules and suppositories.

2.2.7 UV-visible light absorption spectroscopy [33]

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 (λ_{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. The spectra of carotenoids are quite characteristic between 400 and 500 nm. The λ_{max} values of the main carotenoids are given in table 2.8. Also, there are overall spectral shifts according to the solvent used.

Table 2.8 Light adsorption maxima of some carotenoids

Carotenoids	**λ_{max} (nm)**	**Solvent**
Canthaxanthin	466	light petroleum
	474	ethanol
	482	chloroform
α-Carotene	422 444 473	light petroleum
	423 444 473	ethanol
	424 448 476	acetone
	433 457 484	chloroform
β-Carotene	425 449 476	light petroleum
	450 476	ethanol
	429 452 478	acetone
	435 461 485	chloroform
	455	tetrahydrofuran
γ-Carotene	437 462 494	light petroleum
	440 460 489	ethanol
	439 461 491	acetone
	446 475 509	chloroform
δ-Carotene	431 456 489	light petroleum
	440 470 503	chloroform
Lycopene	444 470 502	light petroleum
	446 472 503	ethanol
	448 474 505	acetone
	458 484 518	chloroform
Zeaxanthin	424 449 476	light petroleum
	428 450 478	ethanol
	430 452 479	acetone
	433 462 493	chloroform

2.3 Clay mineral

2.3.1 Introduction

Clay minerals are layer silicates that are formed usually as products of chemical weathering of other silicate minerals at the earth’s surface. They are found most often in shales, the most common type of sedimentary rock. In cool, dry or temperate climates, clay minerals are fairly stable and are an important component of soil. Clay minerals act as “chemical sponges” which hold and dissolved plant nutrients weathered from other minerals. This results from the presence of unbalanced electrical charged (and thus attract negatively charged ions), while other surfaces are negatively charged (attract positively charged ions). Clay minerals also have the ability to attract water molecules. Because this attraction is a surface phenomenon, it is called adsorption (which is different from adsorption because the ion and water are not attracted deep inside the clay grains).

From prehistoric times, clay has been indispensable in architecture, industry and agriculture. As a building material, it is used in the form of brick, either sun-dried or fired. Clays are also of great industrial importance, e.g., in the manufacture of tile, porcelain and pipe for drainage and sewage. Highly adsorbent, bentonite is much used in foundry work for facing the molds and preparing the molding sands for casting metals.The less absorbent bentonites are used chiefly in the oil industry as filtering and deodorizing agents in the refining of petroleum, drilling muds to protect the cutting bit while drilling. Other uses are in clarifying water and wile, purifying sewage. There are many types of known clay minerals. Some of the more common types and their economic uses are described here [34].

- *Kaolinite*

This clay mineral is the weathering product of feldspars. It has a white, powdery appearance. Kaolinite is named after a locality in China called Kaolin, which invented porcelain (known as china) using the local clay mineral. The ceramics industry uses it extensively. Kaolinite is electrically balanced. Its ability of adsorb ion is less than that of other clay minerals. Still, kaolinite was used as the main ingredient for the original formulation of the diarrhea remedy, Kaopectate.

- *Illite*

It is the weathering product of feldspars and felsic silicates. It is named after The state of Illinois and is the dominant clay mineral in Midwestern soils.

- *Chlorite*

This clay mineral it the weathering product of mafic silicates and is stable in cool and dry or temperate climates. It occurs along with illite in Midwestern soils. It is also found in some metamorphic rocks, such as chlorite schist.

- *Vermiculite*

This clay mineral has the ability to adsorb water, but not repeatedly. It is used as a soil additive for retaining moisture in potted plants, and as a protective material for shipping packages.

- *Smectite*

This clay mineral is the weathering product of mafic silicates, and is stable in arid, semi-arid, or temperate climates. It was formerly known as *montmorillonite*. Smectite has the ability to adsorb large amounts of water, forming a water-tight barrier. It is used extensively in the oil drilling industry, civil and environmental engineering (where it is known as bentonite), and the chemical industry.

- *Attapulgite*

This mineral actually resembles the amphiboles more than it does clay minerals, but has a special property that smectite lacks-as a drilling fluid, it stable in salt water environments. When drilling for offshore oil, conventional drilling mud falls apart in the presence of salt water. Attapulgite is used as a drilling mud in these instances. Incidentally, attapulgite is the active ingredient in the current formula of Kaopectate [35].

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 sample. Most of the important clay minerals are made of two basic types of units.

Two units are involved in the atomic structure of most of the clay minerals. One unit consists of two sheets of closely packed oxygens or hydroxyls in which aluminum, iron or magnesium atoms are embedded in octahedral coordination, so that they are equidistant from six oxygens or hydroxyls (figure 2.6). When aluminum is present, only two-third of the possible positions are filled to balance the structure, which is the gibbsite structure and has the formula $Al_2(OH)_6$. When magnesium is present, all the positions are filled to balance the structure, which is the brucite structure and has the formula $Mg_3(OH)_6$. The normal oxygen to oxygen distance is 2.60 A, and a common hydroxyl to hydroxyl distance is about 3 A; in this structural unit, however, the OH to OH distance is 9.24 A, and the space available for the atom in octahedral coordination is about 0.61 A. The thickness of the unit is 5.05 A in clay mineral structures [34].

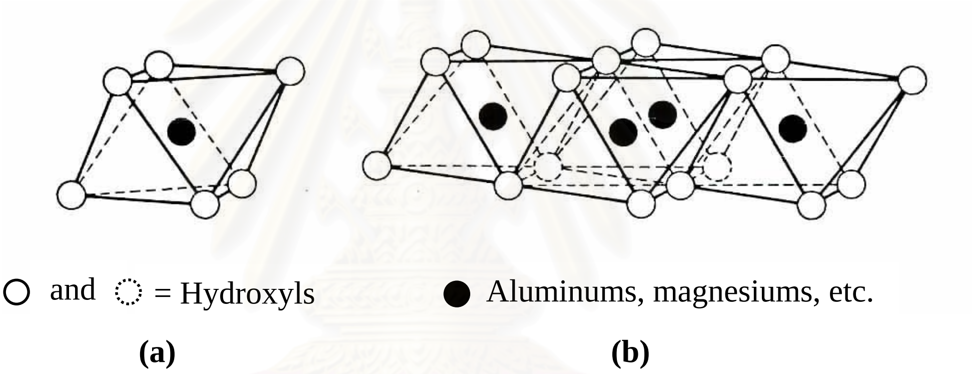


Figure 2.6 Diagrammatic sketch showing (a) a single octahedral unit and (b) the sheet structure of the octahedral units.

The second unit is built of silica tetrahedrons. In each tetrahedron a silicon atom is equidistant from four oxygens, or hydroxyls if needed to balance the structure, arranged in the form of a tetrahedron with the silicon atom at the center. The silica tetrahedral groups are arranged to form a hexagonal network, which is repeated indefinitely to form a sheet of the composition $Si_4O_6(OH)_4$ (figure 2.7). The tetrahedrons are arranged so that the tips of all of them point in the same direction, and the bases of all tetrahedrons are in the same plane (there may be exceptional cases in which some of the tetrahedrons are inverted). The structure can be considered as made of a perforated plane of oxygens which is the plane of the base of the tetrahedral groups; a plane of silicon atoms with each silicon in the cavity at the function of three oxygent atoms and therefore forming a hexagonal network; and a plane of hydroxyl atoms with each hydroxyl directly above the silicon at the tip of the tetrahedrons. The open hexagonal network can be considered as composed of three strings of oxygen atoms intersecting at angles of 120 ^{0}. The oxygen to oxygen distance in the silica tetrahedral coordination is about 2.55 A. The thickness of the undistorted unit is 4.65 A in clay-mineral structures. Each of these units presents a center-to-center height of about 2.1 A [34].

○ and ◌ = Oxygens o and • = Silicons

(a) **(b)**

Figure 2.7 Diagrammatic sketch showing (a) a single silica tetrahedron and (b) the sheet structure of silica tetrahedrons arranged in a hexagonal network.

Some of the clay minerals are fibrous and are composed of different structural units from those noted above. These minerals resemble the ambhiboles in their structural characteristics, and the basic structural unit is composed of silica tetrahedrons arranged in a double chain of the compostion Si_4O_{11}, as shown in figure 2.8. The structure is similar to that of the sheet of silical tetrahedrons in the layer minerals except that it is continuous in only one direction. In the other direction it is restricted to a width of about 11.5 A.

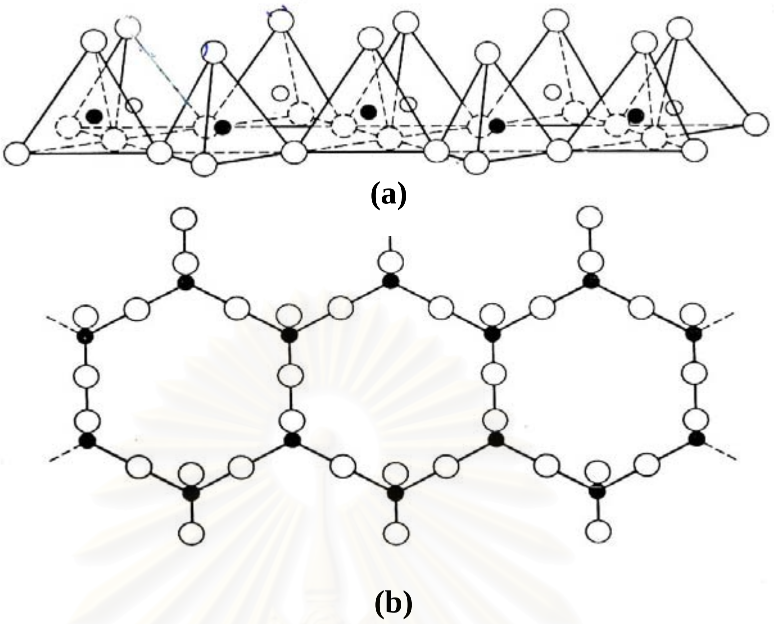


Figure 2.8 Diagrammatic sketch of double chains of silica tetrahedrons, as in the emphibole structure type of clay minerals : (a) in prespective; (b) projected on the plane of the tetrahedrons.

The chains are bound together by atoms of aluminum and/or magnesium placed so that each such atom is surrounded by six “active” oxygen atoms. The active oxygens are those with only one link to silica and hence are those at the edges of the chains and at the tips of the tetrahedrons. Detailed structural data permitting specific statements are not available, but it appears likely that some distortions similar to those structural units [34].

2.3.2 Ion exchange of clays [34]

The clay minerals have the property of sorbing certain anions and cations and retaining them in an exchangeable state; i.e., these ion 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.

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, clay material carrying sodium frequently has very different plastic properties than material the same in every way except that calcium is exchangeable cation.

2.3.2.1 Cation exchange of clays [23, 34]

Clay minerals have the ablility to adsorb and exchange cation form 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^+ cation per 100 g of clay, whilst a kaolinite only adsorbs a few tens of millimoles.

The ideal structure 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.

A typical montmorillonite will have Na^+, Ca^{2+} or Mg^{2+} cations in the interlayer space. These cations are hydrated, usually hexa(apua), 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 form an aqueous solution.

Very small cations such as 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 cation reside depends upon the hydration of the clay, the cation and whether edge or layer charges are involved. Na^+ and 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 equation sheres.

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:

$$M^+ << M^{2+} < H_3O^+ = K^+ = NH_4^+ < M^{3+} < M^{4+}$$

$$\xrightarrow{\hspace{8cm}}$$

Increasing exchange power

(Decreasing ease of exchange)

Figure 2.9 The exchange properties of cations with clays.

There are three causes of cation-exchange capacity of the clay minerals

1. Broken bonds around the edges of the silica-alumina unit would give rise to unsatisfied charges, which would be balanced by adsorbed cations. The broken bonds would tend to be on noncleavage surfaces and hence on the vertical plans and on horizontal planes of the layer clay minerals. The number of broken bonds, hence the exchange capacity due to this cause, would increase as the particle size decrease. Also lattice distortions would tend to increase broken bonds, and the exchange capacity would be excepted to increase as the degree of crystallinity decreased. In smectites and vermiculites, broken bonds are responsible for a relatively small

portion (20%±) of cation-exchange capacity, the remainder probably resulting from substitution within the lattice.

2. Substitutions within the lattice structure of trivalent aluminum for quadtrivalent 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 clay minerals. Sometimes such substitutions are balanced by other lattice changes, for example, OH for O, or by filling more than two-thirds of the possible octahedral positions, but frequently they are balance by adsorbed cations. Exchangeable cations resulting from lattice substitutions are to be found mostly on cleavage surface, e.g., the basal cleavage surfaces of the layer clay minerals. Since the charges resulting from substitutions in the octahedral sheet would act through a greater distance than the charges resulting from substitutions in the tetrahedral sheet, it would be expected that cations held because of the latter substitutions would be bonded by a stronger force than those held by forces resulting from substitutions in the octahedral sheet. In some cases, cations held by forces due to substitutions of aluminum for silicon seem to be substantially nonexchangeable, e.g., the potassium in the micas. In the clay minerals, replacements in the octahedral layer are probably the major substitution causing cation-exchange capacity.

3. The hydrogen of expose 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 basal-plane 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 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.

2.3.2.2 Anion exchange clays [34]

A series of clay minerals of lesser importance, whose charge imbalance or layer substitution pattern has givent them positively charge layers, are known. Such minerals as hydrotalcite ($Mg_{4.5}AlO_{7.5}$) and xonotlite ($Ca_6Si_6O_{17}(OH)_2$) can be used as solid “carriers” of hydroxyl and t-butoxy anions, respectively.

2.3.3 Acidity of clays [19, 28]

Several measurement 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 unit 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 ammonium ions and calcining at 200-300 ^{0}C to expel ammonia. The exchanged proton can migrate into vacancies on the octahedral layer of dioctahedral clays where they protonate bridging oxygen.

2.3.4 Acid Activation of clays [23]

Smectite clays are often treate 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 Fe^{3+} cations which cause the clay to be a gray or yellow colour.

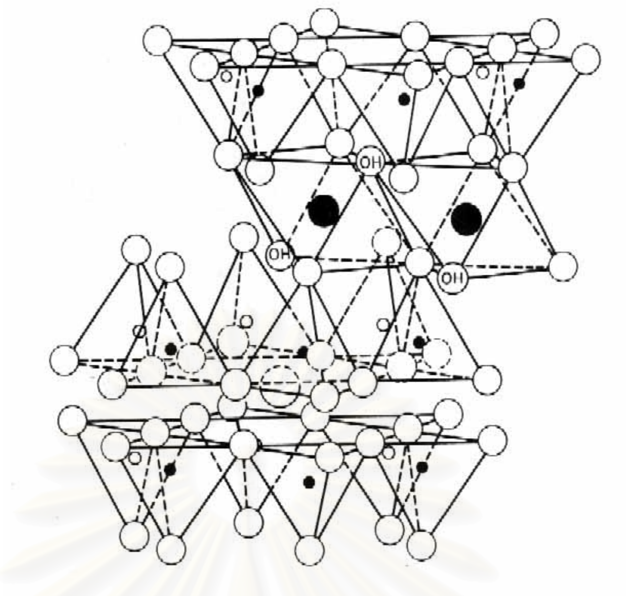
When natural clay is treated with acid having H_2SO_4 nomality up to 2N, beyond which the de-alumination of clay starts with acid affecting octahedral aluminium sheet. The acid activation process is often quite severe and destroys much of the clay layer structure as it removed iron, aluminium and magnesium from the octahedral layer. Scanning electron microscopy shows that the edges of the clay particles become very disordered. By acid leaching, some of the organic matter or impurities covering the active adsorption sites are leached. This increases the pore area and hence exposes more of the active adsorption sites.

In acid activated montmorillonite, the surface acidity of clay is explained in terms of its structure. The structure of montmorillonite clay comprises two tetrahedral silicon layers surrounded by a central octahedral aluminium layer. Substitution of Mg^{2+} for Al^{3+} in octahedral layer and Al^{3+} for Si^{4+} in the tetrahedral layer result in the development of a negative charge in the silicates layer, which is normally neutralized by the hydrated cations present between the sheets. Consequently, clay will have two types of cations, viz., exchangeable extra lattice cations like Na^+, Ca^{2+}, etc. and octahedral Al^{3+}, Mg^{2+} and Fe^{3+} cation. The majority of the exchangeable cations Na^+, K^+, Ca^{2+} get leached out when treated with acid or exchanged by other cations. Lewis acidity generally arises from exposed Al^{3+}, Fe^{3+} ions at the crystallite edge. Bronsted acidity in clays arises from H^+ ions occupying exchanged sites on the surface.

2.3.5 Bentonite

Bentonite is a clay mineral derived from the alteration, over geological time periods, of glassy material emitted from volcanoes-tuff and ash or from alteration of silica bearing rocks such as granite and basalt. The environment requirements for the formation of the clay that is the main component found in bentonite are only approximately known and different climatic and hydrological environments together with the different ages and depths of occurrence produce minor variations in this clay [30].

Bentonite is a naturally occurring material consisting predominantly of the clay mineral montmorillonite and a variety of accessory minerals. These may include lesser amounts of other clay minerals such as attapulgite, kaolin, mica and illite as well as non-clay minerals like quartz, feldspar, calcite and gypsum. Montmorillonite are three-layer clay minerals. They consist of two tetrahedral layers of interconnected SiO_4-tetrahedrons which enclose a central $M(O,OH)_6$-octahedron layer (M=Al, Fe, Mg and others). The silicate layers have slightly negative charge that is compensated by exchangeable ions in the intermediate layers. The charge is so weak that the cations (in natural form, predominantly Ca^{2+}, Mg^{2+} or Na^+) can be adsorbed with an associated hydrate shell. An essential characteristic of all montmorillonite is their ability to absorb tremendous amounts of water and other liquids into their sheet structures. This gives bentonite extraordinary swelling and adhesive properties that are exploited commercially by many industries. It is used as a binder of sand in the foundry industry, an additive to drilling mud in the oil drilling industry, a clarifier in the wine and fruit juice industry and others [37, 38, 39].



○ Oxygens, (OH) Hydroxyls, ● Aluminum, ◯ Potassium
o and • silicons (one fourth replaced by aluminums)

Figure 2.10 Atomic structure of a layer of bentonite, made of a sheet of Al(O,OH)$_6$ octahedral between two sheets of SiO$_4$ tetrahedral

2.4 Literature reviews

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

Barnett *et al.* [7] revealed a method of extracting carotene from oils or fats. According to their invention, oil is extracted with suitable volatile solvent, such as benzene. Afterward, the carotene solution was evaporated in order to remove excess solvent. During evaporation, some of the carotene separate or be deposited in form of crystals, which may be removed in the usual manner, such as in a centrifuge or by filtration. By this simple method, carotene is recovered from oil without loss of carotene.

Keat *et al.* [8] disclosed a process for recovery of carotenes and the production of carotene concentrates from palm oil. Crude palm oil was tranesterified with 79 to 120 parts by weight of methanol and 0.5 to 1.0 parts by weight of a base catalyst. After transesterification reaction, the ester was separated from the glycerol, and wased with water until the washing was neutral. The ester was then dried using drying agents or vacuum. The dried ester was then added with refined and deodorized red palm oil. The resulting mixture was then passed through a vacuum distillation column at a pressure of 0.020 to 0.025 Torr and a temperature 90^0C. The concentrate obtained after the distillation was 6570 ppm.

Ooi *et al.* [9] studied the recovery of carotenoids from palm oil. The carotenoids were recovered through a two-stage process involving transesterification of palm oil followed by molecular distillation of the ester. The crude palm oil was transesterified with methanol/ethanol at a 2:1 molar ratio of oil to alcohol, catalyzed by 0.5 % (w/w) sodium hydroxide to alkyl esters. The carotenes in the transesterfied mixture were recovered by distilling off the esters at 110-170 ^{0}C under high vacuum. The carotenoid fraction contained more than 80,000 ppm.

Nische *et al.* [10] disclosed a process for recovery of carotene from palm oil in five process steps in an economical industrial process. The process began with transesterification. The crude palm oil was catalytically reacted in known manner with an alkanol containing up to 4 carbon atoms to form fatty acid alkyl ester and glycerol. The ester phase was subjected to distillation to remove the fatty acid alkyl ester at 130-150 ^{0}C under high vacuum. The distillation residue was saponified with the sodium hydroxide solution at 105 ^{0}C for 4 hours. The carotene was extracted from the soap solution obtained in the saponification step with the solvent mixture of one part of n-hexane and two parts of acetone. The extract phase was concentrated by evaporation. A yield of carotene at least 80 % was achieved.

Tan and Saley [11] showed a process for recovery of carotenoids and tocotrienols from oil which consists of transesterification and solvolytic micellization. The crude palm oil was transesterified to form an ester-rich layer and glycerol-rich layer. The ester-rich phase was contacted with a lower alkyl alcohol and water under conditions sufficient to cause solvolytic micellization of the ester-rich layer without destroying the carotenoids and tocotrienols, thereby forming a carotenoid-rich layer. The ester-rich layer was separated from the carotenoid-rich layer under conditions sufficient to prevent destroying the tocotrienols in the ester-rich layer. The carotene-rich layer was evaporized to remove residual methanol. The concentration of the carotenoids in the carotenoid-rich layer was about 100,000 ppm.

Kunst *et al.* [12] revealed preparation of concentrates of coloring agents. Concentrates of natural coloring agents such as carotene are prepared from organic media, particulary from palm oil, by a process in which the oil, together with a volatile solvent, is subjected to gel permeation chromatography. For separation with gel permeation the molecular weight of the components to be separated should ideally be within the useful molecular weight working on range of the gel bed. Suitable column materials include cross-linked polystyrene polymers, cross-linked dextrans, cross-linked agaroses, cross-linked celluloses, silica gel and modified silica gel. The concentrated coloring agent may then be used in food products such as margarine and ice cream.

Khachik [13] disclosed a procedure for isolation and crystallization of carotenoids in high purity from crude palm oil which has 2 % to almost 30 % carotene content. An organic solution of palm oil with about 2 % carotene content in solvents such as tetrahydrofuran, tert-butylmethyl ether (TBME) or similar ethers was first saponified e.g. with about 10 % ethanolic or methanolic potassium or sodium hydroxide and after work up, carotenoids were obtained in about 49-55 % purity. Crystallization of this crude product with acetone and an alcohol or tetrahydorfuran and an alcohol at about -40 ^{0}C or lower increases the purity of carotenes to about 76-80 %. The purity of the carotenes could be increased to about 93% or greater with the fore-mentioned solvents. In the case of palm oil with about 20 % to about 30 % carotene content, the saponification step may be eliminated and combinations of organic solvents such as tetrahydrofuran, acetone, and an alcohol may be used as crystallization solvents. In a typical process, palm oil carotene was dissolved in tetrahydrofuran and acetone and the mixture of carotenoids were then crystallized by addition of an alcohol followed by cooling at low temperature (about -40 ^{0}C or lower). The purified mixture of carotenoids obtained by this method was free from oils and could be readily formulated into nutritional supplements for human use as well as proving a suitable and effective color additive for foods.

Baharin *et al.* [14] studied the separation of carotene from crude palm oil by adsorption chromatography with a synthetic polymer adsorbent. The synthetic adsorbents in this experiment were synthetic highly porous resin (HP10-50) and styrene-divinyl benzene copolymer, which were obtained from Mitsubishi Chemicals

Company (Tokyo, Japan). Isopropanol was used as the initial elution solvent because of reverse – phase chromatography. The second solvent was n-hexane, which was preferable for elution the carotene adsorbed on the hydrophobic surface of the adsorbents. The results show that the maximum adsorption capacity of HP-20 was about 10 times that of the other adsorbents. This result indicates that the HP-20 resin has superior ability to adsorb palm carotene in the initial solvent, isopropanol. Carotene was concentrated to about 10^5 ppm solution, which was about 160 times the original concentration in crude palm oil. 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.

Latip *et al.* [15] studied the extraction of carotene from crude palm oil with different type of the synthetic adsorbents in the batch system. The adsorbents in this experiment were a synthetic highly porous resin (HP-20), the synthetic porous resin (SP 850, SP 825 and SP 207) and a styrene-divinyl benzene copolymer (SDVB), were obtained from Mitsubishi Chemical corporation (Tokyo, Japan) and the synthetic adsorbents (Relite Exa 31, Relite Exa 32 and Relite Exa 50) were obtained from Resindion (Milano, Italy). The adsorption process was maintained at 50-55 ^{0}C for 1 hour. After the adsorption process had been completed, the crude palm oil/adsorbent slurry was subjected to soxhlet extractor to extract the crude palm oil from the adsorbent using isopropanol. The isopropanol extraction time was about 2.5 hours at 80-85 ^{0}C. Hexane was then used to extract the carotene from the adsorbent. The carotene extraction process was carried out at 60-65 ^{0}C and was continued until the adsorbent became clear (about 4 hours). The results show that SP 850 gave the highest percentage of carotene extracted. Carotene was concentrated to about 15,000 ppm, which was about 25 times the original concentration in crude palm oil. Carotene recovery varied from 30 to 62 % depending on the process conditions.

Siew *et al.* [16] carried out the experiment by using silica for bleaching of palm oil. The amount of bleaching earth required in the physical refining process of palm oil depends on the activity of earth, quality of the oil and final color specification of the refined products. The use of silica (Trisyl) in combination with bleaching clay in palm oil refining has been investigated. The optimum conditions required for Trisyl and bleaching clay were 95-105 ^{0}C for a period of 30-40 minutes. Improvements in color performance for palm oil products were achieved with the addition of small quantities of Trisyl (0.06-0.24 %) to the bleaching clay. Addition of 0.12 % Trisyl to 0.4 % bleaching clay improved the color of the refined oil by as much as 1.7 red lovibond units. Lower phosphorus level (18.4 and 16.9 ppm) were obtained in the refined oils with an addition of 0.12 and 0.24 % Trisyl, respectively, as compared to a level of 36.2 ppm of phosphorus when no silica was added to the earth. Better color stability was also obtained with oil treated with Trisyl.

Hussein *et al.* [17] studied carotene removal of red palm oil using kaolin-carbon adsorbents which were prepared with and without sulfuric acid pretreatment followed by activation-carbonization at 500 ^{0}C. The mixture of red palm oil and adsorbent was stirred for 40 minutes at room temperature for carotene removal. The adsorbed carotene was extracted with soaking in toluene. The results show that a maximum removal of carotene from red palm oil was obtained at 20 % kaolin loading for both adsorbents prepared with and without sulfuric acid pretreatment with about 45 and 65 % carotene removal, respectively. About 3-4 % of carotene adsorbed could be recovered from both types of adsorbents under optimum condition, in which the percentage recovered decreased with the increase in kaolin loading.

The study by Thanwongjathroengit [18] shows 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 reveal that the factors affecting degradation of β-carotene extraction in palm oil were heat and antioxidant. 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 ^{0}C adsorption temperature for 30 minutes and the appropriate condition for elution of adsorbed carotene from activated carbon was carried out at 25 ^{0}C by using toluene as an eluent which 30 % yield. In column system, the recovery yield was 60 % with 61 % adsorption and 98 % elution.

Ma *et al.* [19] studied the adsorption kinetic of β-carotene from soy oil using regenerated clay. The regeneration of the spent clay (Tonsil Optimum 233 activated clay was obtained by TTET Union Corp., Taiwan) was conducted in box furnace at 500 ^{0}C for 50 minutes. Adsorption process was performed in the batch system under 12 mmHg. Experimental results indicate that the adsorption efficiency and regeneration efficiency was 90.50 and 94.40 %, respectively. The adsorption rate was found to be increased by increasing the ratio of clay to oil, agitation speed or temperature. The rate was also found to be increased by decreasing the particle size of the clay.

Nkpa, *et al.* [20] studied the possibility of using local Nigerian clays in refining the Nigerian crude palm oil. Results show that the Okija clay may posses some potential as an adsorptive cleanser in the refining process. 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 cleanser in refining of palm oil. Measurements of peroxide values, para-anisidine values, carotene content and stabilities of the oils using the active oxygen method were used in assessing the relative performance of the clays. The good results were obtained at acid concentrations of 2 M and 3 M. Bentonite proved better than Okija clay when the leaching was performed with 2 M H_2SO_4, but both clays had comparable effectiveness when 3M H_2SO_4 was used for leaching. The Nsu clay gave the least encouraging results. In another series of experiments, the results showed that for various dosages of clay, up to 3% by weight of the oil to be refined, the oxidative stability of the processed oil was reasonably good for oil which was neither deodorized nor treated with antioxidants.

Khoo *et al.* [21] studied the activities of various bleaching earths for removal of β-carotene in acetone solutions were found to be in the decreasing order tonsil, fuller’s earth, silica and kaolin. Experimental evidence indicates that except for silica, in addition to physical adsorption, processes involving chemisorption and subsequent chemical reaction proceeded on the surface of the clay minerals. Mattallic ion such as Fe^{3+}, were suggested to be the active sites for chemisorption. Oxygen was not essential for activity.

Liew *et al.* [22] studied the adsorption on cation exchange bleaching clays. The X-ray diffraction patterns of commercial bleaching earths, i.e., Filtrol 105, Fulmont and Galleon V2 from Palmex and Magri palm oil Refineries, Malaysian, indicate that they consists mainly of montmorillonite mixed with smaller amount of kaolinites as well as mica and quartz and cristobalite. The active sites in these earths for the adsorption of β-carotene were identified through ion exchange and found to be the protonic sites and some metallic ions. The efficiencies of the various cation in imparting activities to the earths were found to be in the order $Mg^{2+} > Fe^{3+} > H^+ > Ca^{2+} > Na^+$. The apparent adsorption isotherms for β-carotene by filtrol, Fulmont and Galleon were the Langmuir type. The most active clay of adsorption of β-carotene was Fulmont, followed by Filtrol and Galleon. The decrease in activity after Na^+ ion exchange indicates that Na^+ions have very little acitivity for adsorption of β-carotene.

Poopunpanich [23] studies the separation of carotenoids from plam 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, ion exchanged clays, adsorption temperature, ratio of clay to palm oil, adsorption time and types of eluting solvents were investigated to determine the effect on the percentage of β-carotene adsorption and β-carotene recovery, respectively. The results show that the optimum conditions for β-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 ^{0}C adsorption temperature for 70 minutes could adsorb 99.78 % β-carotene. The suitable condition for β-carotene desorption from clay was carried out at room temperature by using tetrahydrofuran as an eluting solvent resulted in 73.73 % β-carotene desorption. Overall β-carotene from crude palm oil correspond to 73.56 % by weight of carotenoids in crude palm oil.

Sarier and Guler [24] studied the mechanism of β-carotene adsorption on activated montmorillonite. The variations in the structure of mineral during adsorption have been examined comparing the results of X-ray diffraction (XRD), differential thermal analysis (DTA), thermogravimetry (TG) and infrared (IR) spectroscopy of the acid activated montmorillonite clay mineral before and after adsorption of β-carotene. Based on the results, it was concluded that β-carotene attaches to the clay surface in the form of carbonium ions either by forming hydrogen bonds with Bronsted sites or by forming coordination bonds with Lewis sites of the activated clay mineral.

Wei *et al.* [2] studied degumming and bleaching effects on selected constituents of palm oil. The purpose is the removal of gums, trace metals, pigments, peroxide, oxidation products and other breakdown products in the crude oil by adsorption on the active surface of the bleaching earth to improve colour and stability of the final oil. Results show that valuable palm minor componets, i.e. tocols (tocopherol and tocotrienols), were retained; no significant change occurred in the total phytosterols, squalene, composition of acylglycerol and free fatty acids by using both acid-activated and neutral clays up to 1.0 %. However, acid-activated clay reduced the carotene content. Both clays gave markedly improved oxidative stability with induction period > 30 hr at 120 ^{0}C. Impurities such as pro-oxidant iron, copper and phosphorus were reduced by both clays. The bleaching effect of neutral clay was relatively poor compared to acid-activated clay in the removal of unwanted compounds.

The invention related to analysis of complex lipid sample and cholesterol esters, base on the number of double bonds in their molecule using Ag ion chromatography, are the followings:

Christie *et al.* [40] analyzed the complex lipid from the black sea invertebrates. The methyl ester derivatives of fatty acid from complex lipid samples was isolated to clean fractions differing in degree of unsaturation, with zero to six double bonds, by high-performance liquid chromatography on a stable silver ion column. Each fraction was identified combination of gas chromatography analysis of the methyl ester on capillary columns coated with polar phases, and gas chromatography-mass spectrometry of the picolinyl ester derivatives. The results show that the fatty acids from the Black Sea invertebrates *Mytilus galloprovincialis* and *Lapana thomasiana* werer achieved of most constituents present at levels of about 0.1 % of the total and above. The fatty acids found included mono- and multi-methyl branced isomers, mono- and polyunsaturated fatty acids of the (n-1), (n-3), (n-4), (n-5), (n-6), (n-7), (n-8), (n-9), (n-11) and (n-13) families, and dienoic acids with several methylene groups between the double bonds, ranging in chain length from C_{13} to C_{22}.

Hoving *et al.* [41] separated human and sheep plasma cholesterol esters by silver ion chromatography using high-performance liquid chromatography (HPLC) or solid-phase extraction columns packed with a bonded sulphonic acid phase. Two methods for the separation of cholesterol esters, base on the number of double bonds in their fatty acid moieties, were presented. Separation on a bonded sulphonic acid phase loaded with silver ions was achieved with cholesterol esters containing up to six double bonds in their fatty acid moieties. No cross-contamination between fractions with different number of double bonds was detected with the HPLC method, as was demonstrated by subsequent gas chromatographic analysis of the fatty acid moieties, following transmethylation.

CHAPTER III

EXPERIMETAL

3.1 Materials and chemicals

1. Clays were obtained from Cernic international Co.,Ltd. as followings :
    1.1 Bentonite clay (China)
    1.2 Bentonite clay (Japan)
2. Crude palm oil was supplied from Olene Co., Ltd. The oil was used without further refining.
3. β-carotene
    Analytical grade; Fluka Chemie A.G., Switzerland
4. Sulphuric acid (96%)
    Analytical grade; Carlo Erba, Italy
5. Silver nitrate
    Analytical grade; Mallinckrodt, USA
6. Sodium Chloride
    Analytical grade; Merck, Germany
7. Ethylenediaminetetra-acetic acid (EDTA)
    General purpose grade; BDH, England
8. Sodium Hydroxide
    Analytical grade; Lab-Scan, Ireland
9. Tetrahydrofuran
    Analytical grade; Lab-Scan, Ireland
10. n-Hexane
    Analytical grade; Merck, Germany
11. Toluene
    Analytical grade; Carlo Erba, Italy
12. Chlorofrom
    Analytical grade; Merck, Germany

13. Dichloromethane
    Analytical grade; Merck, Germany
14. Ethyl acetate
    Analytical grade; Merck, Germany
15. Buthylated Hydroxytoluene (BHT)
    Analytical grade; Fluka Chemie A.G.; Switzerland
16. Triethylamine
    Analytical grade; Merck, Germany
17. Acetonitrile
    HPLC grade; Merck, Germany
18. Dichloromethane
    HPLC grade; Carlo Erba, Italy
19. Methanol
    HPLC grade; Fluka Chemie A.G., Switzerland

3.2 Instruments and apparatus

1. UV-visible Spectrophotometer
   Spectronic 21 Milton Roy
2. X-ray Fluorescence Spectrometer (XRF)
   Philips PW 2400
3. Scanning Electron Microscope (SEM)
   JEOL JSM 6400
4. Surface Area Analyzer (BET)
   Micromeritics Flowsorb II 2300
5. High Performance Liquid Chromatography (HPLC)
   Varian
6. Vacuum pump
7. Rotary evaporator
8. Magnetic stirrer

3.3 Experimental procedure

3.3.1 The acid activation of clays [23]

Fifty grams of each clay was refluxed with 250 ml of 4N H_2SO_4 in a 500 ml round bottom flask at 90 ^{0}C for 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 deionized water until the filtrate was almost neutral to pH indicator paper. The washed clay was dried at 120 ^{0}C in an oven for 6 hours and ground to fine powder before use.

3.3.2 Preparation of the Ag-treated clays

3.3.2.1 Cation exchange method

a) Ag-treated clay (1-step)

Twenty five grams of clay was stirred with 500 ml of 10 % $AgNO_3$ solution at 60 ^{0}C for 4 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 deionized water until it free of residual silver ion (test by NaCl solution). The washed clay was dried at 120 ^{0}C in an oven for 6 hours and ground to fine powder before use.

b) Ag^+-treated clay (2-steps)

Twenty five grams of acid activated clay from topic 3.3.2.1 was stirred with 500 ml of $AgNO_3$ solution at 60 ^{0}C for 4 hours. The quantity of silver was varied in the range of 2.5 – 30 % by weight. 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 deionized water until it free of residual silver ion (test by NaCl solution). The washed clay was dried at 120 ^{0}C in an oven for 6 hours and ground to fine powder before use.

c) AgO-treated clay (2-steps)

The Ag-treated clay (2-steps) from topic 3.3.2.1 b) was calcinated at 300 ^{0}C for 6 hours in an oven.

d) AgCl-treated clay (2-steps)

Ten grams of Ag-treated clay (2-steps) from topic 3.3.2.1 b) was stirred with 100 ml 5 % NaCl solution at room temperature for 1 hour. The slurry was filtered through a buchner funnel using a vacuum pump. The filter cake on filter paper was repeatedly washed with deionized water. The washed clay was dried at 120 ^{0}C in an oven for 6 hours and ground to fine powder before use.

3.3.2.2 Reduction with EDTA method

a) Ag^{0}-treated clay [42]

A mixture containing of EDTA solution and NaOH solution was heated to boiling until the clear solution was obtained. The mole ratio of EDTA to Ag^{+} is 2:1 and the pH of solution was maintained about 13. Five grams of acid activated clay from topic 3.3.1 and 20 % $AgNO_3$ solution were added under vigorous stirring. The mixture was stirred for 4 hours. The slurry was filtered through a buchner funnel using a vacuum pump. The filter cake on filter paper was repeatedly soaked in 0.05 M NaOH. Then it was washed with deionized water until a constant pH was obtained. The washed clay was dried at 120 ^{0}C in an oven for 6 hours and ground to fine powder before use.

3.3.3 Determination of clay properties

3.3.3.1 Structure of clays

The structure of clay was previously characterized by X-ray Fluorescence Spectrometer and Scanning Electron Microscope.

3.3.3.2 Surface area

Clays were dried in an oven at 120 ^{0}C for 6 hours before they were determined the surface area with surface area analyzer (BET).

3.3.3.3 Acidity [23]

Acidity of clays was determined by volumetric titration. In this method, 0.5 g of the clay, previously dried at 120 ^{0}C for 6 hours, was taken in a conical flask to which 15 ml of 0.1 N NaOH was added. After stirring the flask for 10 minutes, excess NaOH was titrated with 0.1 N H_2SO_4. Acidity was determined as milliequivalents of NaOH used per 100 g of clay.

3.3.3.4 The adsorption isotherm of β-carotene by AgCl-treated bentonite

The concentration of β-carotene in THF solution were prepared in the range of 50-800 ppm. Then, 1g of adsorbent, AgCl-treated bentonite (China) treated bentonite treated with BHT, was added to 10 ml of β-carotene solution and the mixture was stirred for 1 hour at room temperature. After adsorption, the mixture was centrifuged and the residual solution was measured β-carotene content by UV-visible spectrometry. The adsorption isotherm was plotted by the experimental data.

3.3.4 Adsorption of carotene from palm oil on the prepared clays [23]

Crude palm oil (CPO) was directly used without further refining. Adsorbents were dried in oven for about 30 minutes before use. The adsorption process was initiated by adding crude palm oil to the adsorbent at ratio of clay to palm oil as 1:5. The mixture was heated to 60 ^{0}C and stirred for 60 minutes. Then, the mixture was filtered through a buchner funnel which was connected to a vacuum pump. The oil 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 (THF) 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 λ_{max} = 455 nm.

3.3.5 The influence of parameters for adsorption step

3.3.5.1 Effect of type of adsorbents

Each adsorbent (as shown in table 3.1) was used along with crude palm oil and followed the experiment as in topic 3.3.4.

Table 3.1 The prepared clays for study of effect of type of adsorbents

Bentonite from China	**Bentonite from Japan**
1. Original bentonite	14. Original bentonite
2. Acid activated bentonite	15. Acid treated bentonite
3. Ag-treated bentonite (1-step)	16. Ag-treated bentonite (1-step)
4. 0.5 % Ag$^+$-treated bentonite (2-steps)	17. 0.5 % Ag$^+$-treated bentonite (2-steps)
5. 1.0 % Ag$^+$-treated bentonite (2-steps)	18. 1.0 % Ag$^+$-treated bentonite (2-steps)
6. 1.5 % Ag$^+$-treated bentonite (2-steps)	19. 1.5 % Ag$^+$-treated bentonite (2-steps)
7. 0.5 % AgO-treated betonite (2-steps)	20. 0.5 % AgO- treated betonite (2-steps)
8. 1.0 % AgO-treated bentonite (2-steps)	21. 1.0 % AgO-treated bentonite (2-steps)
9. 1.5 % AgO-treated bentonite (2-steps)	22.1.5 % AgO-treated bentonite (2-steps)
10. 0.5 % AgCl-treated betonite (2-steps)	23. 0.5 % AgCl-treated betonite (2-steps)
11. 1.0 % AgCl-treated bentonite (2-steps)	24.1.0 % AgCl-treated bentonite (2-steps)
12. 1.5 % AgCl-treated bentonite (2-steps)	25. 1.5 %AgCl-treated bentonite (2-steps)
13. Ag^0-treated bentonite	26. Ag^0-treated bentonite

3.3.5.2 Effect of adsorption time

Each adsorbent was used along with crude palm oil at various times (as shown in table 3.2) and followed the experiment as in topic 3.3.4.

Table 3.2 Effect of adsorption time

Adsorbents	Adsorption time	Ratio of clay to oil	Adsorption temperature	Eluting solvent
0.5 % Ag$^+$-treated bentonite China (2-steps)	30 minutes	1:5	60 ^{0}C	THF
	60 minutes	1:5	60 ^{0}C	THF
	90 minutes	1:5	60 ^{0}C	THF
0.5 % AgO-treated betonite China (2-steps)	30 minutes	1:5	60 ^{0}C	THF
	60 minutes	1:5	60 ^{0}C	THF
	90 minutes	1:5	60 ^{0}C	THF
0.5 % AgCl-treated betonite China (2-steps)	30 minutes	1:5	60 ^{0}C	THF
	60 minutes	1:5	60 ^{0}C	THF
	90 minutes	1:5	60 ^{0}C	THF

3.3.5.3 Effect of ratio of clay to palm oil

Each adsorbent was used along with crude palm oil at various ratio of clay to palm oil (as shown in table 3.3) and followed the experiment as in topic 3.3.4.

Table 3.3 Effect of ratio of clay to palm oil

Adsorbents	Adsorption time	Ratio of clay to oil	Adsorption temperature	Eluting solvent
0.5 % Ag$^+$-treated bentonite China (2-steps)	60 minutes	1:2	60 ^{0}C	THF
	60 minutes	1:5	60 ^{0}C	THF
	60 minutes	1:8	60 ^{0}C	THF
0.5 % AgO treated betonite China (2-steps)	60 minutes	1:2	60 ^{0}C	THF
	60 minutes	1:5	60 ^{0}C	THF
	60 minutes	1:8	60 ^{0}C	THF
0.5 % AgCl treated betonite China (2-steps)	60 minutes	1:2	60 ^{0}C	THF
	60 minutes	1:5	60 ^{0}C	THF
	60 minutes	1:8	60 ^{0}C	THF

3.3.5.4 Effect of adsorption temperature

Each adsorbent was used along with crude palm oil at various temperature (as shown in table 3.4) and followed the experiment as in topic 3.3.4.

Table 3.4 Effect of adsorption temperature

Adsorbent	**Adsorption time**	**Ratio of clay to oil**	**Adsorption temperature**	**Eluting solvent**
0.5 % Ag^+-treated bentonite China (2-steps)	60 minutes 60 minutes 60 minutes	1:5 1:5 1:5	room 60 ^{0}C 90 ^{0}C	THF THF THF
0.5 % AgO-treated betonite China (2-steps)	60 minutes 60 minutes 60 minutes	1:5 1:5 1:5	room 60 ^{0}C 90 ^{0}C	THF THF THF
0.5 % AgCl-treated betonite China (2-steps)	60 minutes 60 minutes 60 minutes	1:5 1:5 1:5	room 60 ^{0}C 90 ^{0}C	THF THF THF

3.3.6 The influence of parameters for desorption step

3.3.6.1 Effect of type of eluting solvents

Adsorbent was used long with crude palm oil and extraction with various eluting solvents (as shown in table 3.5) and followed the experiment as in topic 3.3.4.

Table 3.5 Effect type of eluting solvents

Adsorbent	Adsorption time	Desorption temperature	Eluting solvent
0.5 % AgCl-treated betonite China (2-steps)	90 minutes	room	THF
	90 minutes	room	Hexane
	90 minutes	room	Toluene
	90 minutes	room	Chloroform
	90 minutes	room	Dichloromethane
	90 minutes	room	Ethyl acetate

3.3.6.2 Effect of desorption temperature

Adsorbent was used along with crude palm oil and extraction at various desorption temperature (as shown in table 3.6) and followed the experiment as in topic 3.3.4.

Table 3.6 Effect of desorption temperature

Adsorbent	Desorption time	Desorption temperature	Eluting solvent
0.5 % AgCl-treated betonite China (2-steps)	90 minutes	room	THF
	90 minutes	40 ^{0}C	THF

3.3.7 The influence of parameter for carotene degradation

3.3.7.1 Effect of antioxidant

Buthylated hydroxytoluene (1 %) 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 (4g) 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 and table 3.7.

Table 3.7 Effect of antioxidant

Adsorbent	**Antioxidant**	**Desorption time**	**Desorption temperature**	**Eluting solvent**
0.5 % AgCl-treated betonite China (2-steps)	treated with BHT	90 minutes	room	THF
	non treated with BHT	90 minutes	room	THF

3.3.8 Regeneration of Ag treated clay

Ten grams of used clay, 0.5 % AgCl-treated betonite China (2-steps) treated with BHT was soaked in THF for 12 hours. Then, the mixture was filtered through a buchner funnel which is connected to a vacuum pump. The used clay was dried in an oven at 70 ^{0}C for 1 hour. After 1 hour, the oven was heated up to 300 ^{0}C for regeneration of the used clay for 2 hours. The first, second, and third regenerated clay was used by following the experiment as in topic 3.3.4. at the optimum condition.

3.3.9 Quantitative analysis of carotene using UV-visible Spectrophotometer [23]

The carotene content in palm oil after adsorption and the extracted carotene were determined by diluting 0.5 ml aliquot of each supernatant with tetrahydorfuran to the appropriate dilution (10 ml) and measuring adsorbance in a UV-visible Spectrophotometer at λ_{max}= 455 nm. Standard β-carotene (>99 %) was used to prepare the calibration curve.

3.3.10 Quantitative analysis of β-carotene using HPLC [23]

The extracted carotene (from topic 3.3.4) was diluted with dichoromethane to the appropriate dilution (10 ml) and injected onto the column under the chromatographic conditions as listed in table 3.9. 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 β-carotene (>97%) was used to prepare the calibration curve.

Table 3.8 HPLC Chromatography condition for analysis of carotene

HPLC Parameter	**HPLC Condition**
Analytical column	reverse phase C_{18} 125 × 4 nm
Mobile phase	acetonitrile : dichloromethane : methanol : water
Flow rate	1.5 ml/minute
Column temperature	25 ^{0}C
Detector	UV 455 nm
Pressure	120 kg/cm^3
Sensitivity	0.2 AUFS
Attenuation	2^4 μvolt / full scale

CHAPTER IV

RESULTS AND DISCUSSION

Separation of carotenoids from palm oil was performed under various conditions of adsorption on various Ag-treated clays.

The influence of parameters, *i.e.* types of adsorbent, adsorption time, ratios of clay to palm oil, adsorption temperature, types of eluting solvent and desorption temperature for adsorption on the prepared clays and desorption from clays were studied. Moreover, regeneration of used clay was studied as a mean of environment protection. 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 chemical compositions of all clays were observed using XRF spectrometer as shown in tables A1-A18. The XRF data reported Ag content in composition of Ag-treated clays. These results indicate that Ag was added to composition of both clays (China, Japan) with cation exchange (1-step, 2-steps) and reduction with EDTA. This assumption is confirmed with the XRF spectrum of all clays as shown in figures A2-A11. Ag peak series appear in XRF spectrum of all Ag treated clays but XRF spectrum of both original bentonites do not appear Ag peak series. The Ag content of Ag-treated clay is concluded in table 4.1.

Table 4.1 The Ag content of various Ag-treated clays

Type of clays	**% Ag for preparation by weight**	**% Ag in clays by weight**
Original bentonite^C	-	-
Acid activated bentonite^C	-	-
Ag-treated bentonite (1-step)^C	10.00	4.393
0.5 % Ag^+-treated bentonite (2-steps)^C	2.50	0.604
1.0 % Ag^+-treated bentonite (2-steps)^C	10.00	1.081
1.5 % Ag^+-treated bentonite (2-steps)^C	30.00	1.621
Ag^0-treated bentonite^C	20.00	30.232
Original bentonite^J	-	-
Acid activated bentonite^J	-	-
Ag-treated bentonite (1-step)^J	10.00	9.579
0.5 % Ag^+-treated bentonite (2-steps)^J	4.00	0.594
1.0 % Ag^+-treated bentonite (2-steps)^J	10.00	1.073
1.5 % Ag^+-treated bentonite (2-steps)^J	30.00	1.641
Ag^0-treated bentonite^J	20.00	24.612

Bentonite^C= Bentonite from China, Bentonite^J= Bentonite from Japan

From table 4.1, results show that Ag treatment with cation exchange (1-step) method gave higher Ag content than cation exchange (2-steps) method. For Ag^0 treated clay, Ag^+ ions were reduced with EDTA to Ag cluster and Ag cluster deposit on clay. Unfortunately, Ag adding of clay with this method causes dissolution of the tetrahedral and octahedral sheet and release of the structural cation. This assumption was confirmed with XRF data in tables A9 and A18. The results show loss of some elements in clay compared to original bentonite.

For cation exchange methods (1-step, 2-steps), Ag^+ ions were added to compostion of bentonite by cation exchange on exchange sites and fixation on the layer of clay. About 1995, Besrest *et al.* [37] studied structural investigation of silver-added montrillonite clay with extended X-ray adsorption fine structure spectroscopy (EXAFS). They conclude that on the basal plane of the layers, some oxygen atoms are fixation points, and donors of electrons, which lead to the reduction of one, two or three of the bounded Ag^+ ions of the clusters, which then contain either two species Ag^0/Ag^+, or only Ag^0 atoms. According to this model, the formation of metallic silver clusters results from the interaction of Ag^+ ions with the clay which acts like an electron donor. Localization of the triangular silver clusters on the basal plane of oxygen atoms of the layer of the montmorillonite was shown in figure 4.1 and cation exchange of both acid activated bentonite with Ag^+ ion at various concentrations was shown in table 4.2.

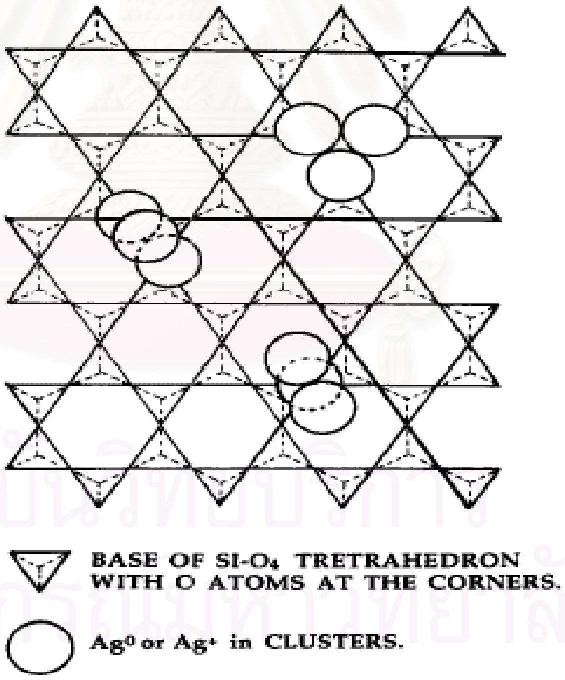


Figure 4.1. Localization of the triangular silver clusters on the basal plane of oxygen atoms of the layers of the montmorillonite. The three possible fixation figures are represented : one, two or three of the oxygen atoms of the base of the SiO_4 tetrahedra.

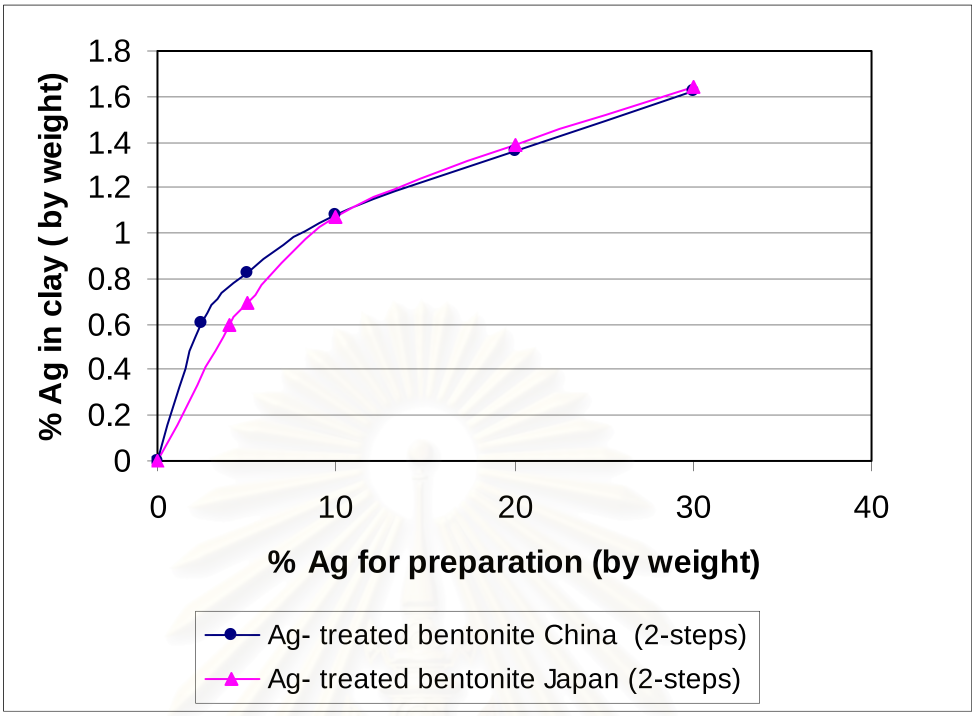


Figure 4.2 Cation exchange of both acid activated bentonite with silver ion at various concentrations

After the Ag treatment, some Ag^+-treated clay (2-steps) was calcinated at 300 ^{0}C to form AgO and treated with NaCl to form AgCl. Tables A6-8, A15-17 reported chemical composition of AgCl-treated bentonite at different percentages of Ag in clay and figures A5 and A10 show XRF spectrum of AgCl-treated bentonite. From these results, XRF data report Cl content in composition of AgCl-treated clay and the XRF spectrum shows Cl peak.

4.1.2 Acidity and surface area analysis

Acidity and surface area of clay were presented in table 4.2. The results show that acidity and surface area of both bentonites increase by acid activation with 4 N H_2SO_4. The increase in acidity of clay is explained in terms of its structure. The structure of bentonite comprises two tetrahedral silicon layers surrounded by a central octahedral aluminum layer. Substitution of Mg^{2+} for Al^{3+} in octahedral layer and Al^{3+} for Si^{4+} in the tetrahedral layer results in the development of a negative charge in silicate layer, which is normally neutralized by the hydrated cations present between the sheet. Consequently, clay will have two types of cations, viz., exchangeable extra lattice cations like Na^+, Ca^{2+}, *etc.* and octahedral Al^{3+}, Mg^{2+} and Fe^{3+} cation. The majority of the exchangeable cations Na^+, K^+, Ca^{2+} get leached out when treated with acid or exchanged by other cations. Partial dissolution of the tetrahedral and octahedral sheets and release of the structure cations such as Mg^{2+}, $Fe^{3+}$and Al^{3+} also arise from acid treatment. Lewis acidity generally arises from exposed Al^{3+}, Fe^{3+} ions at crystallite edge. Bronsted sites are generated by the exchange of interlayer cations with protons [43].

For 0.5 % Ag^+-treated clay (2-steps), results indicate that acidity and surface area of acid activated clay decrease due to Ag treatment. Ag^+ ions were added to chemical compostion of bentonite by cation exchange on exchange sites and fixation on the layer. Ag clusters fixed on clay block part of acid sites on clay. This blocking of Ag cluster is cause of decrease of acidity and surface area of Ag-treated bentonite China (2-steps). For 0.5 % AgCl-treated clay, treatment of Ag^+-treated clay with NaCl causes large decrease in acidity and surface area while calcinations at 300 ^{0}C of Ag^+ treated clay to AgO form slightly change properties of clay.

Table 4.2 Surface area and acidity of clays

Types of clays	Surface area (m^2/g)	Acidity (meg/100 g)
Original bentonite^C	33.30	110.00
Acid activated bentonite^C	170.36	148.00
0.5 % Ag^+-treated bentonite (2-steps)^C	154.08	140.00
0.5 % AgO-treated bentonite (2-steps)^C	-	138.00
0.5 % AgCl-treated bentonite (2-steps)^C	137.03	128.00

Bentonite^C= bentonite from China, Bentonite^J= bentonite from Japan

4.1.3 The adsorption isotherm of β-carotene by AgCl-treated bentonite

Apparent adsoption isotherm was obtained by plotting the mass of adsorbed β-carotene / mass of adsorbent (q) versus the residual mass of adsorbate /volume of solvent or equilibrium concentration (C). Figure 4.3 shows the Langmuir isotherm of AgCl-treated bentonite fitting with a Langmiur equation (1) of the form:

$$\text{Langmuir Isotherm} \quad q = \frac{QbC}{(1+bC)} \ldots\ldots\ldots\ldots\ldots\ldots\ldots(1)$$

q = mass adsorbed β-carotene / mass of adsorbent (mg/kg)

C = residual mass of adsorbate / volume of solvent or equilibrium concentration (mg/l)

b = adsorption equilibrium constant

Q = adsorbate surface concentration when all available adsorption sites are occupied

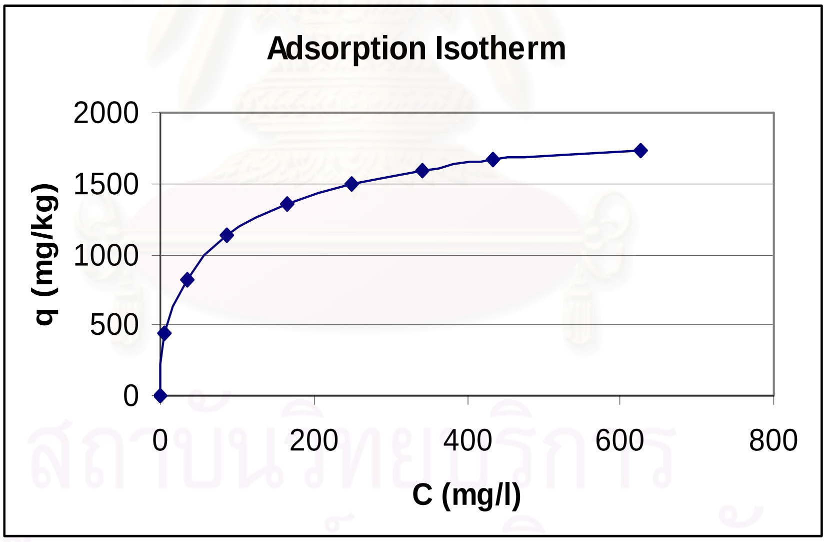
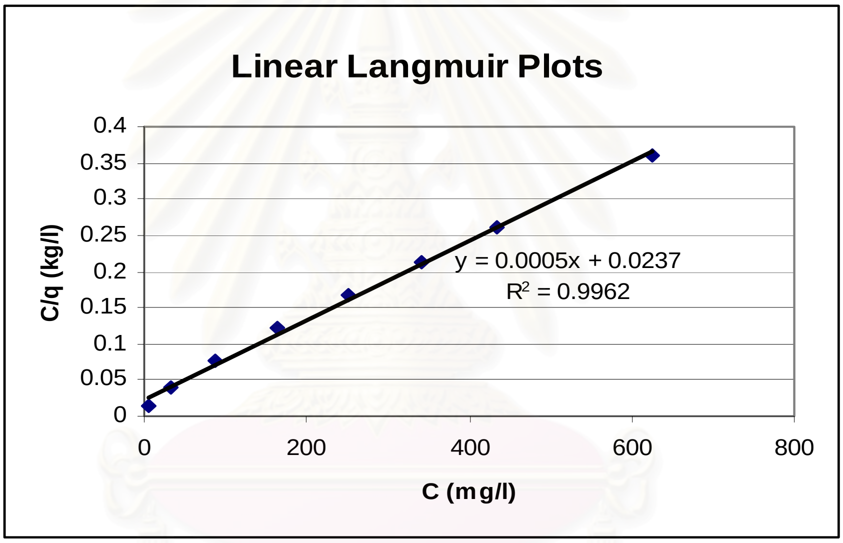


Figure 4.3 Apparent adsorption isotherm of β-carotene by AgCl-treated bentonite

At low concentration, the Langmuir isotherm may appear to be nearly linear. The non-linear form can be evaluated by transforming to the linear equation (2):

$$\frac{C}{q} = \frac{1}{Qb} + \left(\frac{1}{Q}\right)C \text{ ………………………….(2)}$$

Linear Langmuir plots which was obtained with plotting C/q versus C showed in figure 4.4. From the linear equation (2), Q and b constant can be calculated from slope and intercept in linear plots.



Q = 2000   b = 0.021

Figure 4.4 Langmuir isotherm in its linear form

The values for Q and b are inserted into the generic formular (1):

$$q = \frac{42C}{(1+0.021C)} \text{ ……………………….....(3)}$$

In addition, the isotherm which is Langmuir type implied that β-carotene is adsorbed in the form unimolecular layer.

4.2 Quantitative analysis of carotene using UV-visible Spectrophotometer

4.2.1 Adsorption maxima (λ_{max}) of carotene

From the experiment, λ_{max} of standard β-carotene by using tetrahydrofuran as a blank and measuring in a UV-visible spectrophotometer is 455 nm.

4.2.2 Calibration Curve

Standard β-carotene at various concentrations were used for measuring absorbance at λ_{max} = 455 nm to prepare calibration curve as shown in figure B1. This calibration curve was used to calculate carotene content, % adsorption and % recovery of carotene from palm oil.

4.3 Adsorption of carotene from palm oil on various clays

The study of influence of parameters for separation of carotene from palm oil in this topic was performed to determine the optimum condition. Carotene content, % adsorption, % desorption and % recovery of carotene from palm oil was determined by UV-visisble spectrophotometer.

4.3.1 The influence of parameters for adsorption step

4.3.1.1 Effect of type of clays

The various Ag-treated clays (in table 3.1) were prepared for separation of carotenoids from palm oil at the same condition. Results are shown in table C1 and figures 4.3 and 4.4.

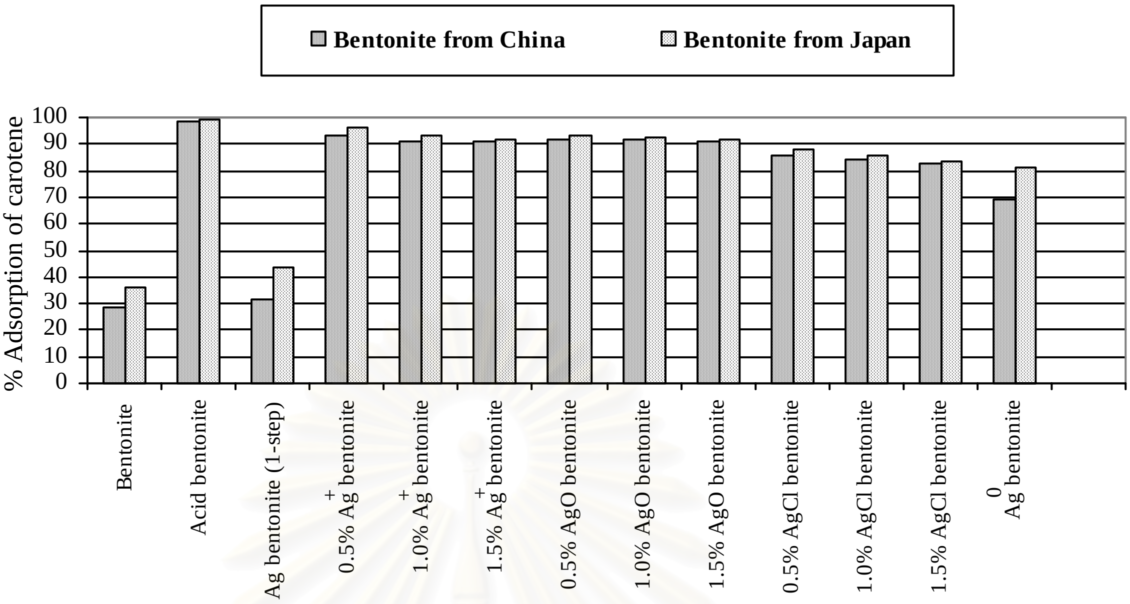


Figure 4.5 Effect of various Ag-treated clays on the % adsorption of carotene by using at 60^{0}C, 60 minutes, ratio of clay to palm oil = 1:5 and THF was used as eluting solvent

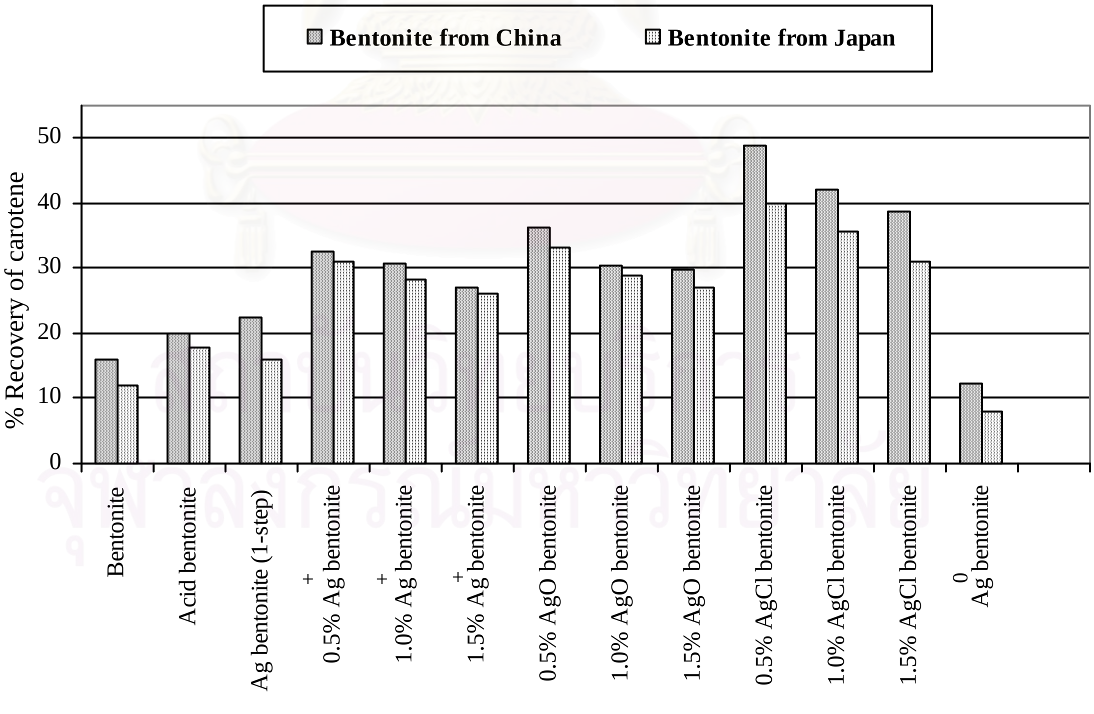
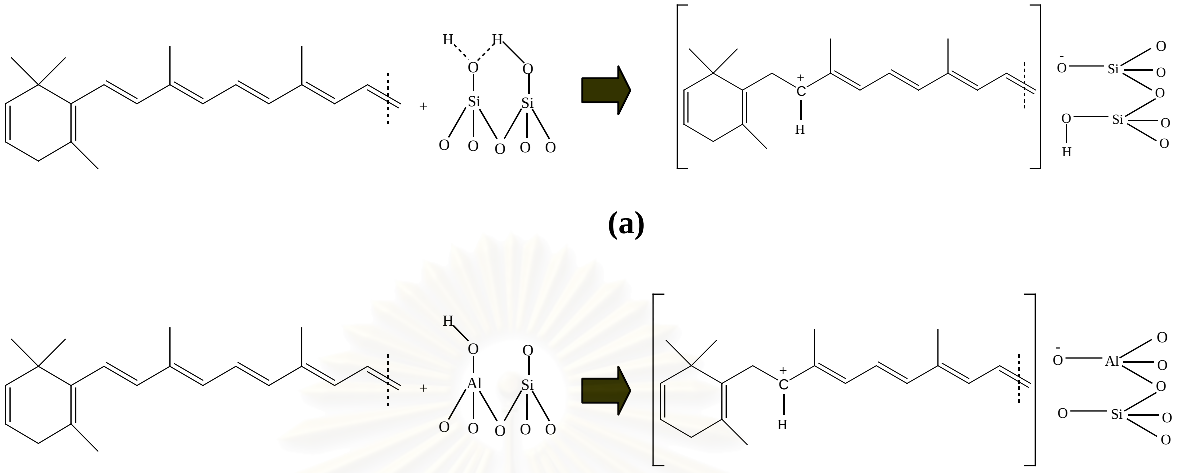


Figure 4.6 Effect of various Ag-treated clays on the % recovery of carotene by using at 60^{0}C, 60 minutes, ratio of clay to palm oil = 1:5 and THF was used

Results show that bentonite from Japan gave higher percentage of carotene adsorption compared to bentonite from China. Acid activated bentonite and Ag-treated bentonite (2-steps) have better adsorption ability than other adsorbents. It was explained that clay undergoes considerable changes to its chemical composition and physical structure during acid activation. After acid activation, clays have higher surface area because of leaching of organic matter and impurity in the clay. The consequence of acid leaching tends to expose active sites for adsorption. Acid leaching removed Al^{3+} and any ions causes charge deficiency in the clay lattice and increase acid sites on clay. These increases in acidity and surface area promote the adsorption property of clay [38]. This assumption is confirmed with results of acidity in table 4.2 and scanning electron photomicrographs in figure A1 (a-f). From scanning electron photomicrographs, acid activated bentonite and Ag-treated bentonite (2-steps) are porous particle with rough surface. Unfortunately, carotenoids are sensitive to heat, acid and light. β-Carotene was adsorbed on the surface either by forming a hydrogen bond with Bronsted sites (figure 4.5) or by forming a coordination bond with Lewis sites (figure 4.6). Thus, β-carotene attaches to the surface in the form of a carbonium ion. This ion can be decomposed only when it is heated or exposed to light with oxidation and isomerization [24]. So, acid activated bentonite gave low % recovery of carotene from palm oil. After treatment with Ag, the clay gave higher % recovery whereas adsorption ability of clay is inferior to acid activated clay. Ag^{+} ions were added to compostion of bentonite by cation exchange on exchange sites and fixation on the layer. Ag clusters which are fixed on clay block the part of acid sites on clay. Because of blocking of Ag clusters, carotenes were adsorbed on Ag clusters with physisorption, which is without degradation of carotene. According to the adsorption concept, the formation of weak reversible complexs between carotene and silver cluster arises from electron donation from occupied 2pπ orbitals of C-C multibonds to the free 5s and 5p orbitals of Ag, as well as to backbonding interaction between occupied 4d orbitals of silver and unoccupied antibonding $2p\pi^{*}$ orbitals of carotene. In conclusion, Ag-treated clay gave the high % recovery of carotene from palm oil due to physisorption of carotene on Ag clusters and decrease in acidity of clay.



(a)

(b)

Figure 4.7 The chemisorption of β-carotene on Bronsted acid sites

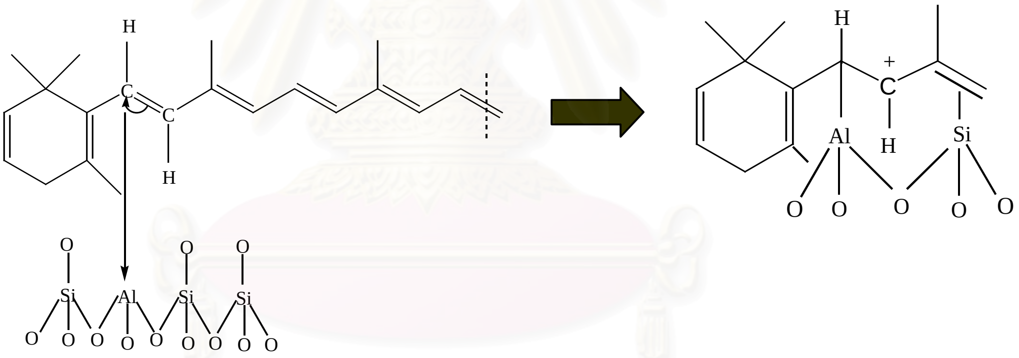


Figure 4.8 The chemisorption of β-carotene on Lewis acid sites

This experiment also studied effect of Ag-treated clay in various forms (Ag^+ form, AgO form, AgCl form and Ag^0 form) at different Ag content (0.5 %, 1.0 % and 1.5%) in clay. The results indicate that 0.5 % AgCl-treated bentonite China (2-steps) gave the highest % recovery (48.74%) of carotene but it gave less % adsorption (86.06 %) than Ag^+ and AgO adsorbents. Ag^0-treated bentonites which were prepared from acid activated clay gave low % adsorption. From figure A1 (b and g), surface of Ag^0-treated clays are not the same rough as acid activated clay. It indicated that the surface of Ag^0-treated clays is coated with Ag clusters. Therefore, adsorption capability of these clays decreases by blocking the part of acid site with Ag cluster and decrease in surface area of clays. Finally, 0.5% Ag^+, AgO and AgCl-treated bentonite China (2-steps) are good adsorbents. They gave high % adsorption and high % recovery. So, these adsorbents were selected to determine the optimum condition in the next step.

4.3.1.2 Effect of adsorption time

The effect of adsorption time was studied at 30, 60 and 90 minutes. The results are presented in table C2 and figures 4.7 and 4.8. They indicate that suitable adsorption time is 60 minutes which is saturated point of adsorption of carotenoids on clay. The 0.5 % AgCl-treated betonite China (2-steps) gave the highest % recovery. The adsorption at the less time gave low % adsorption due to unreachable saturated point. On the other hand, the longer adsorption time gave highest % adsorption but it gave lower % recovery. Because, some β-carotenes are destroyed by heat during adsorption process.

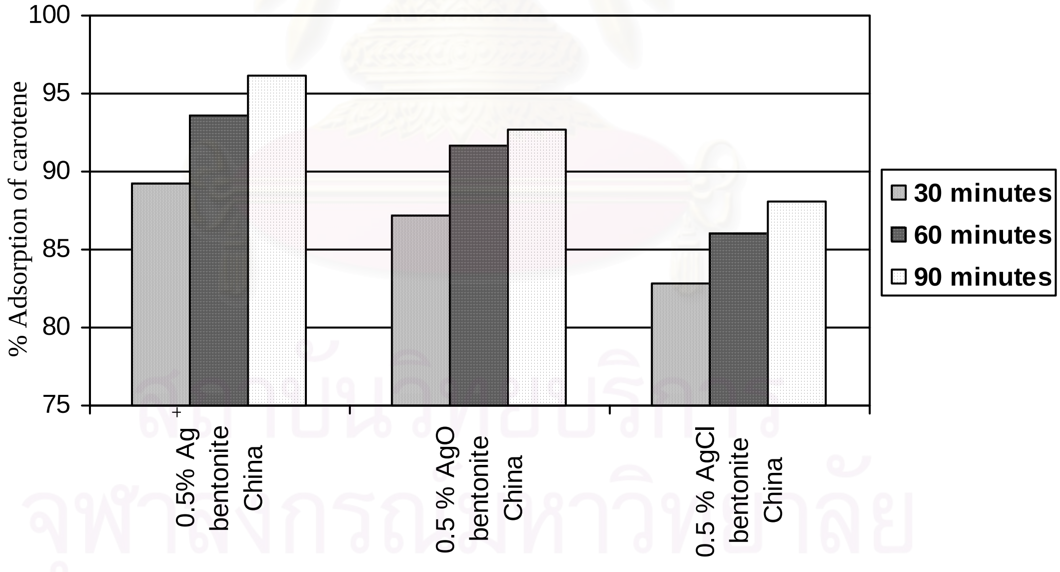


Figure 4.9 Effect of different adsorption time on the % adsorption of carotene by using various Ag-treated clays as adsorbent at 60^0C, ratio of clay to palm oil = 1:5 and THF was used as eluting solvent

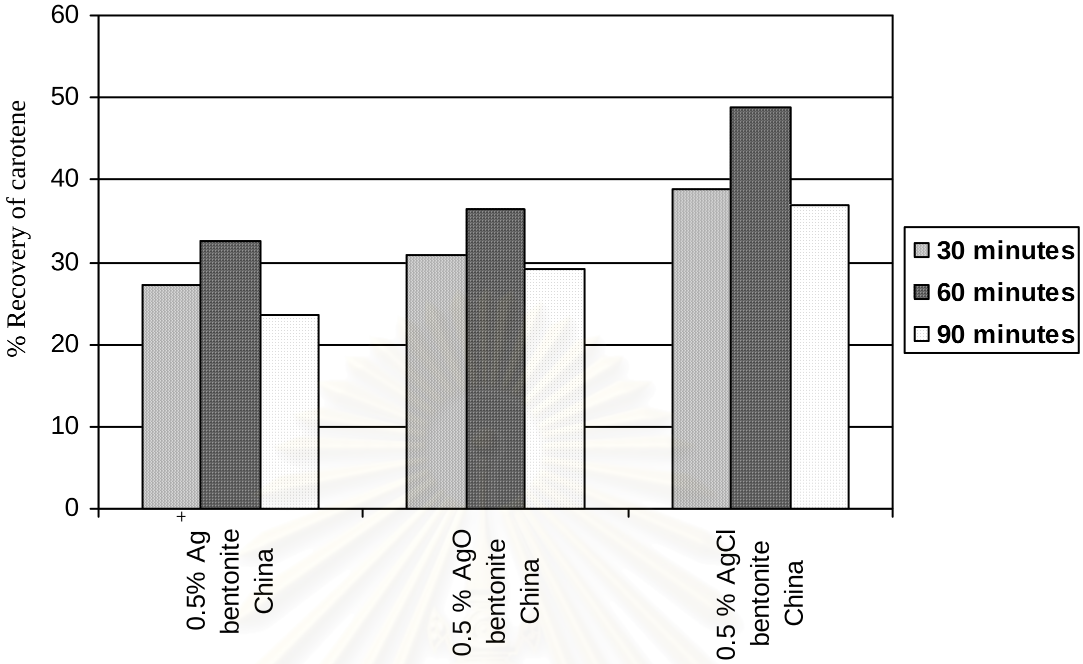


Figure 4.10 Effect of different adsorption time on the % recovery of carotene by using various Ag-treated clays as adsorbent at 60^0C, ratio of clay to palm oil = 1:5 and THF was used as eluting solvent

4.3.1.3 Effect of ratio of clay to palm oil

This experiment studied the optimum ratio of clay to palm oil for the highest yield of carotene extraction. The ratios of clay to palm oil were studied at 1:2, 1:5 and 1:8. The results are shown in table C3 and figures 4.9 and 4.10. They show that the % recovery of carotene increases by the increase in ratio of clay to palm oil as more carotene was adsorbed on the surface of the adsorbent. This is due to increase binding site availability. For these experiments, the suitable ratio of clay to palm oil is 1:5 because it gave the highest % recover of carotene.

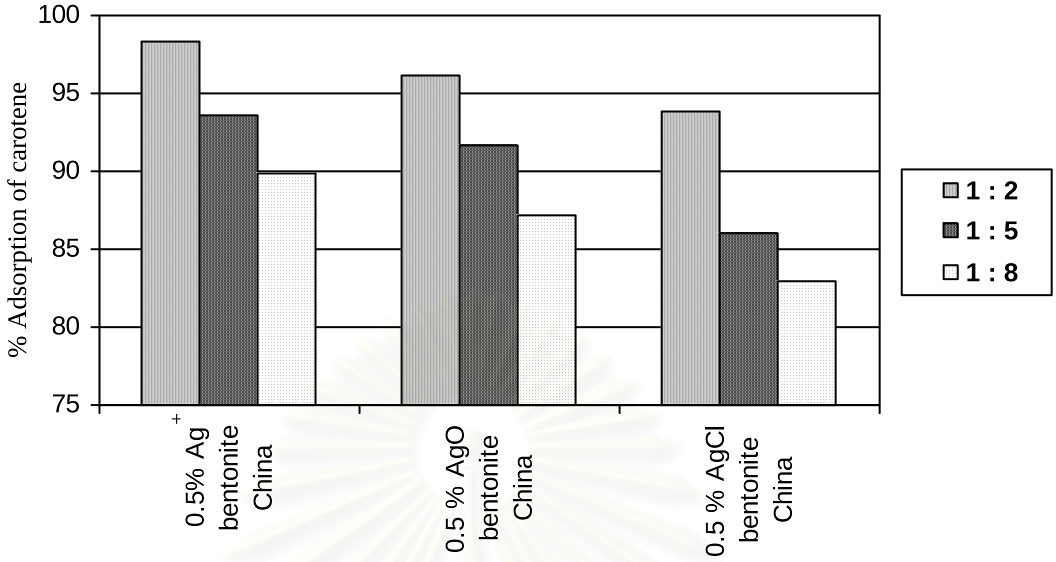


Figure 4.11 Effect of different ratio of clay to palm oil on the % adsorption of carotene by various Ag-treated clays as adsorbent at 60^{0}C, 60 minutes and THF was used as eluting solvent.

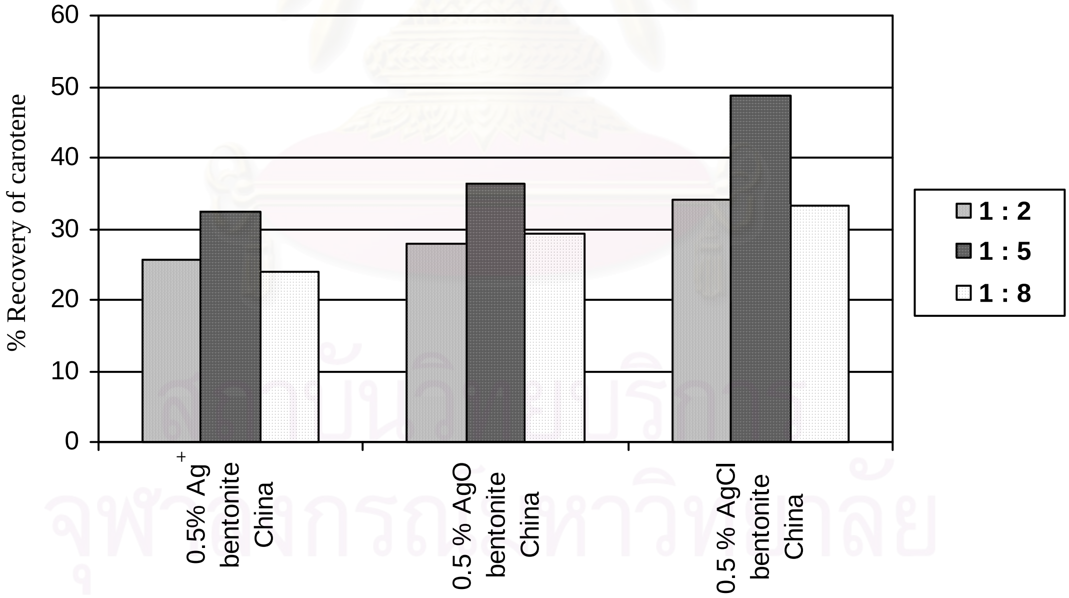


Figure 4.12 Effect of different ratios of clay to palm oil on the % recovery of carotene by various Ag treated clays as adsorbent at 60^{0}C, 60 minutes and THF was used as eluting solvent.

4.3.1.5 Effect of adsorption temperature

The effect of adsorption temperature was studied in this section. The temperature of adsorption was varied at room temperature, 60 ^{0}C and 90^{0}C. Normally, temperature in the bleching process in the refining palm oil industry is about 80-130^{0}C but adsrorption at high temperature promote degradation of β-carotene, which is oxidation of carotene. In this experiment, adsorption process at low temperature was studied in order to decrease loss of β-carotene. The results are shown in table C4 and figures 4.11 and 4.12. From the results, it was concluded that the suitable temperature is 60^{0}C because degradation of carotene at elevated temperatures was possible.

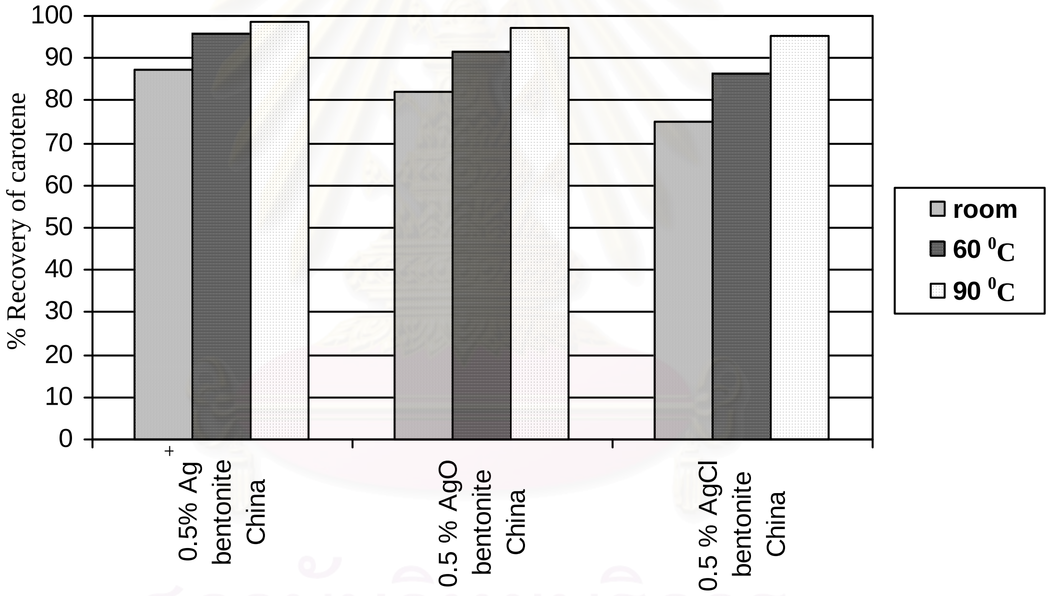


Figure 4.13 Effect of different adsorption temperature on the % adsorption of carotene by using various Ag-treated clays as adsorbent for 60 minutes, ratios of clay to palm oil = 1:5 and THF was used as eluting solvent.

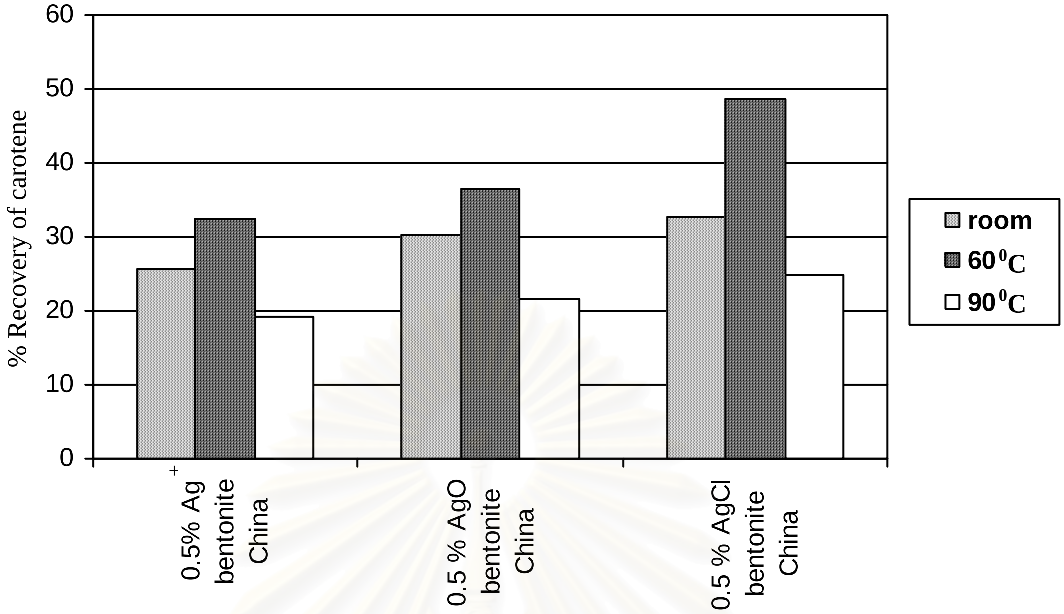


Figure 4.14 Effect of different adsorption temperature on the % recovery of carotene by using various Ag-treated clays as adsorbent for 60 minutes, ratios of clay to palm oil = 1:5 and THF was used as eluting solvent.

4.3.2 The influence of parameters for desorption step

4.3.2.1 Effect of type of eluting solvents

Eluting solvent was one of the important factors in desorption step. In this experiment, hexane, toluene, chloroform, dichloromethane, tetrahydrofuran, and ethyl acetate were used as eluting solvent for the evaluation of β-carotene extraction from crude palm oil. Results are shown in table C5 and figure 4.13. The % recovery of carotene in hexane, toluene, chloroform, dichloromethane and ethyl acetate less than that from tetrahydrofuran. In 1992, Craft and Soares studied relative solubility, stability of β-carotene in organic solvents. They conclude that the solubility of β-carotene was greatest in tetrahydrofuran. The study on the stability of β-carotene in various solvents found that β-carotene has the most stability in tetrahydrofuran [31].

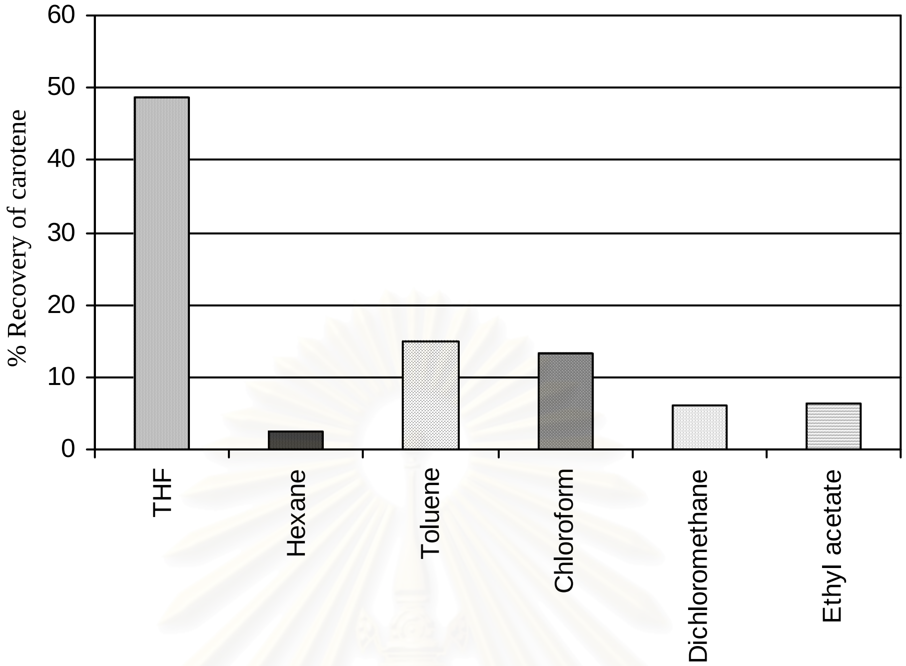


Figure 4.15 Effect of different type of eluting solvents on % recovery of carotene by using 0.5 % AgCl-treated bentonite China (2-steps) as adsorpbent at 60 ^{0}C, 60 minutes and ratio of clay to palm oil = 1:5

4.3.2.2 Effect of desorption temperature

The effect of desorption temperature was studied in this section. The temperature of desorption was varied at room temperature and 40 ^{0}C. The results are shown in table C 6 and figure 4.14. From the results, it was concluded that the suitable temperature is at room temperature because desorption at 40^{0}C promote degradation of β-carotene.

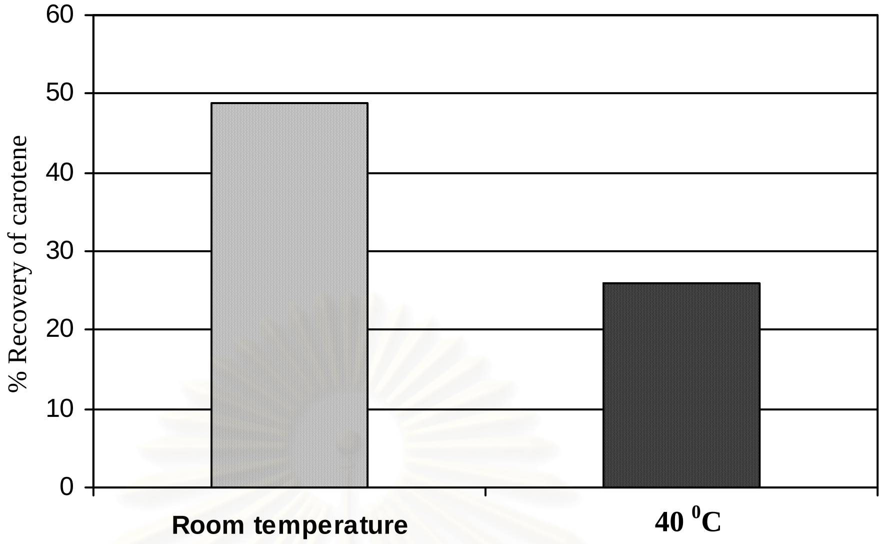


Figure 4.16 Effect of different desorption temperature on % recovery of carotene using 0.5% AgCl-treated bentonite China (2-steps) as adsorbent at 60 ^{0}C, 60 minutes, ratio of clay : palm oil = 1:5 and THF was used as eluting solvent.

4.3.3 The influene of parameter for carotene degradation

4.3.3.1 Effect of antioxidant

The effect of antioxidant was studied in this section. The antioxidant used in this experiment is butylated hydroxytoluene (BHT). The 0.5% AgCl-treated bentonite China (2-steps) treated with BHT was used in comparison with the nontreated BHT bentonite. Results are shown in table C7 and figure 4.15. The 0.5% AgCl-treated bentonite China (2-steps) treated with BHT gave higher % recovery of carotene than nontreated BHT adsorbents because BHT can protect oxidation which was one of the important of the degradation of the β-carotene.

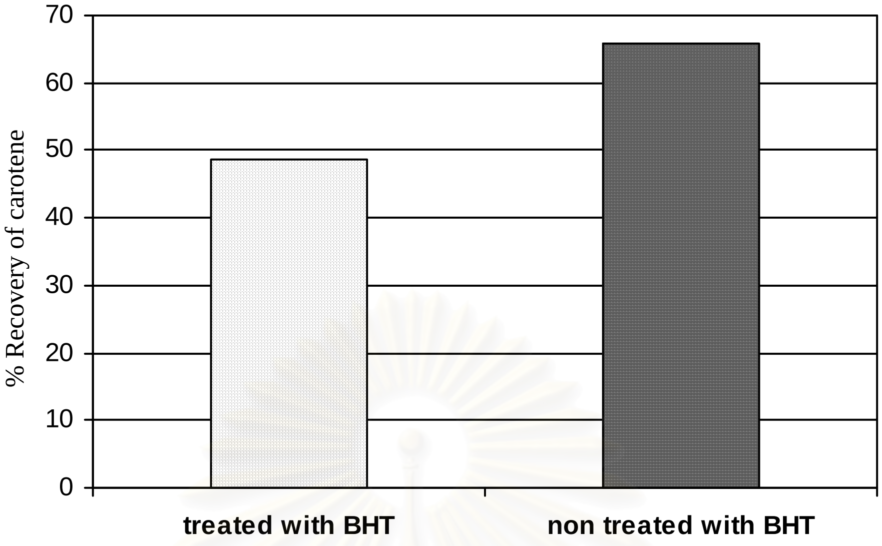


Figure 4.17 Effect of antioxidant on % recovery of carotene at 60 ^{0}C, 60 minutes, ratio of clay to palm oil = 1:5 and THF was used as eluting solvent.

4.3.4 Regeneration of adsorbent

This experiment studied the regeneration of adsorbent and efficiency of regenerated clay at the optimum condition. The adsorbent studied in the experiment is 0.5%AgCl- treated bentonite China (2-steps) treated with BHT. The used clay was regenerated with soaking in THF followed by regeneration into oven at 300^{0}C. Results were presented in table C 8 and figures 4.16 and 4.17. The results showed that the regenerated adsorbent gave lower % adsorption than fresh adsorbent and it also gave lower % recovery. It explained that soaking used clay in tetrahydrofuran only extracts in some parts of adsorbed carotene and other adsorbed organic matters. Regeneration of clay at high temperature does not only burn organic matter depositing on clay but also calcinate Ag clusters to from AgO which is inactive to adsorption. Therefore, the clay has poor adsorption capability due to blocking of carbon on adsorption sites and decreasing of carotene 455adsorption ability of Ag clusters. Moreover, BHT decomposes during regeneration at high temperature. This decomposition results in decrease antioxidation property. So, the used clay gave low % recovery of carotene.

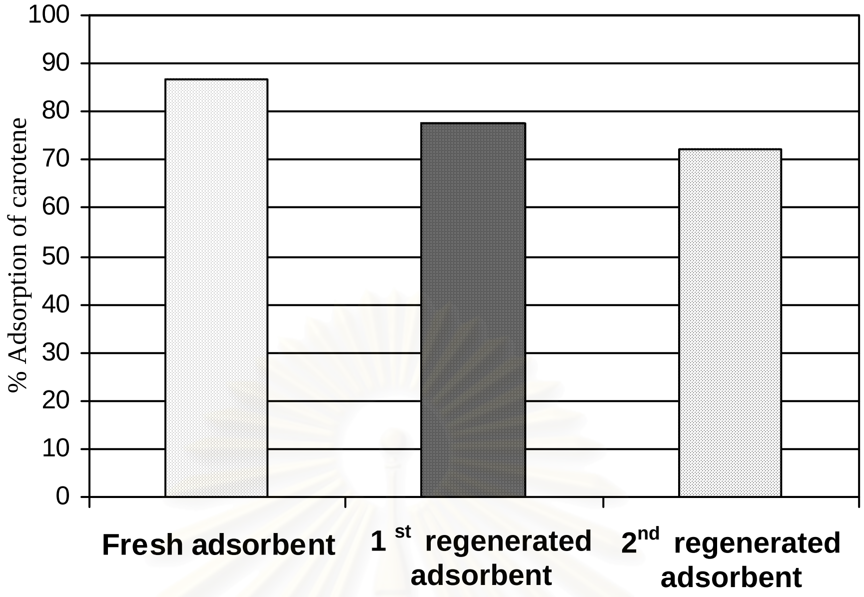


Figure 4.18 Effect of fresh adsorbent and regenerated adsorbents on % adsorption of carotene at 60 ^{0}C, 60 minutes, ratio of clay to palm oil = 1:5 and THF was used as eluting solvent.

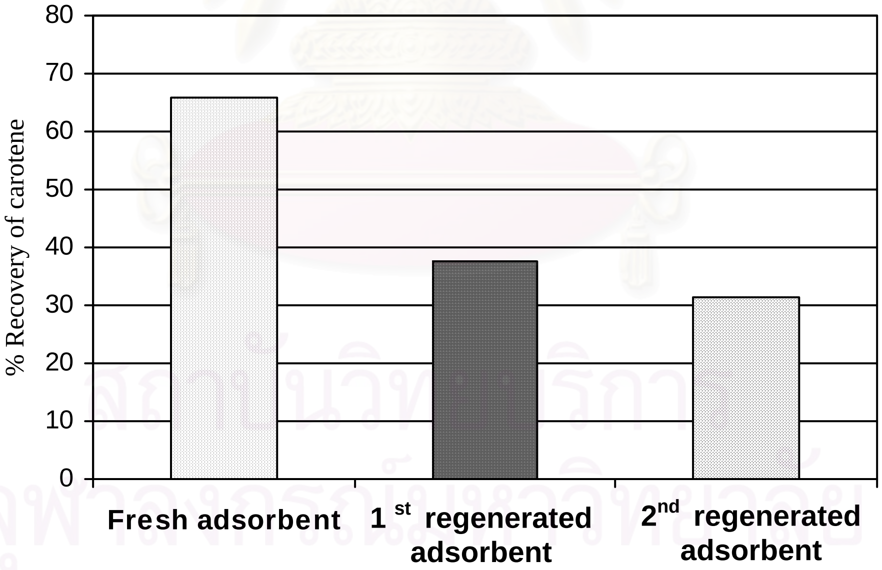


Figure 4.19 Effect of fresh adsorbent and regenerated adsorbents on % recovery of carotene at 60 ^{0}C, 60 minutes, ratio of clay to palm oil = 1:5 and THF was used as eluting solvent.

4.4 Quantitative analysis of β-carotene using HPLC

Standard β-carotene at various concentrations were used to prepare calibration curve as shown in figure B2. This calibration curve was used to calculate β-carotene content in the extracted carotene.

The chromatograms of standard β-carotene, the extracted carotene and the spiking technique by using HPLC are shown in figure B3. The spiking technique was used to confirm that the extracted carotene matched with standard β-carotene which was indicated that the peak area using the spiking technique was higher than the other peaks.

Form the experiment under chromatographic conditions (table 3.9), the extracted carotene was separated from the column at 8.162 minutes, while the standard β-carotene was separated from the column at 7.990 minutes as shown in figure B3. The calculation of β-carotene content, compared with the calibration curve (figure B2), indicated that β-carotene content in the 2.01 g of carotene extract was 2.31 mg corresponded to 37.68 % by weight of all extracted carotenes in crude palm oil.

4.5 Carotene recovery

The optimum conditions for carotene recovery which use 0.5% AgCl-treated bentonite China (2-steps) treated with BHT as adsorbent, 60^0C adsorption temperature, 60 minutes adsorption time, 1:5 ratio of clay to palm oil, THF as eluting solvent was used in this section. The initial crude palm oil (CPO) has carotene at 465.74 mg/kg palm oil. The experiment used 20.02 g of palm oil, and the initial palm oil contained 9.32 mg of carotene. After the eluting solvent was evaporated, 2.01 g of the carotene extract which has 6.13 mg carotene content is obtained. Therefore, overall carotenes which can be recovered from this experiment is 6.13 mg corresponded to 65.77 % by weight of carotene in crude palm oil. The carotene extract was about 2,990.24 ppm of carotene, which is more than 6 times the original concentration of crude palm oil.

CHAPTER V

CONCLUSION AND SUGGESTION

5.1 Conclusion

This research investigated the preparation of various Ag-treated clays: Ag-treated bentonite (1-step), Ag-treated bentonite (2-steps) at different Ag content in Ag^+ form, AgO form, AgCl form and Ag^0 treated bentonite. Both clays from China and Japan were treated with two methods as cation exchange and reduction with EDTA. All Ag-treated clays were prepared as adsorbents for separation of carotenoids from palm oil. The influence of parameters was studied for determination of the optimum condition. Results indicate that AgCl-treated clay (2-steps) which was prepared with acid activation, cation exchange with Ag^+ and treatment with NaCl is the best adsorbent because it has good adsorption properties and gave the highest percentage of recovery of carotene. The Ag-treated clay (2-steps) can improve recovery of carotene with decrease of β-carotene degradation during adsorption and desorption process. Ag^+ ions were added to compostion of bentonite by cation exchange on exchange sites and fixation on the layer of clay. Ag clusters fixed on clay block part of acid sites on clay. This blocking of Ag cluster increases in recovery of carotene because degradation of adsorbed β-carotene decreases by physisorption on Ag clusters and decrease in acidity of clay. In addition, treatment Ag-treated clay (2-steps) with NaCl to form AgCl also resulted in increasing of percentage of recovery of carotene while treatment with calcination to form AgO do not change property of clay.

Finally, the optimum condition for adsorption on adsorbents were carried out by using 0.5 % AgCl-treated bentonite (2-steps) China at a ratio of 1:5 by weight of clay to palm oil, at 60 ^{0}C adsorption temperature for 60 minutes. Higher temperature was found to be one of the factors affecting degradation of β-carotene. Besides, the treatment of antioxidant before adsorption process gave higher percentage of recovery of carotene. The suitable condition for carotene desorption was performed at room temperature by using THF as an eluting solvent. Beside, regeneration of used clay was also studied in this research. From the results, regenerated clay has poor adsorption property and it gave low % recovery of carotene.

Under the above conditions, overall carotene recovery was 6.13 mg corresponding to 65.77 % by weight of carotene in crude palm oil which was 9.32 mg. The results of this study point out that Ag treated clay was suitable for using as adsorbent in the bleaching process and useful to recovery adsorbed pigment such as β-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 for commercial and scale up for industrial scale. The choice of the correct bleaching earth to be used in any specific application must take into consideration the removal of all undesirable impurities. 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 other Thai clays, will continue to be consideration for possible application as bleaching earths such as in the edible oil industries.

REFERENCES

1. Goh, S.H., Choo, Y.M. Minor constituents of palm oil J. Am. Oil Chem. Soc. 62 (1985): 237-240.
2. Wei, P., May, C., Ngan, M., Hock, C. Degumming and bleaching: Effect on selected constituents of palm oil J. Oil Palm Res. 16 (2004): 57-63.
3. Ouyang, J.M., Daun, H., Chang, S.S., Ho, C.T. Formation of carbonyl compounds from β-carotene during palm oil deodorization J. Food Sci. 45 (1980): 1241-1217
4. Swoboda, P.A.T. Chemistry of refining J. Am. Oil Chem. Soc. 62 (1985): 287-292.
5. Stage, H. The physical refining process J. Am. Oil Chem. Soc. 62 (1985): 299-308.
6. ปาล์มน้ำมัน [online]. Available from: http://www.doae.go.th/plant/palm.htm [2004, Dec 15]
7. Barnett, H., Heights, F., F.O., A. Method of extracting carotene. United States Patent. US 2,032,165, 1936.
8. Keat, O.,May, C., Hock, A. Recovery of carotenoids. United State Patent. US 5,019,668, 1991.
9. Ooi, C.K., Choo, Y.M., Yap, S.C., Basiron, Y., Ong, A.S.H. Recovery of carotenoids from palm oil J. Am. Oil Chem. Soc. 71 (1994): 423-426.
10. Nitsche, M., Johannisbauer, W., Jordan, V. Process for obtaining carotene from palm oil. United States Patent. US 5,902,890, 1999.
11. Tan, B., Saleh, M. Integrated process for recovery of carotenoids and tocotrienols from oil. United States Patent. US 5,157,132, 1992.
12. Kunst, A., Hakkaart, M. Preparation of concentrates of coloring agents. United States Patent. US 5,246,722, 1993.
13. Khachik, F. Process for purification and crystallization of palm oil carotenoids. United States Patent. US 0158455 A1, 2003.
14. Baharin, B.S., Rahman, K., Karim, M.I., Oyaizu, T., Tanaka, K. Tanaka, Y., Takagi, S. Separation of palm carotene from crude palm oil by adsorption

chromatography with a synthetic polymer adsorbent J. Am. Oil Chem. Soc. 75 (1998): 399-404.

15. Latip, R.A., Baharin, B.S., Man, Y.B., Rahman, R. Evaluation of different types of synthetic adsorbents for carotene extraction from crude palm oil J. Am. Oil Chem. Soc. 77 (2000): 1277-1281.
16. Siew, W., Tan, Y., Tang, Y. Silica refining of palm oil J. Am. Oil Chem. Soc. 71 (1994): 1013-1016.
17. Hussein, M., Kuang, D. Zainal, Z., Teck, T. Kaolin-carbon adsorbents for carotene removal of red palm oil J. Colloid Interface Sci. 235 (2001): 93-100.
18. Thangwongjathroengit, D. Extraction of β-carotene from palm oil using activated carbon. Master's Thesis, Program of Biotechnology, Faculty of Science, Chulalongkorn University, 1994.
19. Ma, M., Lin, C. Adsorption kinetics of β-carotene from soy oil using regenerated clay Separation of Purification Technology (2004): 1-9.
20. Nkpa, N., Arowolo, T.A., Akpan, H.J. Quality of nigerian palm oil after bleaching with local treated clays J. Am. Oil Chem. Soc. 66 (1989): 218-222.
21. Khoo, L.E., Morsingh, F., Liew, K.Y. The adsorption of β-carotene I. by bleaching earths J. Am. Oil Chem. Soc. 56 (1979): 672-675.
22. Liew, K.Y., Tan, S.H., Morsingh, F., Khoo, L.E. Adsorption of β-carotene: II . On cation exchanged bleacing clays J. Am. Oil Chem. Soc. 59 (1982): 480-484.
23. Poopunpanich, W. Separation of carotenoids from palm oil by adsorption on clays Master's Thesis, Program of Petrochemistry, Faculty of Science, Chulalongkorn University, 2003.
24. Sarier, N., Guler, C. The mechanism of β-carotene adsorption on activated montmorillonite J. Am. Oil Chem. Soc. 66 (1989): 917-923.
25. Heidlas, J., Huber, G., Cully, J., Kohlrausch, U. Process for the extraction of carotenes from natural sources. United States Patent. US 5,714,658, 1998.
26. Maclellan, M. Palm oil J. Am. Oil Chem. Soc. 60 (1983): 320-325.
27. May, C. Palm oil carotenoids [online]. Available from: http://www.unu.edu [2004, Dec 15]

28. Kang, J. Refinery of palm oil [online]. Available from: http://www.andrew.cmu.edu [2004, Dec 3]

29. Tan, B. Palm carotenoids, tocopherols and tocotrienols J. Am. Oil Chem. Soc. (1989): 770-775.

30. Hendry, G.A.F., Houghton, J.D. Natural Food Colorants. 2 nd ed. London: Blackkei, 1996, pp.197-200, 228-230.

31. Craft, N. Relative solubility, stability and adsorptivity of lutein and β-carotene in organic solvents J. Agric. Food Chem. 40 (1992): 431-434.

32. Macdougall, D. Color in Food. New York: Wood Head Publishing Limited, 2002, pp. 193-196.

33. Vargas, F., Lopez, O. Loondor, B. Natural Colorants for Food and Nutraceutical Uses. New York: CRC Press, 2003, pp. 113-116, 141-143.

34. Grim, R.E. Applied Clay Mineralogy. New York: McGraw-Hill Book Company Inc, 1962, pp. 51-57, 185-230.

35. Tong, W. Introduction to clay minerals & soils [online]. Available from: http://servercc.oakton.edu [2004, Dec 15]

36. What is Bentonite? [online]. Available from: http:// www. ima-eu.org/benton.htm [2004, Dec 15]

37. Besrest, F., Benazeth, S., Souleau, Ch. EXAFS structural investigation of a silver-added montmorillonite clay Materials Lett. 24 (1995): 17-21.

38. Esmer, K. Electrical conductivity of modified bentonites and FT-IR spectroscopic investigations of some aromatic molecules adsorbed by bentonites Materials Lett. 34 (1998): 398-404.

39. Bentonite [online]. Available from: http://www.ima-eu.org [2004, Dec 15]

40. Christie, W.W., Brechany, E., Stefanov, K. Silver ion high-performance liquid chromatography and gas chromatography mass spectrometry in the analysis of complex fatty acid mixtures : application to marine invertebrates Chem. Phys. Lipids 46 (1988): 127-135.

41. Hoving, E., Muskiet, F. Separation of cholesterol esters by silver ion chromatography using high-performance liquid chromatography or solid-phase extraction columns packed with a bonded sulphonic acid phase J. Chromatography 565 (1991): 103-110.

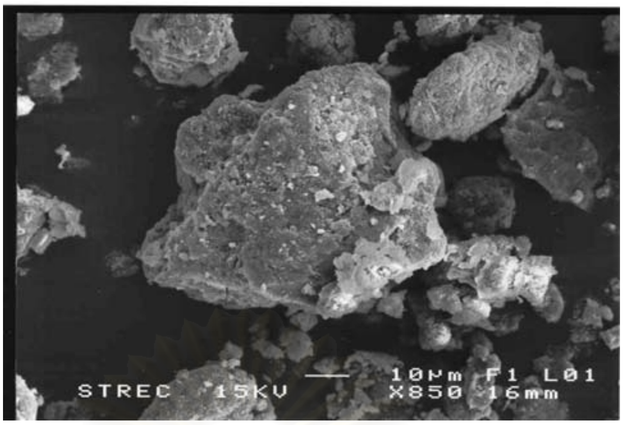
42. Heard, S., Grieser, F., Barraclough, C. The characterization of Ag sols by electron microscopy, optical adsorption, and electrophoresis J. Colloid Interface Sci. 93 (1983): 545-555.

43. Yadav, M., Chudasama, C., Jasra, R. Isomerisation of α-piene using modified montmorillonite clays J. Molecular Catalysis A: Chemical 216 (2004): 51-59.

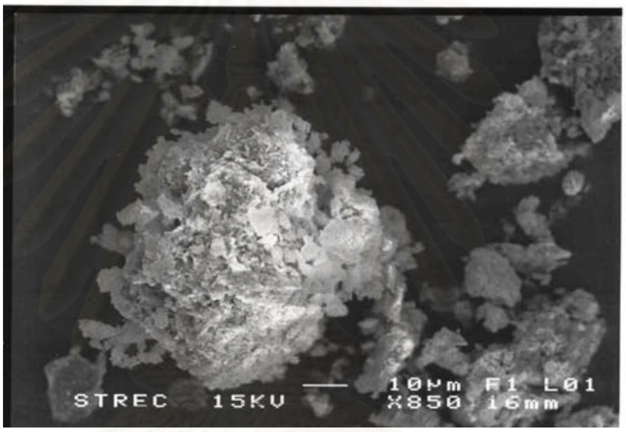
44. Kheok, S.C., Lim, E.E. Mechanism of palm oil bleaching by montmorillonite clay activated at various acid concentrations J. Am. Oil Chem. Soc. 59 (1982): 129-131.

APPENDIX A

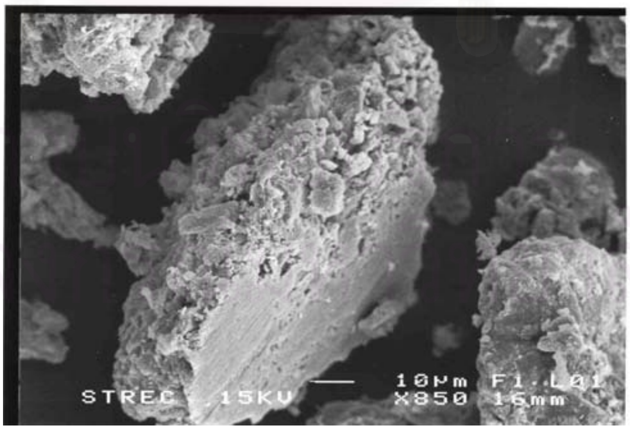
The SEM of Clay Sample
Chemical Composition and XRF Spectrum of Clay Sample



(a) Bentonite China

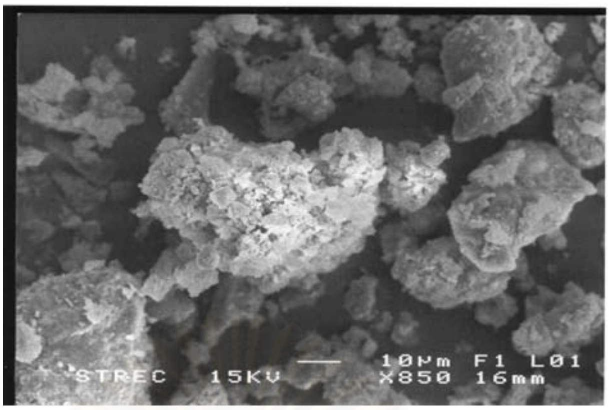


(b) Acid activated bentonite China

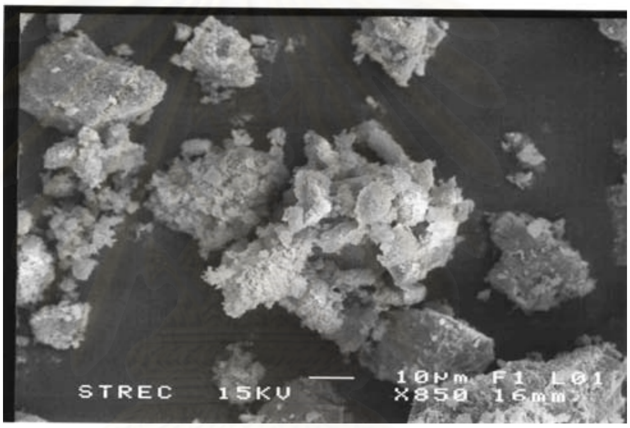


(c) Ag-treated bentonite China (1-step)

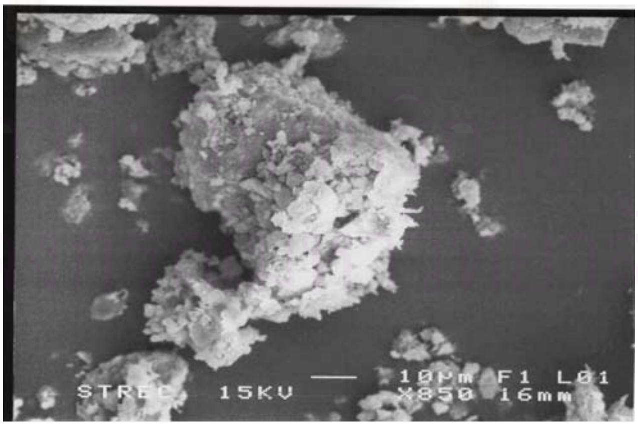
Figure A1 Scanning electron photomicrographs of clay samples



(d) 0.5 % Ag^+-treated bentonite China (2-steps)

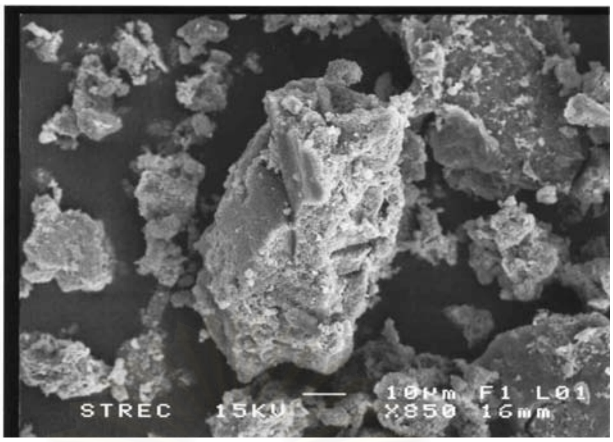


(e) 0.5 % AgO-treated betonite China (2-steps)



(f) 0.5 % AgCl-treated betonite China (2-steps)

Figure A1 (continued) Scanning electron photomicrographs of clay samples



(g) Ag^0-treated bentonite China

Figure A1 (continued) Scanning electron photomicrographs of clay samples

Table A1 Chemical composition of Bentonite China

No.	**Compound**	**Concentration % by weight**	**No.**	**Compound**	**Concentration % by weight**
1.	Na_2O	3.469	7.	CaO	2.031
2.	MgO	3.731	8.	TiO_2	0.110
3.	Al_2O_3	14.243	9.	MnO_2	0.073
4.	SiO_2	72.874	10.	Fe_2O_3	1.316
5.	P_2O_5	<<	11.	SrO	0.057
6.	K_2O	2.096	12.	ZrO_2	<<

% Ag in clay = 0.000 %

Table A2 Chemical composition of Ag-treated bentonite China (1-step)

No.	**Compound**	**Concentration % by weight**	**No.**	**Compound**	**Concentration % by weight**
1.	Na_2O	1.199	7.	CaO	1.853
2.	MgO	4.038	8.	TiO_2	0.112
3.	Al_2O_3	13.385	9.	MnO_2	0.068
4.	SiO_2	70.742	10.	Fe_2O_3	1.416
5.	ZrO_2	0.023	11.	SrO	0.039
6.	K_2O	2.081	12.	AgO	5.045

% Ag in clay = 4.393 %

Table A3 Chemical composition of 0.5 % Ag^+-treated bentonite China (2-steps)

No.	Compound	Concentration % by weight	No.	Compound	Concentration % by weight
1.	Na_2O	1.248	8.	CaO	0.295
2.	MgO	2.524	9.	TiO_2	0.142
3.	Al_2O_3	10.987	10.	MnO_2	-
4.	SiO_2	81.067	11.	Fe_2O_3	0.938
5.	ZnO	0.009	12.	SrO	0.016
6.	K_2O	2.064	13.	P_2O_5	-
7.	ZrO_2	0.016	14.	AgO	0.694

% Ag in clay = 0.604 %

Table A4 Chemical composition of 1.0 % Ag^+-treated bentonite China (2-steps)

No.	Compound	Concentration % by weight	No.	Compound	Concentration % by weight
1.	Na_2O	1.209	8.	CaO	0.306
2.	MgO	2.682	9.	TiO_2	0.114
3.	Al_2O_3	11.793	10.	MnO_2	-
4.	SiO_2	79.619	11.	Fe_2O_3	0.990
5.	ZnO	-	12.	SrO	0.015
6.	K_2O	2.017	13.	P_2O_5	-
7.	ZrO_2	0.014	14.	AgO	1.241

% Ag in clay = 1.081 %

Table A5 Chemical composition of 1.5 % Ag^+-treated bentonite China (2-steps)

No.	Compound	Concentration % by weight	No.	Compound	Concentration % by weight
1.	Na_2O	1.213	8.	CaO	0.278
2.	MgO	2.740	9.	TiO_2	0.111
3.	Al_2O_3	11.592	10.	MnO_2	-
4.	SiO_2	79.090	11.	Fe_2O_3	1.013
5.	ZnO	-	12.	SrO	0.015
6.	K_2O	2.065	13.	P_2O_5	-
7.	ZrO_2	0.015	14.	AgO	1.861

% Ag in clay = 1.620 %

Table A6 Chemical composition of 0.5 % AgCl-treated bentonite China (2-steps)

No.	Compound	Concentration % by weight	No.	Compound	Concentration % by weight
1.	Na_2O	1.245	8.	CaO	0.515
2.	MgO	2.527	9.	TiO_2	0.142
3.	Al_2O_3	9.332	10.	MnO_2	-
4.	SiO_2	82.068	11.	Fe_2O_3	0.936
5.	ZnO	0.008	12.	SrO	0.016
6.	K_2O	2.284	13.	AgO	0.650
7.	ZrO_2	0.018	14.	Cl	0.265

% Ag in clay = 0.566 %

Table A7 Chemical composition of 1.0 % AgCl-treated bentonite China (2-steps)

No.	**Compound**	**Concentration % by weight**	**No.**	**Compound**	**Concentration % by weight**
1.	Na_2O	0.661	8.	CaO	0.355
2.	MgO	1.488	9.	TiO_2	0.161
3.	Al_2O_3	11.920	10.	MnO_2	-
4.	SiO_2	79.610	11.	Fe_2O_3	1.484
5.	ZnO	-	12.	SrO	0.026
6.	K_2O	2.545	13.	AgO	1.144
7.	ZrO_2	0.018	14.	Cl	0.592

% Ag in clay = 0.996 %

Table A8 Chemical composition of 1.5 % AgCl-treated bentonite China (2-steps)

No.	**Compound**	**Concentration % by weight**	**No.**	**Compound**	**Concentration % by weight**
1.	Na_2O	0.672	8.	CaO	0.290
2.	MgO	1.437	9.	TiO_2	0.150
3.	Al_2O_3	11.629	10.	MnO_2	-
4.	SiO_2	79.370	11.	Fe_2O_3	1.422
5.	ZnO	-	12.	SrO	0.015
6.	K_2O	2.157	13.	AgO	1.860
7.	ZrO_2	0.018	14.	Cl	0.972

% Ag in clay = 1.619 %

Table A9 Chemical composition of Ag^0 treated-bentonite China

No.	Compound	Concentration % by weight	No.	Compound	Concentration % by weight
1.	Na_2O	2.590	8.	CaO	0.184
2.	MgO	1.419	9.	TiO_2	0.118
3.	Al_2O_3	7.404	10.	MnO_2	-
4.	SiO_2	51.542	11.	Fe_2O_3	0.589
5.	ZnO	-	12.	SrO	-
6.	K_2O	1.525	13.	P_2O_5	-
7.	ZrO_2	-	14.	AgO	34.716

% Ag in clay = 30.232 %

Table A10 Chemical composition of Bentonite Japan

No.	Compound	Concentration % by weight	No.	Compound	Concentration % by weight
1.	Na_2O	6.431	9.	CaO	3.215
2.	MgO	4.860	10.	TiO_2	0.396
3.	Al_2O_3	14.135	11.	MnO_2	0.114
4.	SiO_2	65.863	12.	Fe_2O_3	3.611
5.	ZnO	0.008	13.	SrO	0.025
6.	K_2O	1.045	14.	BaO	0.042
7.	ZrO_2	0.021	15.	P_2O_5	0.131
8.	SO_3	0.060			

% Ag in clay = 0.000 %

Table A11 Chemical composition of Ag-treated bentonite Japan (1-step)

No.	Compound	Concentration % by weight	No.	Compound	Concentration % by weight
1.	Na_2O	2.358	9.	CaO	2.947
2.	MgO	4.860	10.	TiO_2	0.400
3.	Al_2O_3	13.102	11.	MnO_2	0.095
4.	SiO_2	60.367	12.	Fe_2O_3	3.721
5.	ZnO	-	13.	SrO	0.033
6.	K_2O	0.994	14.	BaO	-
7.	ZrO_2	0.020	15.	P_2O_5	0.103
8.	SO_3	-	16.	AgO	11.000

% Ag in clay = 9.579 %

Table A12 Chemical composition of 0.5 % Ag^+-treated bentonite Japan (2-steps)

No.	Compound	Concentration % by weight	No.	Compound	Concentration % by weight
1.	Na_2O	1.960	9.	CaO	1.268
2.	MgO	3.803	10.	TiO_2	0.416
3.	Al_2O_3	13.506	11.	MnO_2	0.041
4.	SiO_2	74.401	12.	Fe_2O_3	2.693
5.	ZnO	-	13.	SrO	0.021
6.	K_2O	0.946	14.	BaO	0.080
7.	ZrO_2	0.014	15.	P_2O_5	-
8.	SO_3	0.043	16.	AgO	0.798

% Ag in clay = 0.695 %

Table A13 Chemical composition of 1.0 % Ag^+-treated bentonite Japan (2-steps)

No.	Compound	Concentration % by weight	No.	Compound	Concentration % by weight
1.	Na_2O	1.943	9.	CaO	1.196
2.	MgO	3.798	10.	TiO_2	0.455
3.	Al_2O_3	13.436	11.	MnO_2	0.043
4.	SiO_2	74.079	12.	Fe_2O_3	2.666
5.	ZnO	0.009	13.	SrO	0.021
6.	K_2O	0.978	14.	BaO	0.055
7.	ZrO_2	0.017	15.	P_2O_5	0.034
8.	SO_3	0.037	16.	AgO	1.232

% Ag in clay = 1.073 %

Table A14 Chemical composition of 1.5 % Ag^+-treated bentonite Japan (2-steps)

No.	Compound	Concentration % by weight	No.	Compound	Concentration % by weight
1.	Na_2O	1.951	9.	CaO	1.003
2.	MgO	3.710	10.	TiO_2	0.431
3.	Al_2O_3	13.352	11.	MnO_2	0.038
4.	SiO_2	74.089	12.	Fe_2O_3	2.528
5.	ZnO	0.011	13.	SrO	0.018
6.	K_2O	0.968	14.	BaO	-
7.	ZrO_2	0.014	15.	P_2O_5	-
8.	SO_3	-	16.	AgO	1.885

% Ag in clay = 1.641 %

Table A15 Chemical composition of 0.5 % AgCl-treated bentonite Japan (2-steps)

No.	**Compound**	**Concentration % by weight**	**No.**	**Compound**	**Concentration % by weight**
1.	Na_2O	0.893	9.	CaO	1.154
2.	MgO	1.692	10.	TiO_2	0.663
3.	Al_2O_3	11.189	11.	MnO_2	0.048
4.	SiO_2	79.218	12.	Fe_2O_3	3.073
5.	ZnO	0.011	13.	SrO	0.024
6.	K_2O	1.152	14.	BaO	-
7.	ZrO_2	0.018	15.	AgO	0.654
8.	SO_3	0.052	16.	Cl	0.159

% Ag in clay = 0.569 %

Table A16 Chemical composition of 1.0 % AgCl-treated bentonite Japan (2-steps)

No.	**Compound**	**Concentration % by weight**	**No.**	**Compound**	**Concentration % by weight**
1.	Na_2O	0.687	9.	CaO	1.238
2.	MgO	1.673	10.	TiO_2	0.595
3.	Al_2O_3	10.988	11.	MnO_2	-
4.	SiO_2	78.294	12.	Fe_2O_3	3.350
5.	ZnO	-	13.	SrO	0.024
6.	K_2O	1.165	14.	BaO	-
7.	ZrO_2	0.021	15.	AgO	1.427
8.	SO_3	-	16.	Cl	0.359

% Ag in clay = 1.243 %

Table A17 Chemical composition of 1.5 % AgCl-treated bentonite Japan (2-steps)

No.	**Compound**	**Concentration % by weight**	**No.**	**Compound**	**Concentration % by weight**
1.	Na_2O	0.957	9.	CaO	1.099
2.	MgO	1.680	10.	TiO_2	0.642
3.	Al_2O_3	10.830	11.	MnO_2	0.050
4.	SiO_2	77.173	12.	Fe_2O_3	3.110
5.	ZnO	-	13.	SrO	0.021
6.	K_2O	1.191	14.	BaO	-
7.	ZrO_2	0.021	15.	AgO	0.758
8.	SO_3	-	16.	Cl	0.689

% Ag in clay = 1.531 %

Table A18 Chemical composition of Ag^0 treated-bentonite Japan

No.	**Compound**	**Concentration % by weight**	**No.**	**Compound**	**Concentration % by weight**
1.	Na_2O	2.728	9.	CaO	0.866
2.	MgO	3.020	10.	TiO_2	0.359
3.	Al_2O_3	9.681	11.	MnO_2	-
4.	SiO_2	52.377	12.	Fe_2O_3	2.040
5.	ZnO	-	13.	SrO	-
6.	K_2O	0.640	14.	BaO	-
7.	ZrO_2	-	15.	P_2O_5	-
8.	SO_3	0.027	16.	AgO	28.263

% Ag in clay = 24.612 %

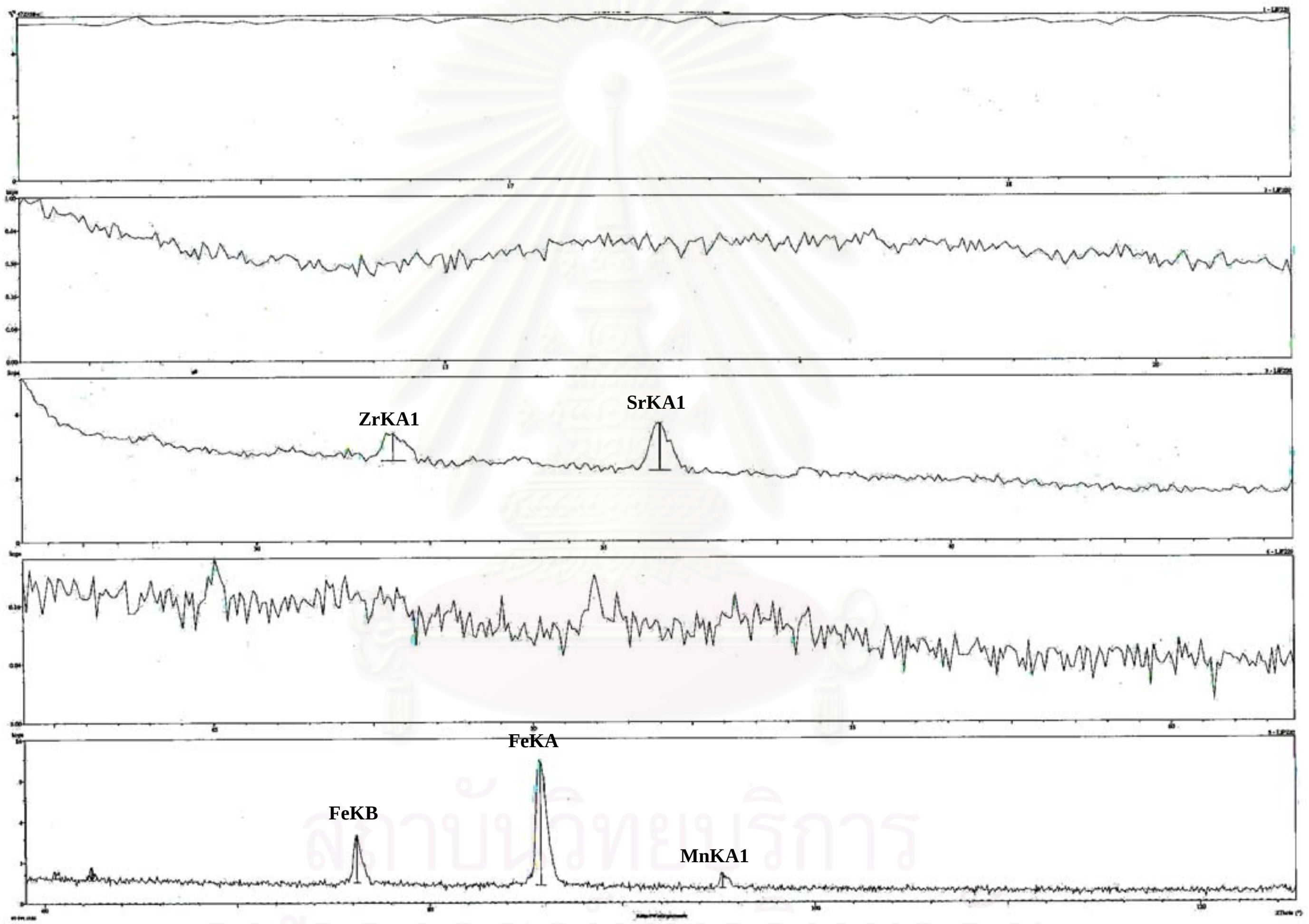


Figure A2 XRF spectrum of bentonite China

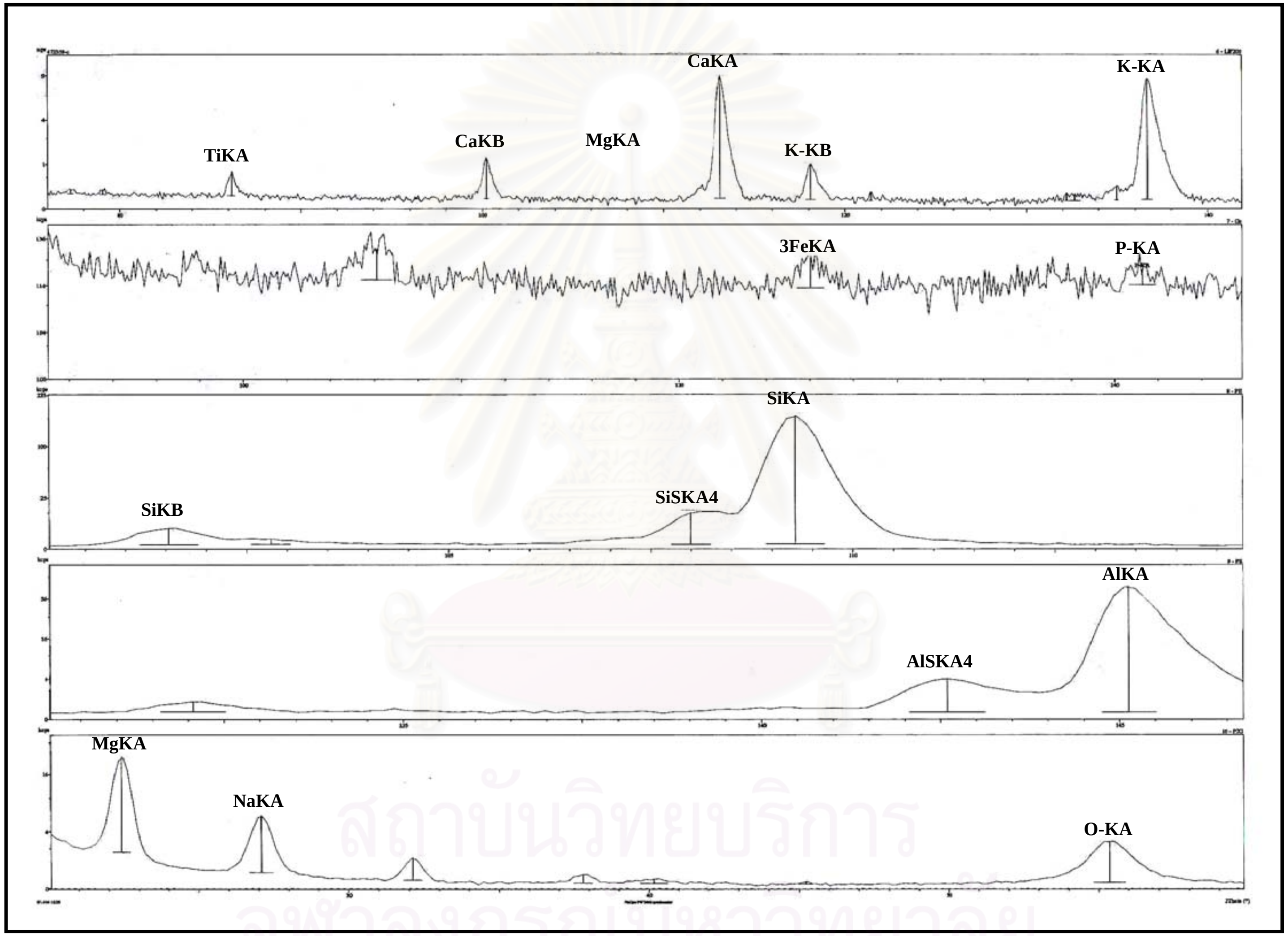


Figure A2 (continued) XRF spectrum of bentonite China

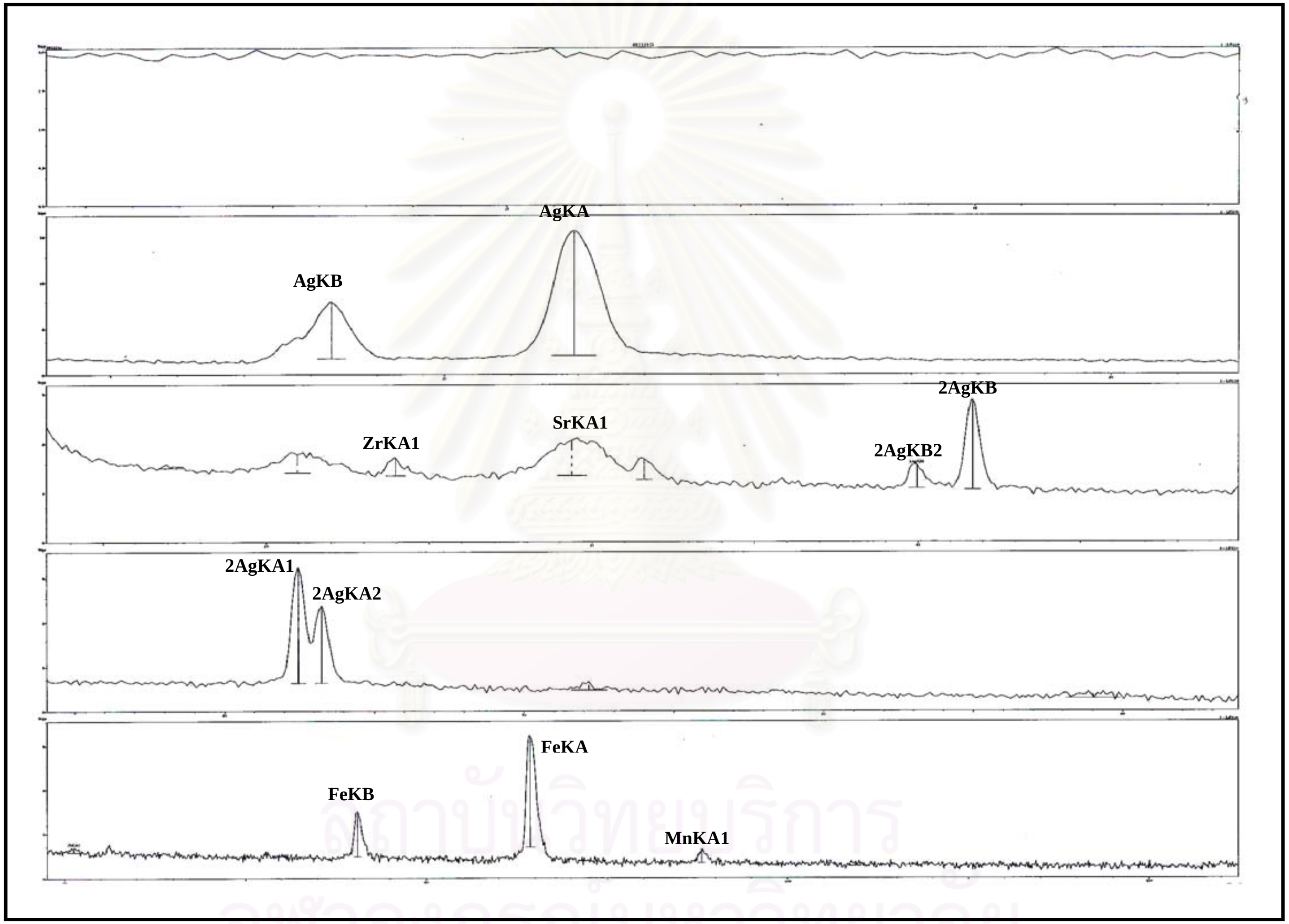


Figure A3 XRF spectrum of Ag-treated bentonite China (1-step)

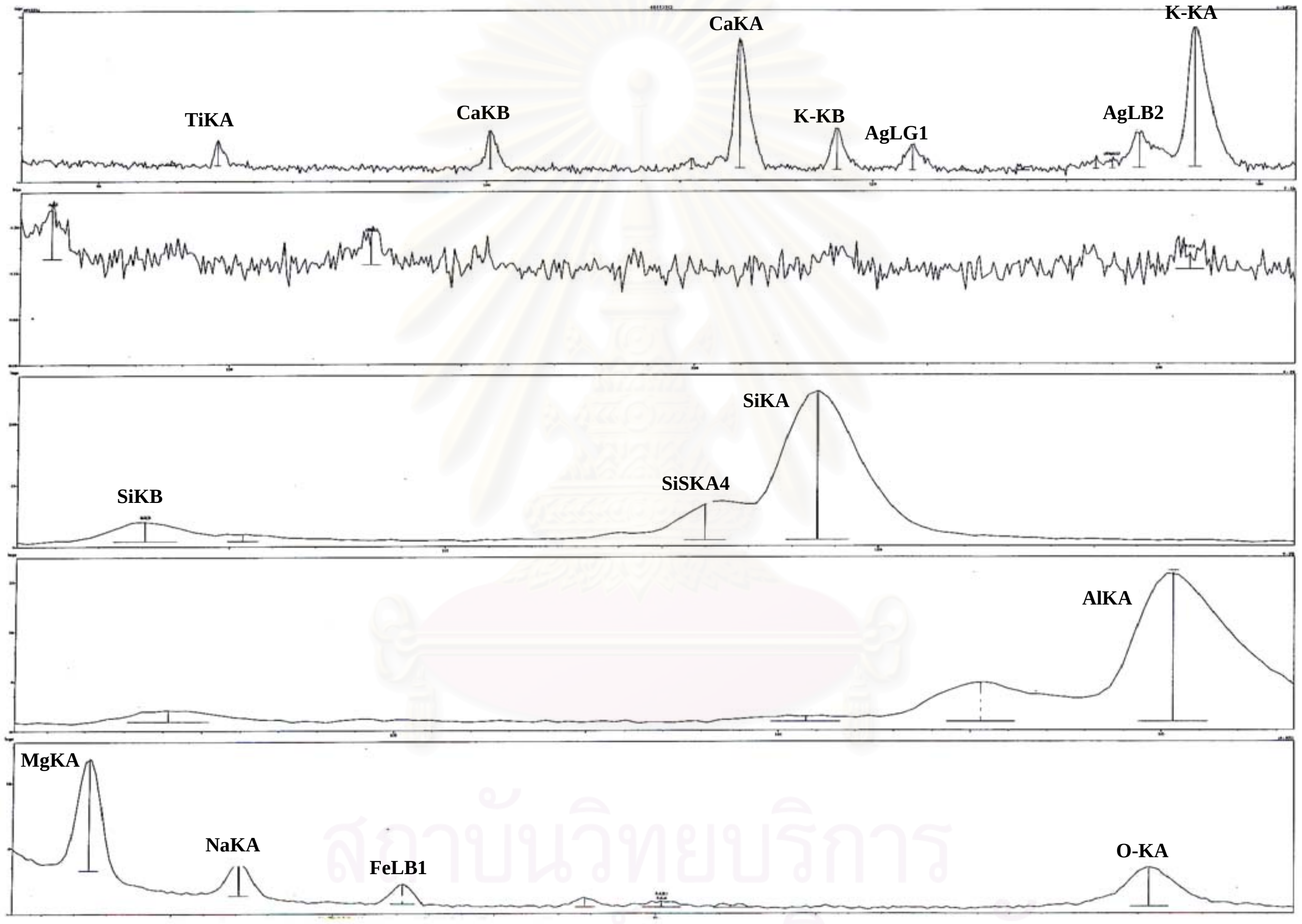


Figure A3 (continued) XRF spectrum of Ag-treated bentonite China (1-step)

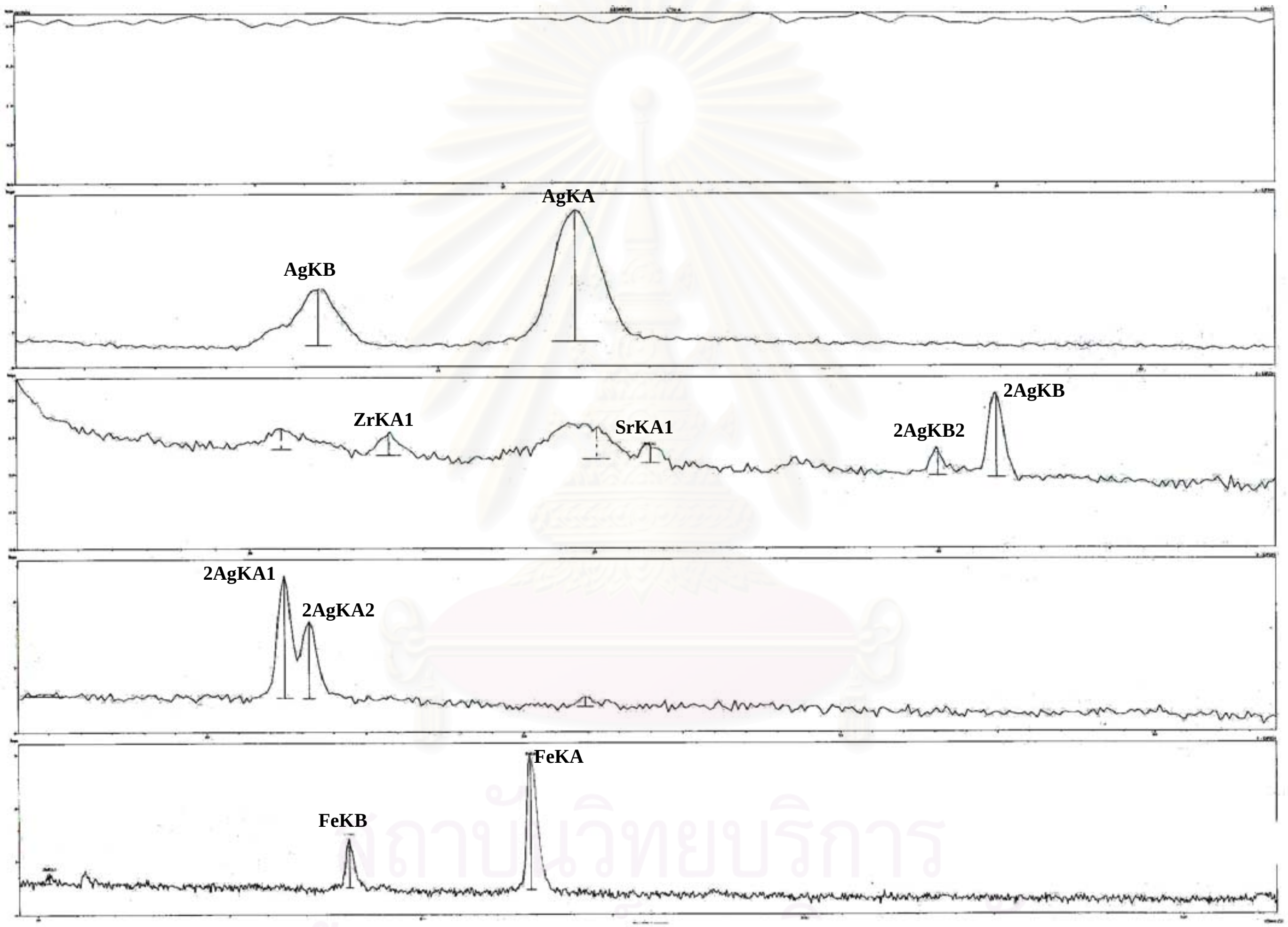


Figure A4 XRF spectrum of Ag$^+$-treated bentotnite China (2-steps)

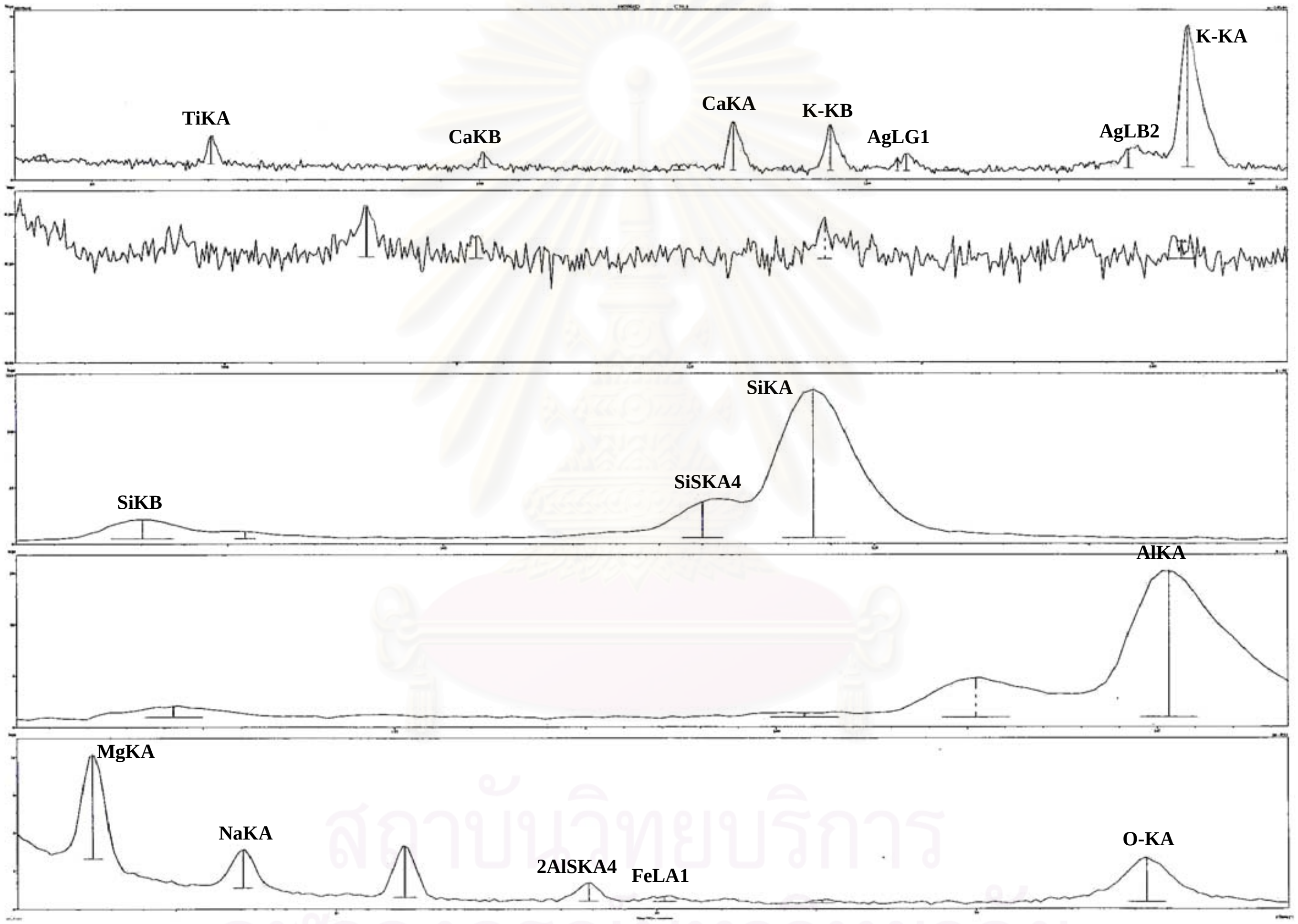


Figure A4 (continued) XRF spectrum of Ag$^+$-treated bentotnite China (2-steps)

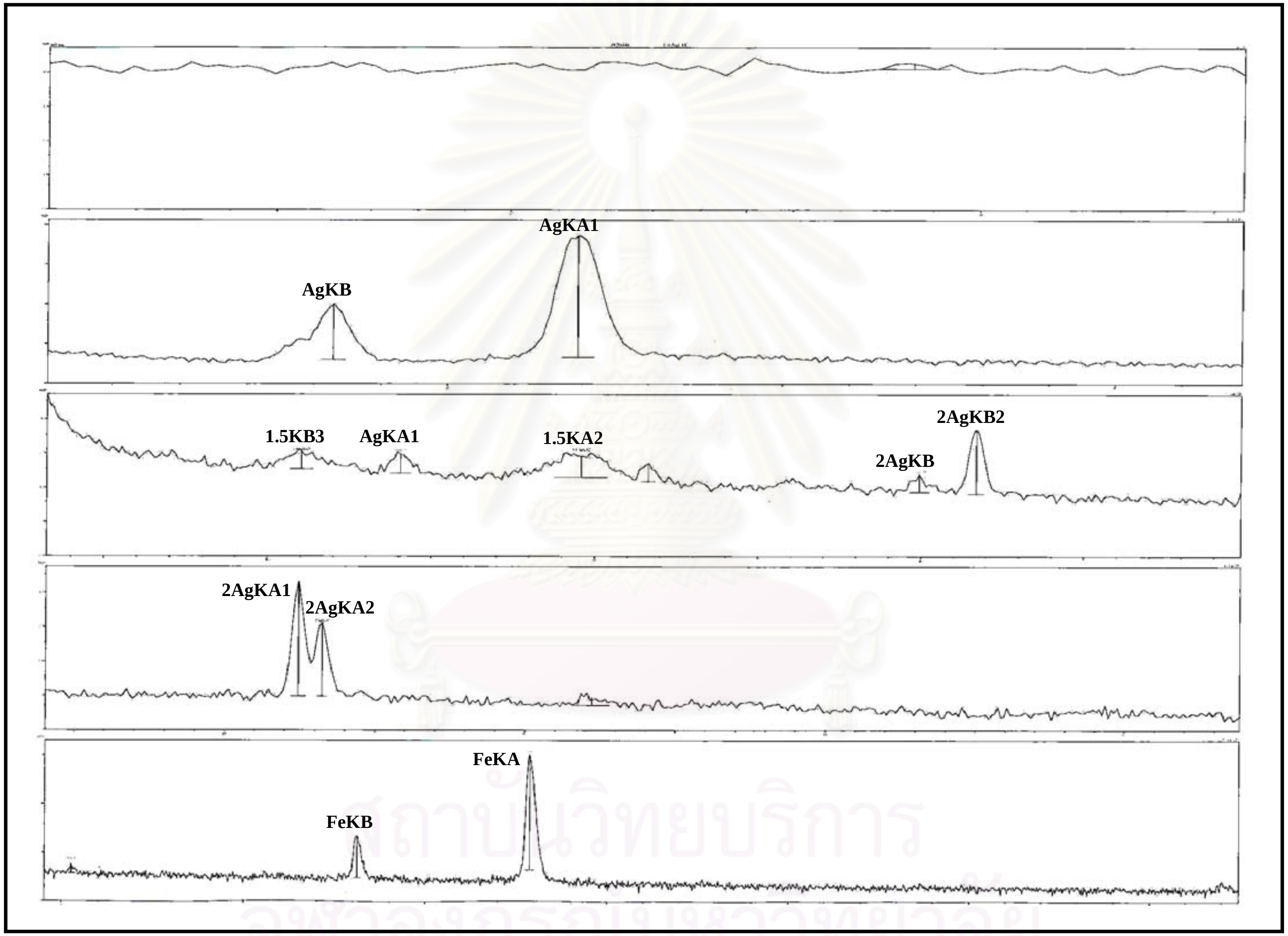


Figure A5 XRF spectrum of AgCl-treated bentotnite China (2-steps)

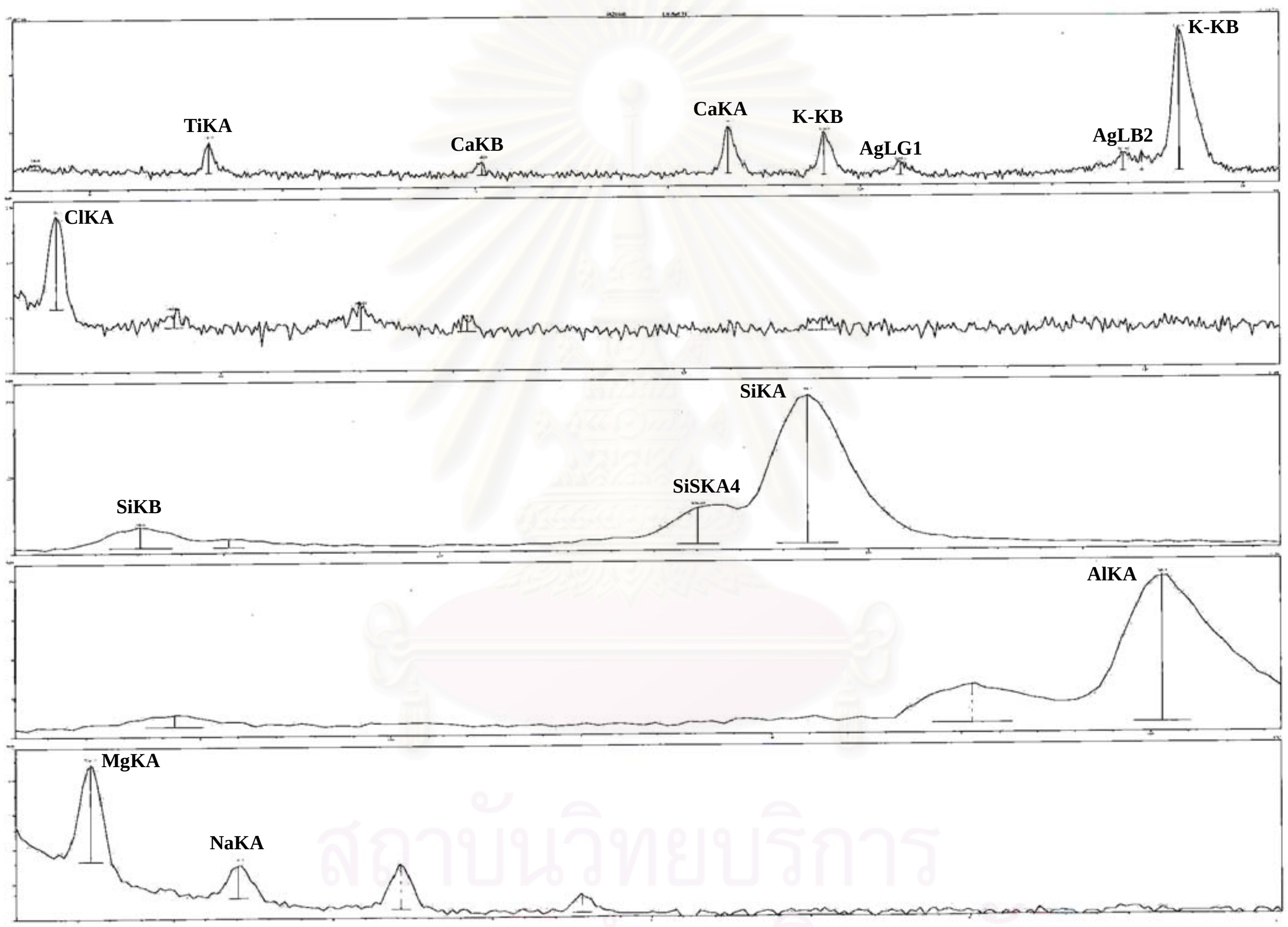


Figure A5 (continued) XRF spectrum of AgCl-treated bentotnite China (2-steps)

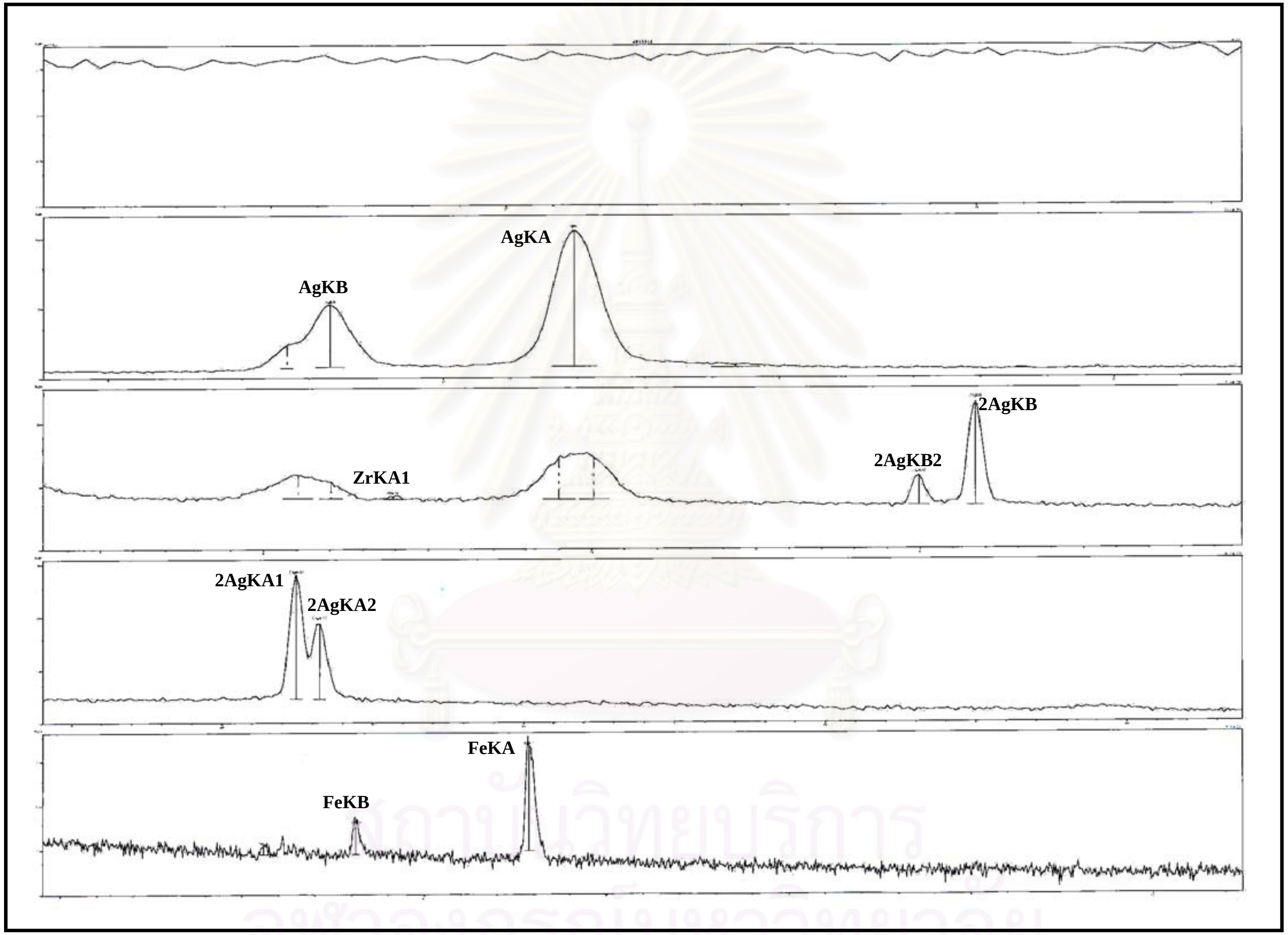


Figure A6 XRF spectrum of Ag^0-treated bentotnite China

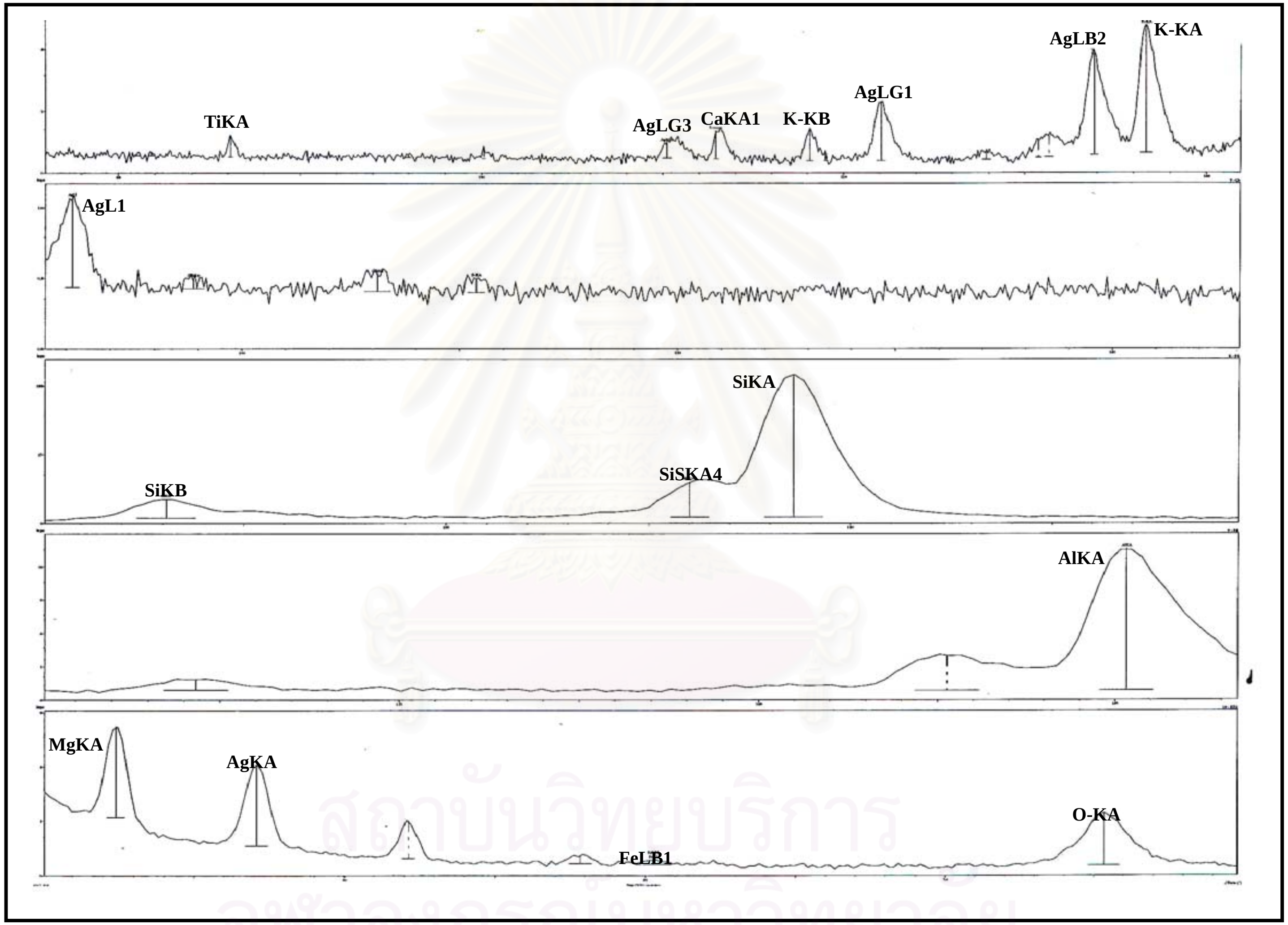


Figure A6 (continued) XRF spectrum of Ag^0-treated bentotnite China

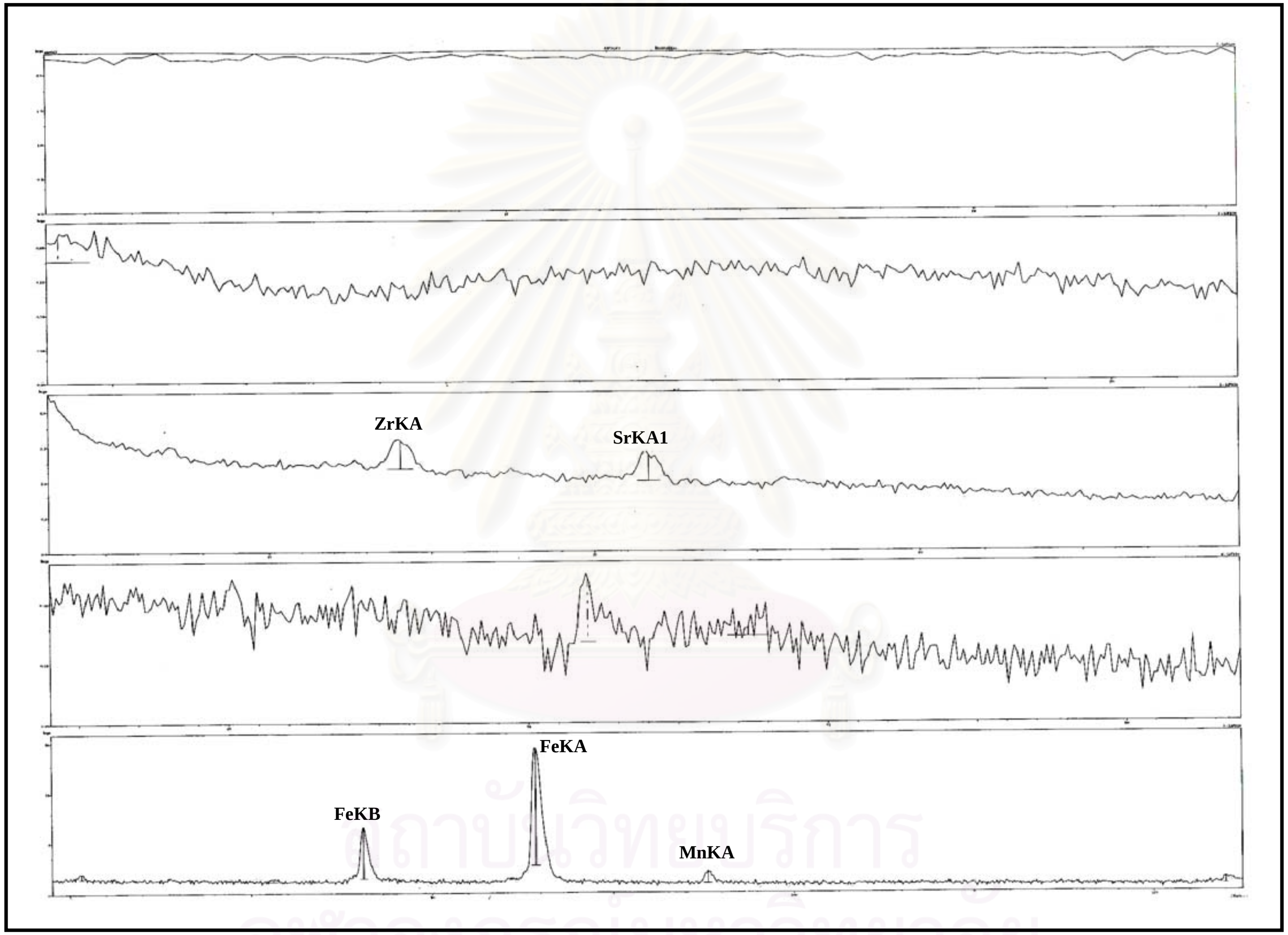


Figure A7 XRF spectrum of Bentonite Japan

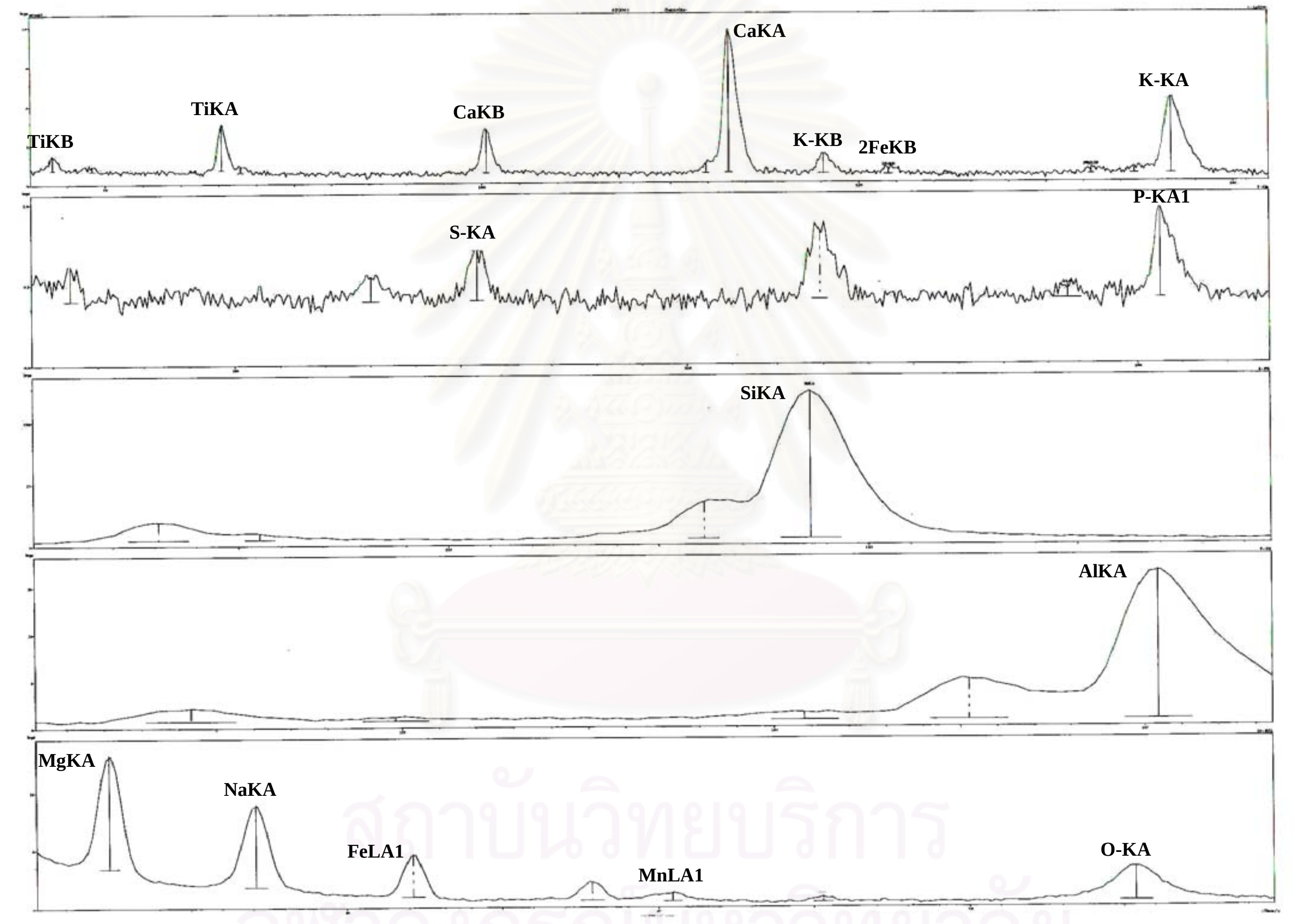


Figure A7 (continued) XRF spectrum of Bentonite Japan

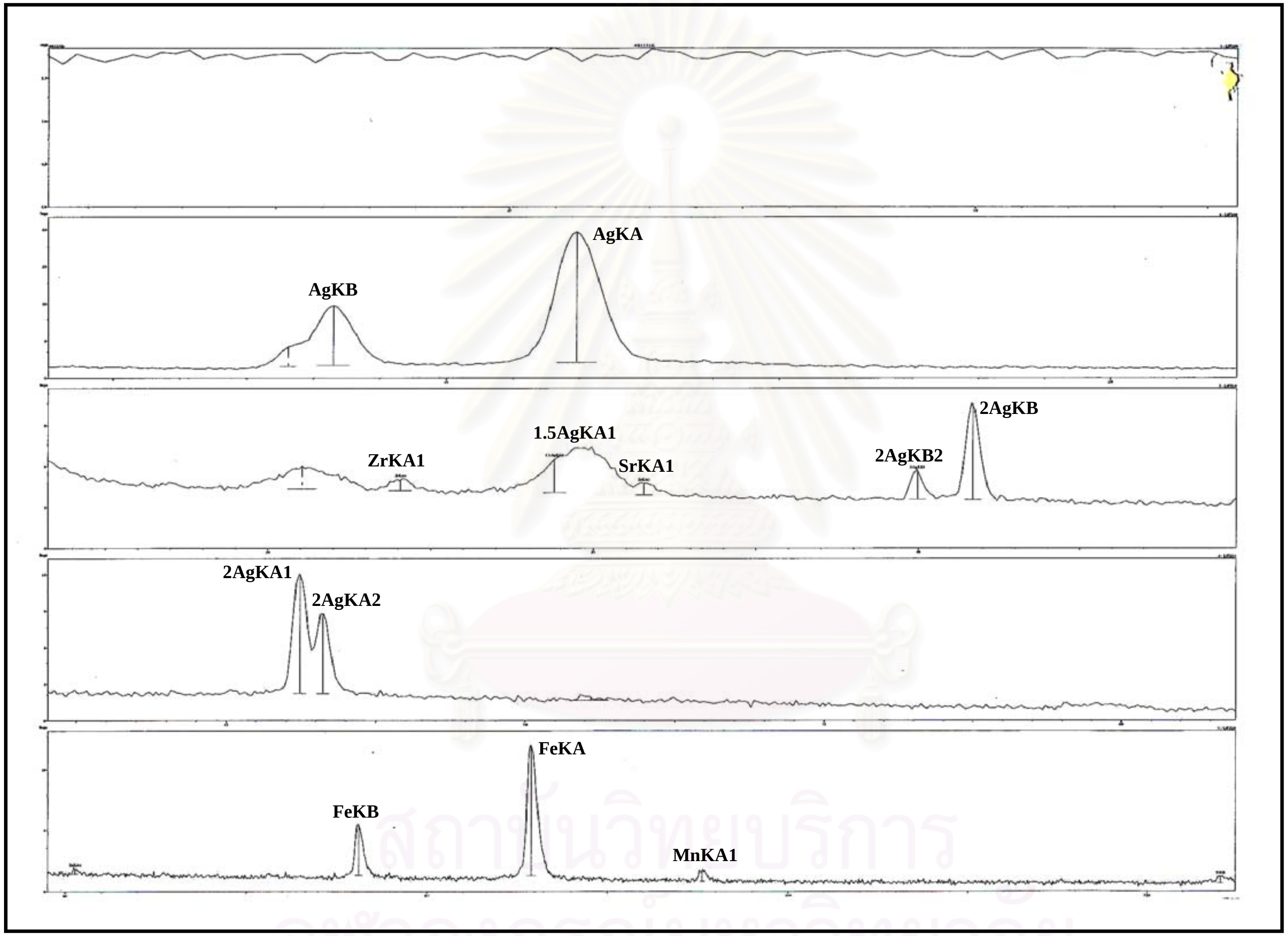


Figure A8 XRF spectrum of Ag-treated bentonite Japan (1-step)

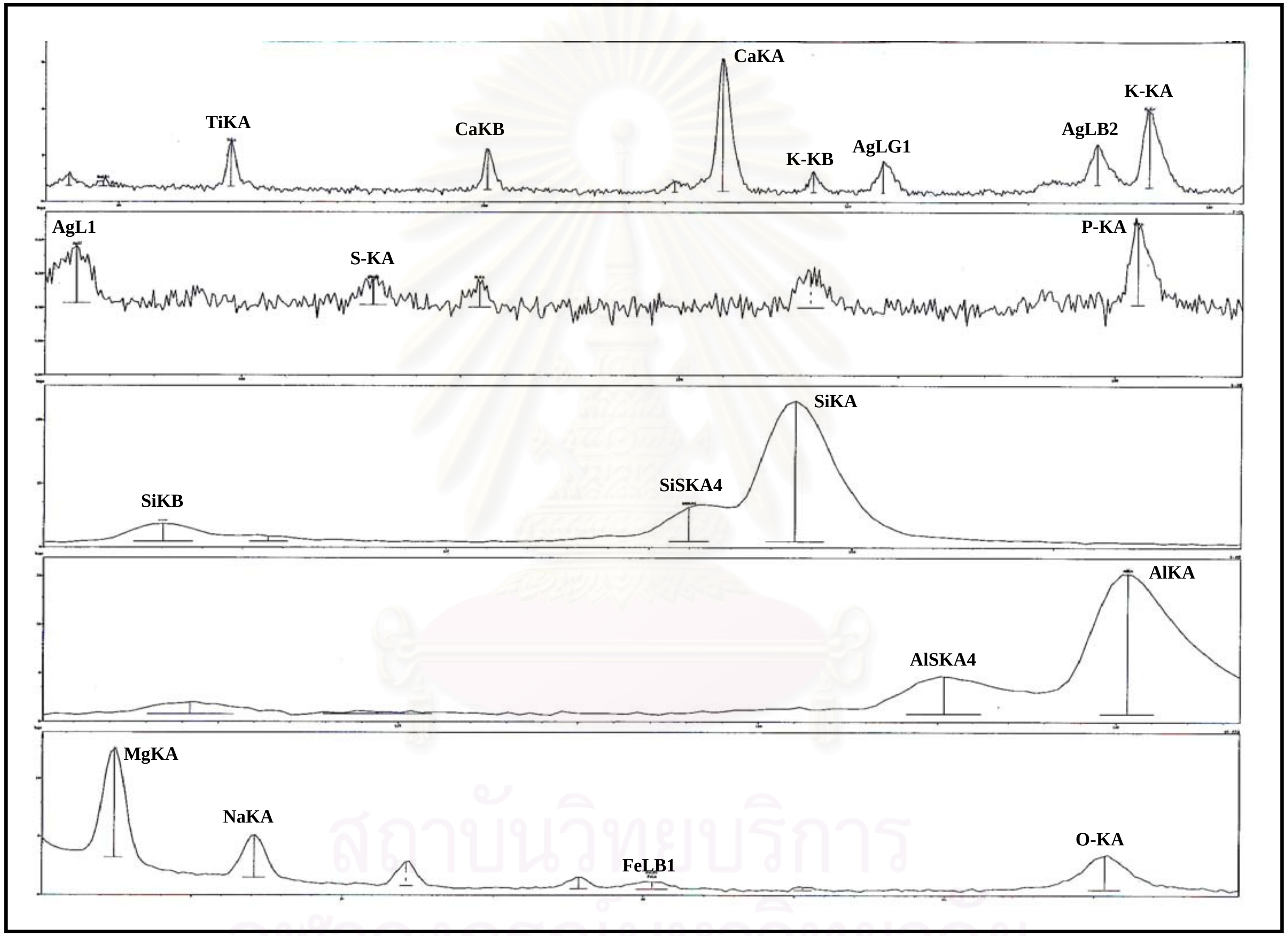


Figure A8 (continued) XRF spectrum of Ag-treated bentonite Japan (1-step)

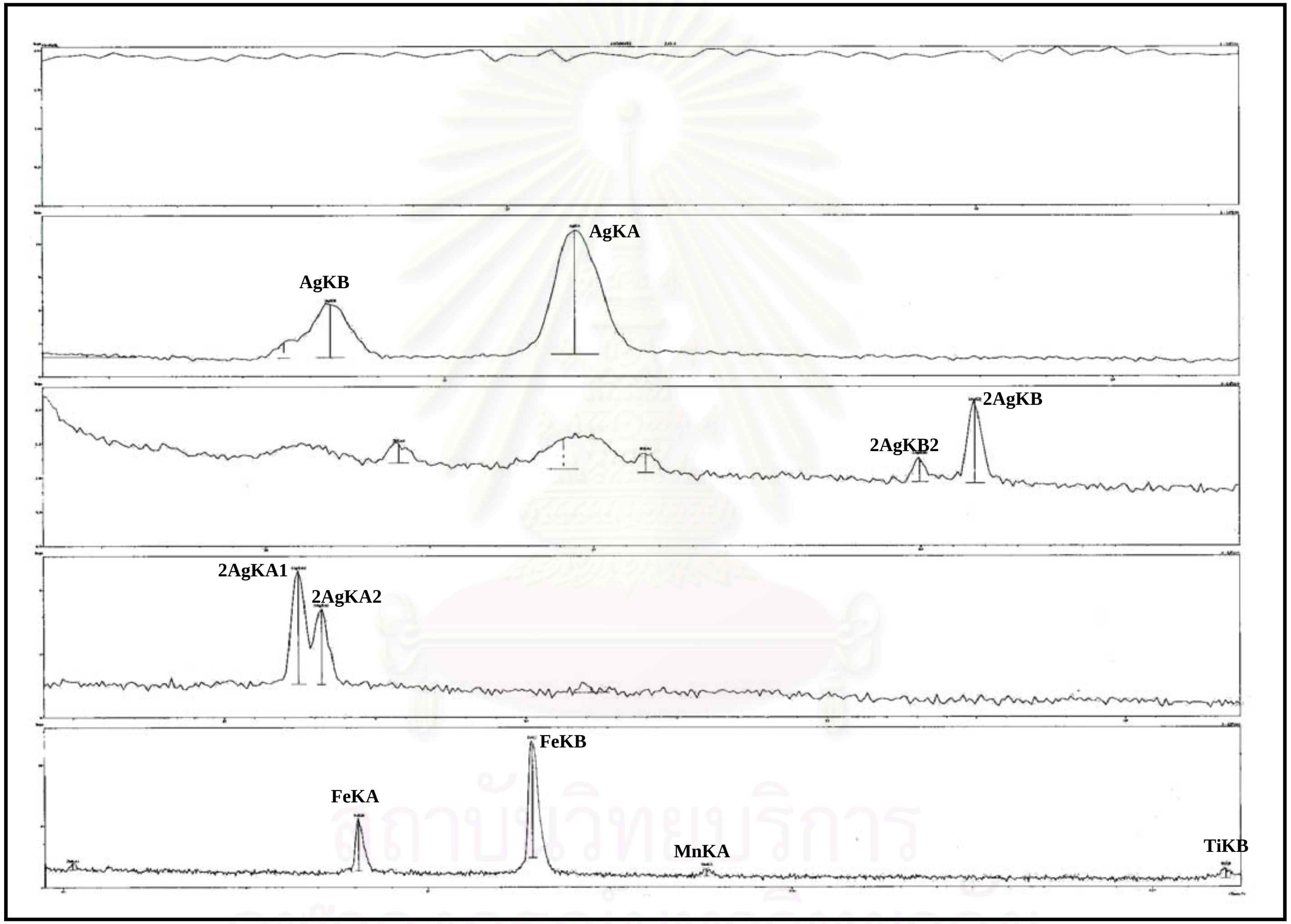


Figure A9 XRF spectrum of Ag$^+$-treated bentonite Japan (2-steps)

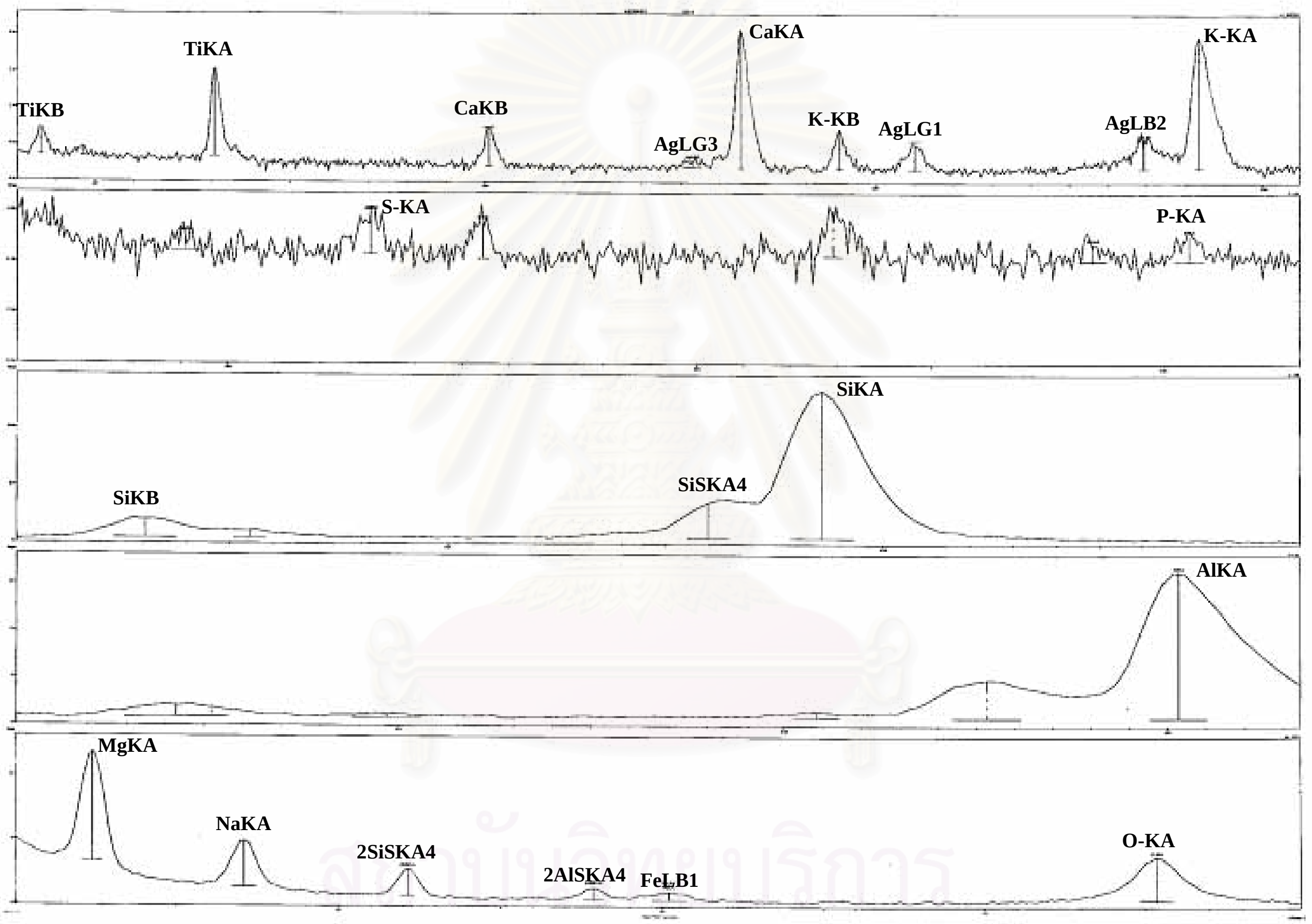


Figure A9 (continued) XRF spectrum of Ag$^+$-treated bentonite Japan (2-steps)

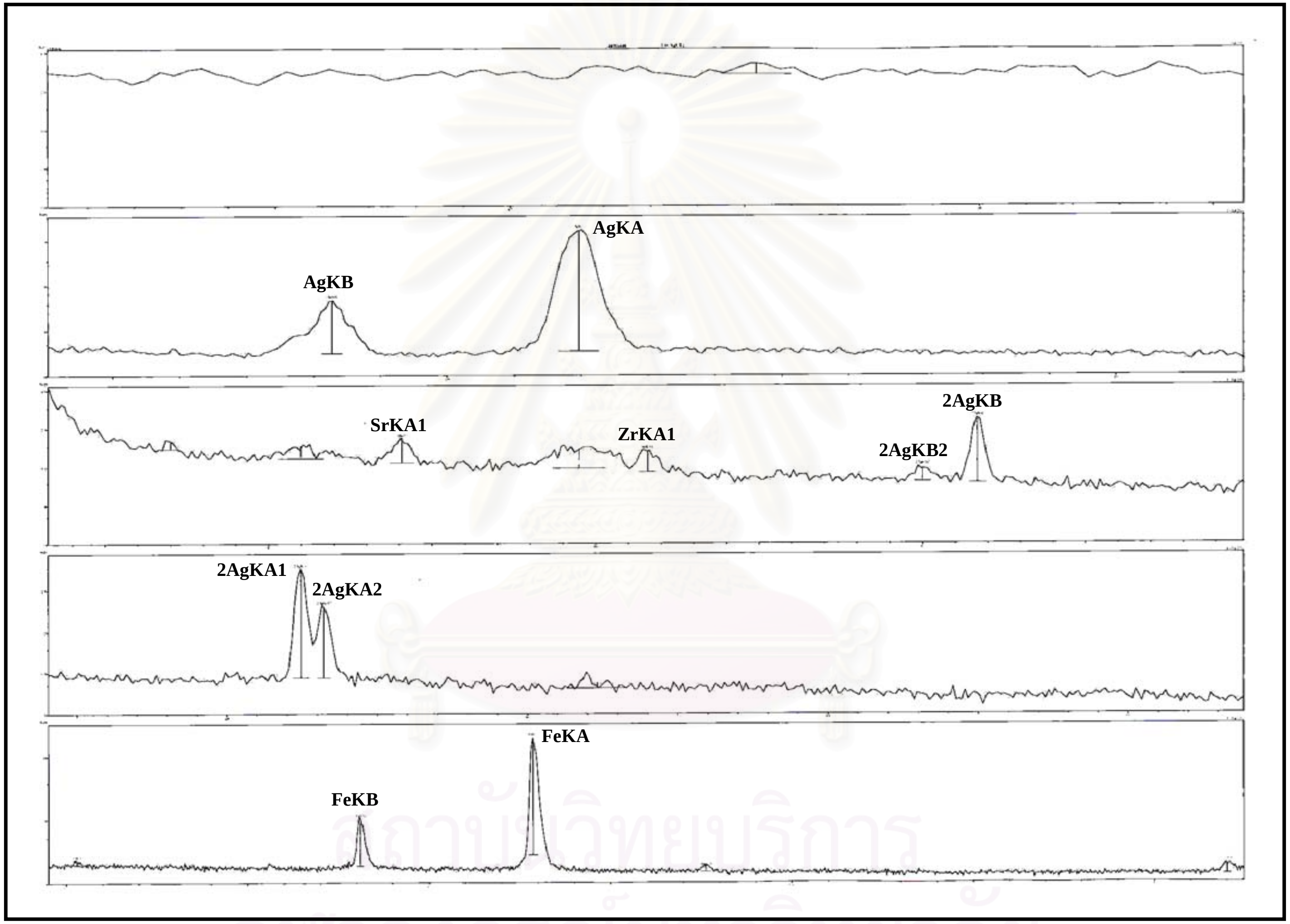


Figure A10 XRF spectrum of AgCl-treated bentonite Japan (2-steps)

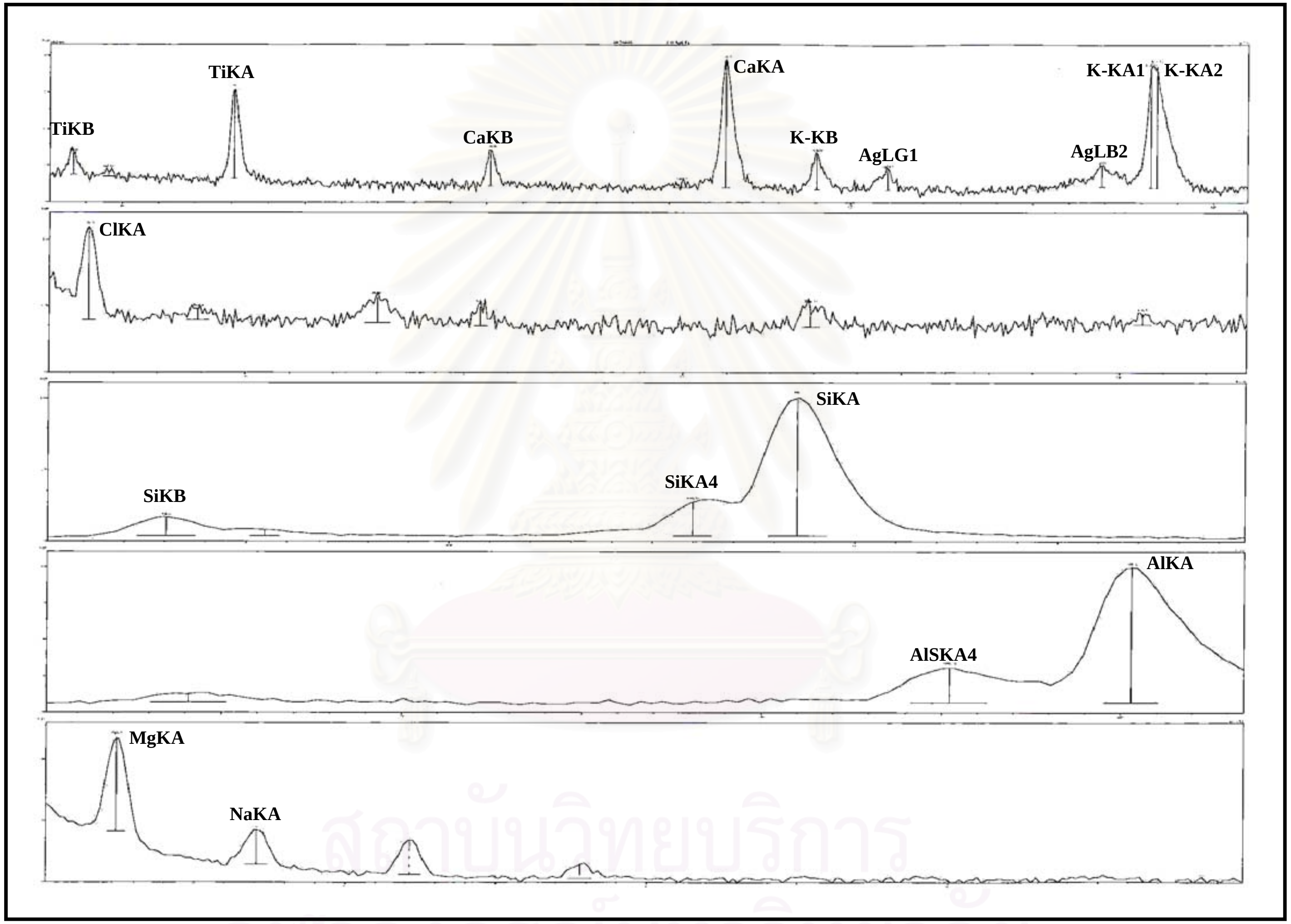


Figure A10 (continued) XRF spectrum of AgCl-treated bentonite Japan (2-steps)

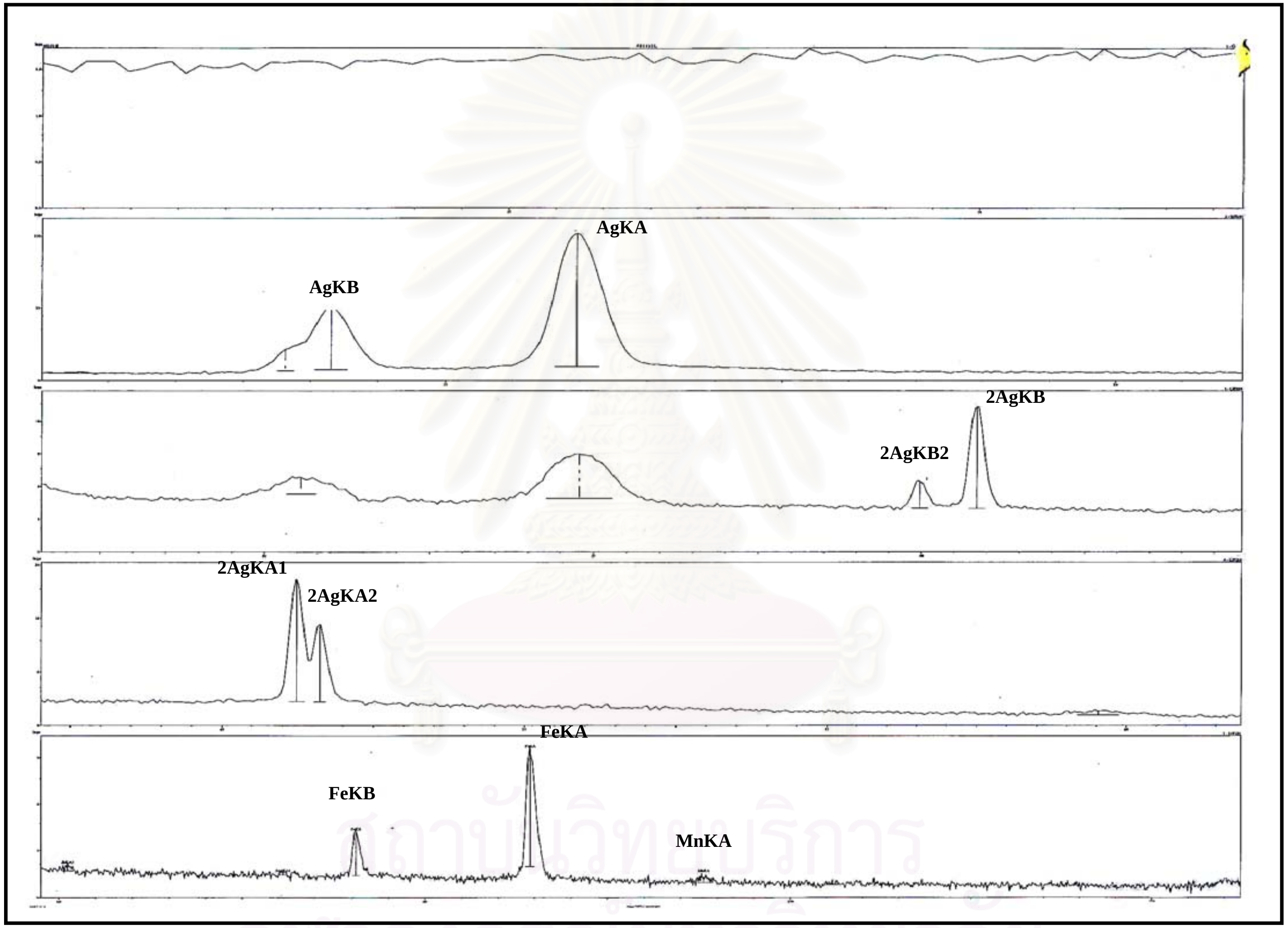


Figure A11 XRF spectrum of Ag^0-treated bentonite Japan

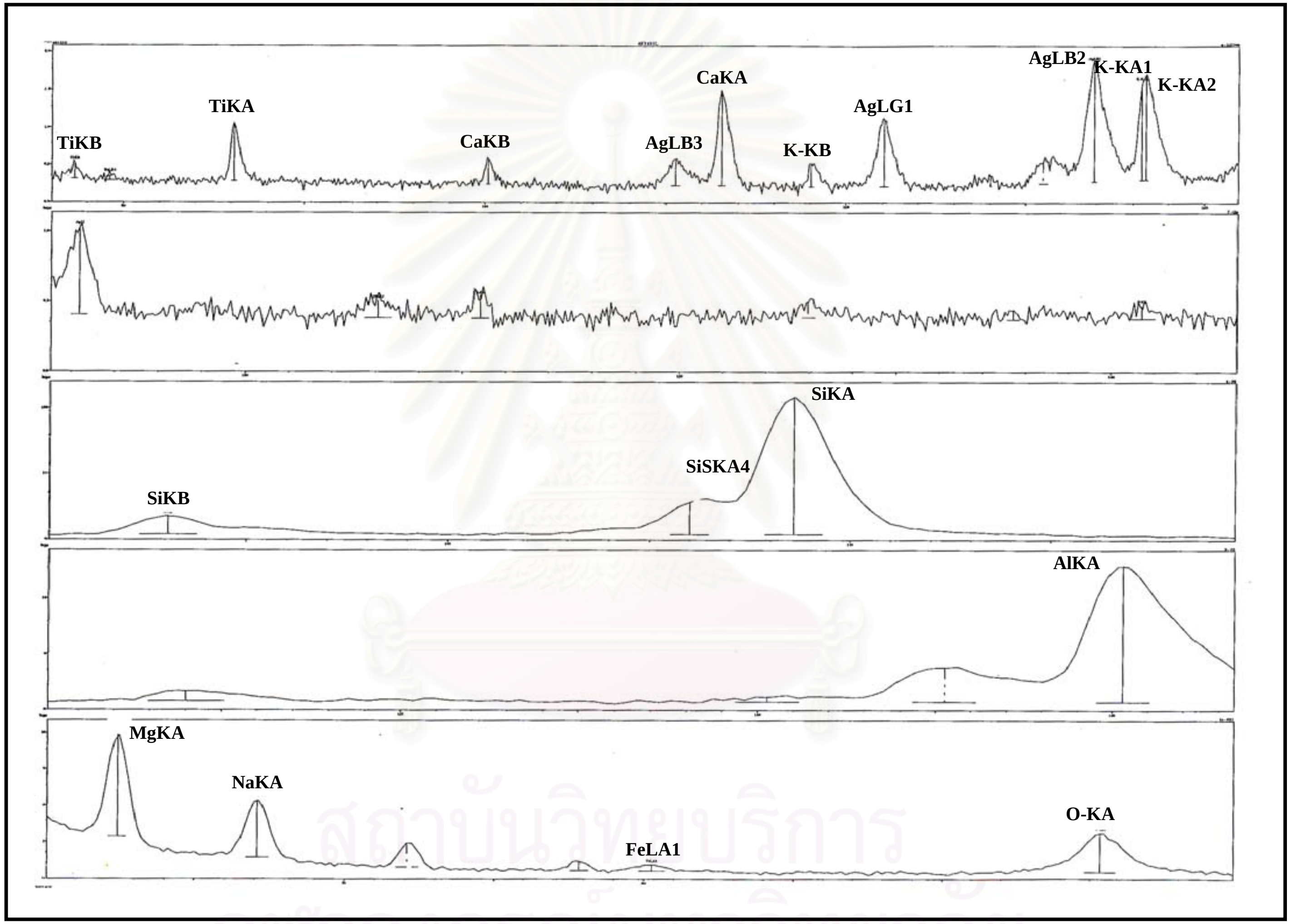


Figure A11 (continued) XRF spectrum of Ag^0-treated bentonite Japan

APPENDIX B

Calibration curve of Standard β-carotene
and
Chromatograms of β-carotene

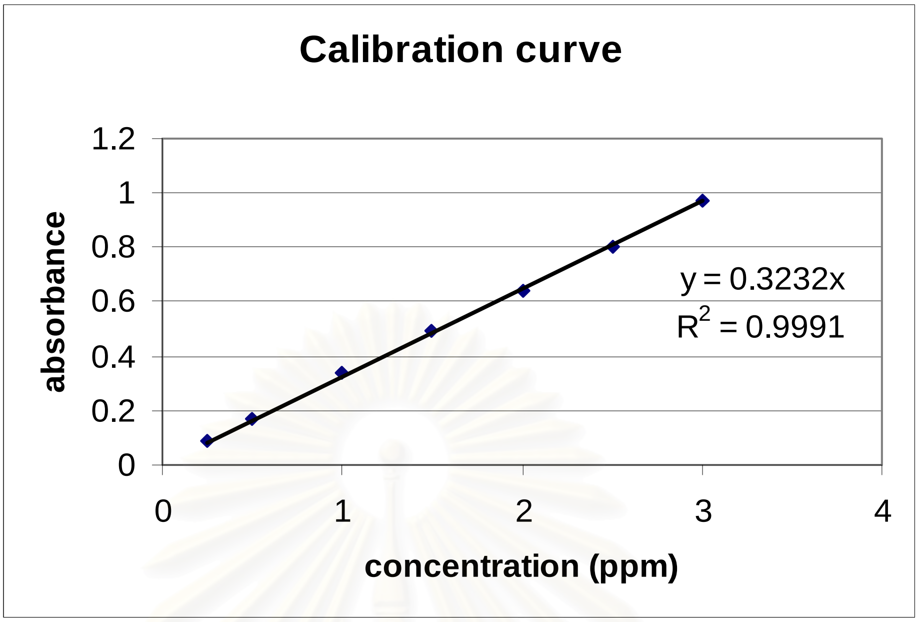


Figure B1 Calibration curve of Standard β-carotene (UV-visible spectrometry)

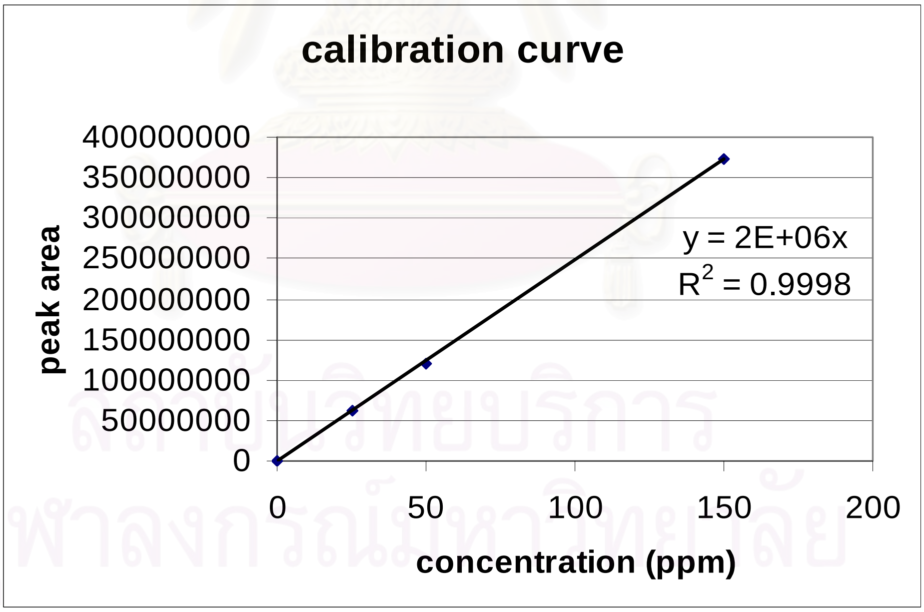
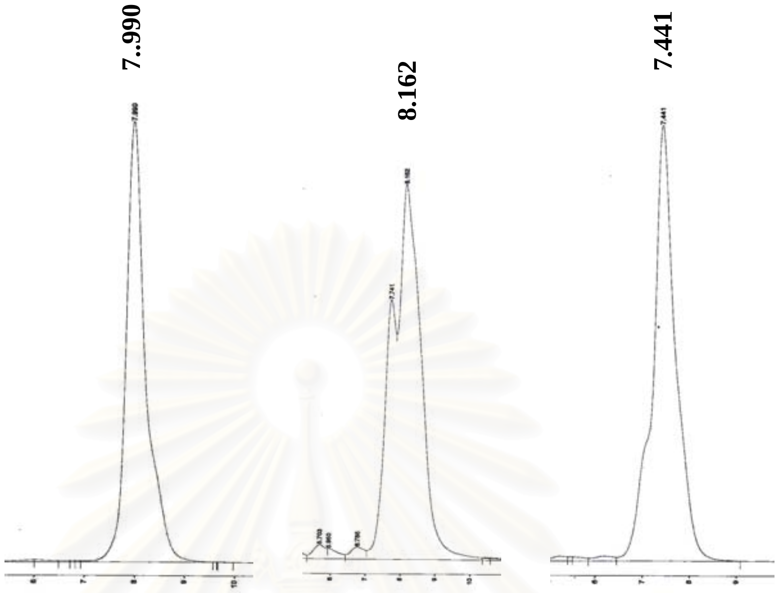


Figure B2 Calibration curve of Standard β-carotene (HPLC)



(a) (b) (c)

Figure B3 Chromatograms of β-carotene using HPLC

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

APPENDIX C

Experimental Data

Table C1 % Adsorption, % desorption and % recovery of carotene of various Ag-treated clays at 60 ^{0}C, 60 minutes, ratio of clay to palm oil = 1:5 and THF was used as eluting solvent

Adsorbents	**% Adsorption**	**% Desorption**	**% Recovery**	**SD**
Original Bentonite^C	28.85	55.21	15.93	± 0.10
Acid activated bentonite^C	98.77	20.15	19.91	± 0.20
Ag-treated bentonite (1-step)^C	31.93	69.80	22.28	± 0.30
0.5 % Ag^+-treated bentonite^C	93.53	34.64	32.46	± 0.69
1.0 % Ag^+-treated bentonite^C	91.04	33.60	30.60	± 0.31
1.5 % Ag^+-treated bentonite^C	91.17	29.75	27.13	± 1.64
0.5 % AgO-treated bentonite^C	91.67	39.66	36.36	± 0.59
1.0 % AgO-treated bentonite^C	91.87	33.14	0.45	± 1.16
1.5 % AgO-treated bentonite^C	91.06	32.83	29.90	± 0.08
0.5 % AgCl-treated bentonite^C	86.06	56.57	48.74	± 0.39
1.0 % AgCl-treated bentonite^C	84.21	50.08	42.18	± 0.37
1.5 % AgCl-treated bentonite^C	82.62	46.92	38.72	± 0.10
Ag^0-treated bentonite^C	69.51	17.85	12.41	± 0.15

Bentonite ^{C} *= Bentonite from China*

Bentonite ^{J} *= Bentonite from Japan*

Table C1 (continued) % Adsorption, % desorption and % recovery of carotene of various Ag-treated clays at 60 ^{0}C, 60 minutes, ratio of clay to palm oil 1:5 and THF was used as eluting solvent

Adsorbents	**% Adsorption**	**% Desorption**	**% Recovery**	**SD**
Original Bentonite^J	36.15	33.13	11.97	± 0.25
Acid activated bentonite^J	99.48	17.79	17.70	± 0.16
Ag-treated bentonite (1-step)^J	43.43	36.59	15.90	± 0.85
0.5 % Ag$^+$-treated bentonite ^{J}	96.17	32.13	30.90	± 0.49
1.0 % Ag$^+$-treated bentonite ^{J}	93.33	30.20	28.19	± 0.44
1.5 % Ag$^+$-treated bentonite ^{J}	91.89	28.39	26.11	± 0.33
0.5 % AgO-treated bentonite ^{J}	93.40	35.43	33.09	± 0.32
1.0 % AgO-treated bentonite ^{J}	92.30	31.37	28.95	± 0.35
1.5 % AgO-treated bentonite ^{J}	91.44	29.52	27.09	± 0.29
0.5 % AgCl-treated bentonite ^{J}	88.18	45.13	39.80	± 0.39
1.0 % AgCl-treated bentonite ^{J}	85.94	41.61	35.76	± 0.94
1.5 % AgCl-treated bentonite ^{J}	83.31	37.25	31.03	± 0.23
Ag^0-treated bentonite^J	61.16	9.98	8.10	± 0.30

Bentonite ^{C} *= Bentonite from China*

Bentonite ^{J} *= Bentonite from Japan*

Table C2 % Adsorption, % desorption and % recovery of carotene of different adsorption time by using various Ag-treated clay (2-steps) as adsorbent at 60 ^{0}C, ratio of clay to palm oil = 1:5 and THF was used as eluting solvent

Adsorbents	Adsorption time	% Adsorption	% Desorption	% Recovery	SD
0.5 % Ag^+-treated bentonite ^{C}	30 minutes	89.23	30.49	27.21	± 0.49
	60 minutes	95.53	34.64	32.64	± 0.69
	90 minutes	96.14	24.59	23.64	± 0.93
0.5 % AgO-treated bentonite ^{C}	30 minutes	87.24	35.49	30.97	± 0.20
	60 minutes	91.67	39.66	36.36	± 0.59
	90 minutes	92.75	31.44	29.16	± 1.29
0.5 % AgCl-treated bentonite ^{C}	30 minutes	82.85	46.96	38.90	± 0.49
	60 minutes	86.06	56.57	48.74	± 0.39
	90 minutes	88.11	41.92	36.95	± 0.93

Bentonite ^{C} = Bentonite from China

Bentonite ^{J} = Bentonite from Japan

Table C3 % Adsorption, % desorption and % recovery of carotene of different ratio of clay to palm oil by using various Ag-treated clay (2-steps) as adsorbent at 60 ^{0}C, 60 minutes and THF was used as eluting solvent

Adsorbents	**Ratio of clay to oil**	**% Adsorption**	**% Desorption**	**% Recovery**	**SD**
0.5 % Ag^+-treated bentonite ^{C}	1 : 2	98.32	26.21	25.77	± 0.53
	1 : 5	95.53	34.64	32.64	± 0.69
	1 : 8	89.90	26.61	23.93	± 0.89
0.5 % AgO-treated bentonite ^{C}	1 : 2	96.15	28.98	27.87	± 0.41
	1 : 5	91.67	39.66	36.36	± 0.59
	1 : 8	87.15	29.17	25.42	± 0.54
0.5 % AgCl-treated bentonite ^{C}	1 : 2	93.84	36.32	34.08	± 0.49
	1 : 5	86.11	52.62	48.74	± 0.39
	1 : 8	82.95	40.01	33.19	± 0.54

Bentonite ^{C} *= Bentonite from China*

Bentonite ^{J} *= Bentonite from Japan*

Table C4 % Adsorption, % desorption and % recovery of carotene of different adsorption temperature by using various Ag-treated clay (2-steps) as adsorbent for 60 minutes, ratio of clay to palm oil = 1 : 5 and THF was used as eluting solvent

Adsorbents	**Adsorption temperature**	**% Adsorption**	**% Desorption**	**% Recovery**	**SD**
0.5 % Ag$^+$-treated bentonite ^{C}	room	87.25	29.50	25.73	± 0.61
	60 ^{0}C	95.53	34.64	32.64	± 0.69
	90 ^{0}C	98.69	19.48	19.22	± 0.81
0.5 % AgO-treated bentonite ^{C}	room	82.06	36.56	30.33	± 0.16
	60 ^{0}C	91.67	39.66	36.36	± 0.59
	90 ^{0}C	96.98	22.22	21.60	± 1.00
0.5 % AgCl-treated bentonite ^{C}	room	75.04	43.75	32.83	± 1.03
	60 ^{0}C	86.11	52.62	48.74	± 0.39
	90 ^{0}C	95.08	26.21	24.92	± 0.69

Bentonite ^{C} *= Bentonite from China*

Bentonite ^{J} *= Bentonite from Japan*

Table C5 % Adsorption, % desorption and % recovery of carotene of different type of eluting solvents by using 0.5% AgCl-treated bentonite (2-steps)^C as adsorbent at 60 ^{0}C, 60 minutes, ratio of clay to palm oil = 1:5

Adsorbent	**Eluting solvents**	**% Adsorption**	**% Desorption**	**% Recovery**	**SD**
0.5 % AgCl-treated bentonite ^{C}	THF	86.06	56.57	48.74	± 0.39
	Hexane	86.08	2.90	2.49	± 0.52
	Toluene	86.89	17.28	15.01	± 0.06
	Chloroform	87.01	15.26	13.28	± 0.76
	Dichloromethane	87.99	7.07	6.22	± 0.81
	Ethyl acetate	88.10	7.14	6.29	± 0.46

Bentonite ^{C} = Bentonite from China

Bentonite ^{J} = Bentonite from Japan

Table C6 % Adsorption, % desorption and % recovery of carotene of different desorption temperature by using 0.5% AgCl-treated bentonite (2-steps)^C as adsorbent at 60 ^{0}C, 60 minutes, ratio of clay to palm oil = 1:5 and THF was used as eluting solvent.

Adsorbents	**Desorption temperature**	**% Adsorption**	**% Desorption**	**% Recovery**	**SD**
0.5 % AgCl-treated bentonite^C	room	86.08	56.57	48.74	± 0.39
	40 ^{0}C	84.95	30.39	25.82	± 0.78

Bentonite ^{C} = Bentonite from China

Bentonite ^{J} = Bentonite from Japan

Table C7 % Adsorption, % desorption and % recovery of carotene of 0.5% AgCl-treated bentonite (2-steps)^C before and after treated with antioxidant at 60 ^{0}C, 60 minutes, ratio of clay to palm oil = 1:5 and THF was used as eluting solvent.

Adsorbent	**Antioxidant**	**% Adsorption**	**% Desorption**	**% Recovery**	**SD**
0.5 % AgCl-treated bentonite ^{C}	non treated with BHT	86.06	56.57	48.74	± 0.39
	treated with BHT	88.82	75.76	65.77	± 0.44

Bentonite ^{C} = Bentonite from China

Bentonite ^{J} = Bentonite from Japan

Table C8 % Adsorption, % desorption and % recovery of carotene of 0.5% AgCl-treated bentonite (2-steps)^C before and after regeneration at 60 ^{0}C, 60 minutes, ratio of clay to palm oil = 1:5 and THF was used as eluting solvent.

Clay	**Regenerated adsorbents**	**% Adsorption**	**% Desorption**	**% Recovery**	**SD**
0.5 % AgCl-treated bentonite ^{C}	Fresh adsorbent	86.82	75.76	65.77	± 0.44
	1st regenerated adsorbent	77.39	48.39	37.43	± 0.93
	2nd regenerated adsorbent	72.06	43.61	31.44	± 1.71

Bentonite ^{C} = Bentonite from China

Bentonite ^{J} = Bentonite from Japan

VITA

Panintorn Dechfoong was born on March 21, 1979, in Bangkok, Thailand. She received her Bachelor’s Degree of Science in Industrial Chemistry, King Mongkut’s Institute of Technology North Bangkok in 2002. She continued the Master Program of Multidisplinary of Petrochemistry and Polymer Science, Faculty of Science, Chulalongkorn University and completed the program in 2006.