Thermal and Structural Characterization of Beeswax Samples and their Chromatographic Fractionated Components

REEM MOHAMED AHMED EBRAHIM

B. Sc. Honors Chemistry-Sudan University of Science and Technology, 2008

A dissertation

Submitted to the University of Gezira in Partial Fulfilment of the Requirements for the Award of the degree of Master of Science 

in

Chemistry

Department of Applied Chemistry and Chemical Technology

Faculty of Engineering and Technology

May 2015
Thermal and Structural Characterization of Beeswax Samples and their Chromatographic Fractionated Components.

Reem Mohamed Ahmed Ebrahim

Supervision committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. EssaEsmail Mohamed Ahmed</td>
<td>Main Supervisor</td>
<td>________________</td>
</tr>
<tr>
<td>Dr. Mustafa Ohag Mohamed</td>
<td>Co-supervisor</td>
<td>________________</td>
</tr>
</tbody>
</table>

Date: May/2015
Thermal and Structural Characterization of Beeswax Samples and their Chromatographic Fractionated Components.

Reem Mohamed Ahmed Ebrahim

Examination Committee:

<table>
<thead>
<tr>
<th>Name</th>
<th>Position</th>
<th>Signature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dr. EssaEsmail Mohamed Ahmed</td>
<td>Chair Person</td>
<td>----------</td>
</tr>
<tr>
<td>Dr. AdilElhag Ahmed</td>
<td>External Examiner</td>
<td>----------</td>
</tr>
<tr>
<td>Dr. Mohammed Osman Babiker</td>
<td>Internal Examiner</td>
<td>----------</td>
</tr>
</tbody>
</table>

Date of Examination: 05/05/2015
Dedication

This work is dedicated to the soul of my beloved father Mohamed Ahmed who was my teacher and friend. I have found more love, pleasure, and fun in your arms. I miss you very much and I wish you could have been with us at this special moment of my achievements. I love you dad forever.

I also dedicate my work to my beloved mother Buthaina Mohameden, I really feel she is my sister and my friend. I cannot find words to express my love. I love you.

Special dedication goes to:

My lovely sister Salma.

My brothers Abass, Aladdin and Amar for their encouragement, kindness, and support throughout this work.

My uncles and aunts.

I love you all.
Acknowledgement

First of all I would like to thank Allah (God) who gave me life, grace, favour, wisdom and good health to complete this research project.

I wish to express my deepest gratitude to my supervisor Dr. Essa Esmail Mohammed Ahmed for his guidance, advice, criticism and encouragements throughout the research. I learned a lot through our communications and working together. Really I cannot find words to express my thanks.

I would like to thanks all my teachers and specially Zein Alabdeen Ahmed Alsheikh for his encouragement during this study. I really feel he is my father.

Special thanks to my teachers Abdalkarim, Afaf Albashir and Ebrahim Khalifa for their help and support.

I would also like to thank my friends Amina and Mr. Adam. They helped me to collect the samples. My thank goes to Nader Osman and Sara Babker who helped me in the analysis of the samples.

Finally, my thanks extend to the staff of the department of chemistry at Sudan University of Science and Technology for allowing me to use their laboratories and facilities.
Thermal and Structural Characterization of Beeswax Samples and their Chromatographic Fractionated Components.
Reem Mohamed Ahmed Ebrahim

Abstract

In recent years, the utilization of materials derived from petroleum origin have become a matter of criticism from governments, academic institutes, some organizations, and research centres due to both environmental and economical concerns. Because of that, attention has been shifted toward materials that are renewable, sustainable, recyclable, environmentally friendly, and of low cost. In this study, structural and thermal properties of two beeswax samples and their column chromatography fractionated constituents were investigated. One sample was collected from Aldamazin local market (Blue Nile State, Sudan) and the other sample was obtained from wild bee colonies from western Sudan (Aljeninah). Two different fractions were isolated by silica gel column chromatography via different solvent systems (pyre n-hexane and pure dichloromethane). The physicochemical parameters of the two samples were determined first and then different techniques include SEM, FT-IR, GC/MS, GC/FID, and TGA were carried out to characterize the samples and their fractions. The physicochemical parameters have shown the presence of ester compounds and degree of unsaturation in both samples. SEM micrographs have revealed smooth surfaces with nodule-like shapes for the bleached samples. FT-IR and GC/MS have demonstrated that these samples are heterogeneous in nature and contain hydrocarbons, esters, and alcohols together with some minor components. TGA results have revealed that beeswax is thermally stable up to 290 °C and completely pyrolyzed at 600°C. Furthermore, the isolation of hydrocarbons, acids, and alcohols fractions were confirmed from their FT-IR spectra (FT-IR and GC/FID for hydrocarbons). Separations of different beeswax fractions of the samples were successfully achieved (as reported in FTIR and GC/MS analyses) although the characterization of the acids and the alcohols fractions by GC/MS was challengeable due to their low volatility and we could not find a high temperature HT-GC chromatograph to overcome this problem. Furthermore, for better understanding of the thermal properties and hence the structural features and the possible applications of the different fractions; TGA and DSC analyses are crucial.
تشخيص الخصائص البنائية والحرارية لعينات من شمع النحل ومكوناته المفصولة كروماتوغرافيا.

ريم محمد أحمد إبراهيم

ملخص البحث

في السنوات الأخيرة، أصبحت هناك انتقادات توجه من قبل الحكومات والمؤسسات الأكاديمية والبحثية، وكذا لبعض المنظمات لاستخدام المواد المشتقة من أصل بترولي وذلك من ناحيتين البيئية والاقتصادية. لهذا السبب تتحول الاهتمام نحو استخدام موادقابلة للتجد ومستدامة ويمكن إعادة تدويرها وكذلك صديقة للبيئة وتتوفر الخصائص البنائية والحرارية لعينتين من شمع النحلومكوناته افصل عن طريق كروماتوغرافيا العود. جمعت العينة الأولى من الأسواق المحلية مدينة الدمازين (ولاية النيل الأزرق، السودان) بينما تم الحصول على العينة الأخرى من مستعمرات النحل البري من غرب السودان (مدينة الجنينة). تم فصل شقين من العينتين بواسطة كروماتوغرافيا العمود المعبأ بالسيليكا جل عبر نظام مختلفين (الهكسان النيوتي والثنائي كلورو ميثان النيوتي). حددت الخصائص الفيزيوكيميائية للعينتين أولا ومن ثم أستخدمت تقنيات مختلفة مثل المجهر الإلكتروني وطيف الأشعة تحت الحمراء وكروماتوغرافيا الغاز/طيف الكتلة وكروماتوغرافيا الغاز/مكشاف التآكل والهيدروكربونات الفيزيوكيميائية ووجود مركبات الاستر ومركبات غير مشبعة في كلا العينتين. وقد أظهرت صور المجهر الإلكتروني أن العينات تمتاز بسطح أملس مع ظهور أشكال عنقودية. أظهرت تحليلات الأشعة تحت الحمراء وكروماتوغرافيا الغاز/مكشاف التآكل أن هذه العينات غير متجمدة في طبيعتها، وتحتوي على المواد الهيدروكربونية والإسترات والكحولات مع وجود بعض المكونات الأخرى بسبب سيئية. بيئة التحليل الوزني الحراري لمكثفات منطقة شمع العسل مستقرة حتى درجة حرارة 290 درجة مئوية وتفكك حراريا تماما في درجة حرارة 600 درجة مئوية. بالإضافة التي ذلك تم التأكد من بنية الهيدروكربونات والأحماض والكحولات المفصولة كروماتوغرافيا بواسطة المجهر الإلكتروني تحت الحمراء وكروماتوغرافيا الغاز/مكشاف التآكل والهيدروكربونات (أعمال (أعمال) 2000) ثم فصل مكونات العينات المختلفة بنجاح (كم مبيع من النتائج) إلأتنته sạchك الأحماض والكحولات تحدت تطبيقاتها المتدنية وعدم وجود جهاز كروماتوغرافيا غاز مع ضروري لفهم أفضل للخصائص البنائية والتطبيقات المختلفة الممكنة لهذه المكونات المفصولة.
# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dedication</td>
<td>III</td>
</tr>
<tr>
<td>Acknowledgement</td>
<td>IV</td>
</tr>
<tr>
<td>Abstract</td>
<td>V</td>
</tr>
<tr>
<td>ملخص البحث</td>
<td>VI</td>
</tr>
<tr>
<td>Table of contents</td>
<td>VII</td>
</tr>
<tr>
<td>List of tables</td>
<td>IX</td>
</tr>
<tr>
<td>List of figures</td>
<td>X</td>
</tr>
<tr>
<td>List of symbols and abbreviations</td>
<td>XI</td>
</tr>
<tr>
<td>Chapter 1: Introduction</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Research objectives</td>
<td>2</td>
</tr>
<tr>
<td>1.2 Thesis organization</td>
<td>2</td>
</tr>
<tr>
<td>Chapter 2: Literature review</td>
<td>3</td>
</tr>
<tr>
<td>2.1 Sources and classification of natural waxes</td>
<td>3</td>
</tr>
<tr>
<td>2.2 Chemical and physical properties of natural waxes</td>
<td>3</td>
</tr>
<tr>
<td>2.3 Uses of natural waxes</td>
<td>4</td>
</tr>
<tr>
<td>2.4 Beeswax</td>
<td>4</td>
</tr>
<tr>
<td>2.4.1 Chemical composition of beeswax</td>
<td>5</td>
</tr>
<tr>
<td>2.4.2 Physical properties and structure of beeswax</td>
<td>8</td>
</tr>
<tr>
<td>2.4.3 Uses of beeswax</td>
<td>10</td>
</tr>
<tr>
<td>Chapter 3: Materials and Methods</td>
<td>12</td>
</tr>
<tr>
<td>3.1 Materials</td>
<td>12</td>
</tr>
</tbody>
</table>
3.1.1 Beeswax samples
3.1.2 Chemicals
3.2 Treatment of the row beeswax
3.3 Physico-chemical parameters of beeswax
  3.3.1 Acid value
  3.3.2 Peroxide value
  3.3.3 Iodine value
  3.3.4 Saponification value
3.4 Fractionation of beeswax by column chromatography
3.5 Hydrolysis of esters compounds
3.6 Scanning electron microscopy (SEM)
3.7 Characterization of beeswax and beeswax-fractions using FT-IR
3.8 Characterization of beeswax using GC/MS
3.9 Characterization of hydrocarbons using GC/FID
3.10 Thermogravimetric analysis (TGA)

Chapter 4: Results and Discussion

4.1 Physico-chemical parameters of beeswax samples
4.2 Scanning electron microscopy of Beeswax
4.3 Fourier transform-infrared (FT-IR) analyses of beeswax samples
4.4 Gas chromatography-mass spectrometry (HT-GC/MS) of beeswax samples
4.5 Thermogravimetric analysis of beeswax samples
4.6 Fractionation of beeswax samples by silica gel column chromatography
  4.6.1 FT-IR analyses of fraction (1)
  4.6.2 Gas chromatography (GC/FID) analysis of fraction 1
4.7 FT-IR analyses of fraction (2)
4.8 Hydrolysis and Characterization of the alkaline components of fraction 2
Chapter 5: Conclusion and Recommendations
Reference
LIST OF TABLES

Table 4.1: Represents the physico-chemical parameters of beeswax samples 20
Table 4.1: Characteristic infrared absorption frequencies of beeswax sample 1 and 22
Table 4.3: Represents the compounds and their percentage of beeswax sample 1 by 27
using GC/MS spectroscopy
Table 4.4: Represents the compounds and their percentages in sample 2 by using 28
GC/MS
Table 4.5: Represents the percentages of fractions of beeswax samples 1 and 2 30
Table 4.6: The summary of the GC/FID chromatography for the n-hexane fraction 32
(fraction 1) of sample 1
Table 4.7: Shows percentages of compounds were resulted from hydrolysis of 34
ester.
LIST OF FIGURES

Figure 1.1: Represents classification of waxes 3
Figure 4.1: SEM micrograph of beeswax sample 1 21
Figure 4.2: SEM micrograph of beeswax sample 2 22
Figure 4.3: FT-IR spectrum of beeswax sample 1 23
Figure 4.4: FT-IR spectrum of beeswax sample 2 24
Figure 4.5: GC/MS spectrum of beeswax sample 1 25
Figure 4.6: GC/MS spectrum of beeswax sample 2 26
Figure 4.7: TGA curve of beeswax sample 1 29
Figure 4.8: TGA curve of beeswax sample 2 30
Figure 4.9: FT-IR spectrum of fraction 1 of samples 1 31
Figure 4.10: FT-IR spectrum of fraction 2 of samples 1 33
Figure 4.11: FT-IR spectrum of alcohols of samples 1 34
Figure 4.12: FT-IR spectrum of acids of samples 1 35
**LIST OF SYMBOLS AND ABBREVIATION**

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>FT-IR</td>
<td>Fourier transform-infrared</td>
</tr>
<tr>
<td>GC/MS</td>
<td>Gas chromatography mass spectroscopy</td>
</tr>
<tr>
<td>HT-GC/MS</td>
<td>Height temperature gas chromatography mass spectroscopy</td>
</tr>
<tr>
<td>ESI–MS</td>
<td>Electrospray ionization mass spectroscopy</td>
</tr>
<tr>
<td>SIM</td>
<td>Spray ionization mass</td>
</tr>
<tr>
<td>MRM</td>
<td>Multiple reaction monitoring</td>
</tr>
<tr>
<td>THM–GC/MS</td>
<td>Thermally assisted hydrolysis and methylation</td>
</tr>
<tr>
<td>XRD</td>
<td>X-ray diffraction</td>
</tr>
<tr>
<td>C^{13}–NMR</td>
<td>C^{13} Nuclear magnetic resonance</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>GC/FID</td>
<td>Gas chromatography flame ionization detector</td>
</tr>
<tr>
<td>TGA</td>
<td>Thermogravimetric analysis</td>
</tr>
<tr>
<td>BSE</td>
<td>Back scattered electron imaging</td>
</tr>
<tr>
<td>SEI</td>
<td>Secondary electron imaging</td>
</tr>
<tr>
<td>EDS</td>
<td>Energy-dispersive X-ray spectrometry</td>
</tr>
</tbody>
</table>
Chapter One

Introduction

Recently, renewable and sustainable natural resources have attracted significant attention due to depletion of petroleum reserves together with growing environmental concerns. Petroleum based materials are non-renewable, non-sustainable, cause wasteful problem, and costly. In this context, renewable materials such as biomass, natural waxes, and vegetable oils have been considerably investigated as alternatives for non-renewable ones [1-3].

Natural waxes have been used by mankind since prehistoric times. Many uses of wax are based on the imitation of its natural functions. Waxes in nature primarily serve to provide protective barriers on the surfaces of living organisms. Their functions are also determined by wax characteristics such as adhesion and cohesion, as well as slip and deformation effects [4]. Natural waxes are also used to preserve food and beverages, forming composite materials, preparation of nano-materials, manufacturing candles, in industrial sector and in medicine [5,6].

Beeswax (white and yellow) is refined wax of honey combs. It is produced in wax glands located in the abdomen of bees [7]. Generally, beeswax consists of a complex mixture of aliphatic hydrocarbons, mono-, di- and poly esters, hydroxy esters, free fatty acids, free fatty alcohols and minor of other compounds [7-11]. It has been reported in the literature that the chemical composition of beeswax depends on its origin, age, and climatic conditions [12]. In the earliest time it was exploited for various purposes such as preservation of mummies, square wax writing tablets, bending agent, and for sealing and waterproofing [13-16]. Although beeswax is now partly replaced by synthetic or fossil products, it played an important role in a number of fields such as polymer technology, symbolic and artistic fields, preparation of cosmetics or medicinal commodities, food, pharmaceutical, and pesticides [17-23].

To the best of our knowledge, there are no published research articles which study elaborately the structural and thermal properties of the individual constituent components of the beeswax. Furthermore, in all of the published research works beeswax has been used as a whole material
for the specified application. Fractionation of beeswax into its different constituents could further extend its application and make it value added material.

1.1 Research objectives

The objectives of this research work were to separate and characterize the different constituent components of two beeswax samples originated from different localities in Sudan. Structural and thermal properties are chosen mainly because they are crucial for understanding the physical and chemical properties as well as the applications of the wax in different fields.

1.2 Thesis organization

Chapter 1: Introduction
Chapter 2: Literature review
Chapter 3: Materials and methods
Chapter 4: Results and discussion
Chapter 5: Conclusion and recommendations
Chapter two

Literature review

2.1 Sources and classification of natural waxes

Waxes are always a mixture of more or less hydrophobic organic substances of medium chain length. According to their source, waxes are divided into natural and synthetic. Natural waxes are a mixture of various long-chain fatty acids and variety of other constituents [24-28]. They are obtained from mineral, animal and plants sources [24].

Natural waxes can be divided into renewable and non-renewable fig (1.1). The renewable waxes are re-growing and can be either chemically unmodified like beeswax, shellac wax, carnauba wax, candelilla wax, ricebrain and sunflower seed wax. The non-renewable fossil waxes are divided into crude and refined-like montan wax and petroleum waxes [24,27].

Figure 1.1 Represents classification of waxes[24]

2.2 Chemical and physical properties of natural waxes

Natural waxes are composed of mixtures of long chain linear and even-numbered aliphatic monoesters, partially varying amount of linear hydrocarbons (candelilla and beeswax), free wax acids (beeswax), free wax alcohols, policosanols (shellac and carnauba wax) and other ingredients
like phytosterols [24]. Akoh and Min.[29] it has been mentioned that most natural waxes consist of triglyceride and carbon acids (stearic acid, oleic acid and palmitic acid). In another study, Kuzeneet et al.[30] have reported that the natural waxes are mixture of esters of long chain fatty acids and long chain alcohols (wax ester), hydrocarbons, and a variety of other lipophilic compounds including the free compounds of the wax esters, aldehydes, ketones and terpenoids. Vincent et al.[31] characterized beeswax, natural candelilla wax, wool wax (lanolin), paraffin wax, and carnauba wax by using thin layer chromatography and gas liquid chromatography. Their findings showed that most natural waxes are composed of n-hydrocarbons, even numbered chain n- alcohols, n-fatty acids, and n-fatty esters.

Natural wax has different physical properties depending on its type of natural waxes (plant waxes, animal waxes and mineral waxes). Akoh and Min. [29] have reported the properties of natural waxes (animal, mineral and plant waxes). They found that natural waxes are soft and tacky to hard and plastic or breakable at 20°C. Their melting points range between 35 °C to 140 °C, and their solubilities and consistency depend strongly on temperature. They have acid values of 2.5–38mg KOH/g, saponification values of 43–147mg KOH/g, ester values of 39–100mg KOH/g, and iodine values of 7–30g I/100g.

2.3 Uses of natural waxes

Natural waxes have found applications in different fields and provide exceptional properties in diverse systems in cosmetic, pharmaceutical, food and household consumer good. Especially properties refer to oil gelling and retention as well as viscosity build-up and structuring [24,27].

2.4 Beeswax

Beeswax is an exudation of the abdominal glands of the honey bee. It is white or colorless when freshly exuded but will become coloured mainly by picking up and storing pollen and honey[25,26,29,31-36].

2.4.1 Chemical composition of beeswax

The chemical composition of beeswax has been reported by many researchers [12,25,26,29,30,34,37-49]. Different techniques such as FT-IR, HT-GC/MS, and GC/MS have been used for characterization purposes.

Krelland Hepburn [25,37] have reported that beeswax contains 14% hydrocarbons, 35% monoesters, 14% diesters, 3% triesters, 4% hydroxymonoesters, 8% hydroxypolyesters, 1% acid esters, 2% acid polyesters, 12% free fatty acids, 1% free fatty alcohols, and 6% unidentified.

Lakshmi et al. [26] studied the chemical composition of beeswax by using FT-IR and GC/MS. They concluded that the beeswax contains heterogeneous compounds. The FTIR analyses demonstrated that beeswax contains functional groups of carbonyl, fatty acids, and esters. GC/MS showed that beeswax contains cyclohexadecane, hexadecanoic acid (palmatic acid), 1-octadecane, heneicosane, 9-octadecenoic acid, 7-octadecenoic acid (stearic acid), N-docosane, tricosane, tetracosane, pentacosane, hexacosane, heptacosane, octacosane, nonacosane, and triacontane.

Kuzeneet al. [30] studied the chemical composition of beeswax by using HT-GC/MS (High temperature GC/MS) and found that beeswax contains 12-14% free fatty acids; most of which are saturated and have a chain length of C_{24}-C_{32}; it was also found to contain 1% free primary fatty alcohols; of a chain length of C_{28}-C_{35}, 35-45% linear wax monoesters as well as hydroxymonoesters; have chain length of C_{40}-C_{48} (palmatic acid, 15-hydroxypalmatic acid and oleic acid). The variation in total chain lengths of the ester is mainly the result of different chain lengths of the alcohols moiety (C_{24}-C_{34}). Furthermore they found that beeswax contains 15-27% complex wax esters which composed of 15% hydroxy palmitic acid or diols, diesters, tri and higher esters, 12-16% odd-numbered straight chain hydroxy carbon have chain length of C_{27}-C_{33}, and alkadienes and trienes.

Tulloch et al. [34] examined mellifera beeswax by using high temperature gas chromatography-mass spectroscopy. It was found that beeswax contains monoesters (35-45%), complex esters (15-27%), long chain hydrocarbons (12-16%) and free fatty acids (12-4%).
Aichholz et al. [38] have studied the chemical composition of beeswax using GC/MS. Their results showed that beeswax contains 40.8% monoesters, 9.2% hydroxymonoesters, 7.4% diesters, 12.8% alkanes, 2.9% alkenes, 18% free fatty acids and 0.6% free fatty alcohols. Monoesters which constitute the most abundant compounds in beeswax with saturated alkyl palmitates (C_{38}–C_{52}) and unsaturated alkyl esters of oleic acid (C_{46}–C_{54}). Hydroxymonoesters are long chain alcohols, esterified by a hydroxyl acid (mainly 15-hydroxypalmitic acid) or a primary hydroxyl group of a diol (mainly palmitic acid). While diesters and hydroxy diesters consist mainly of diesters, acylated hydroxyesters, hydroxypalmitic acid esters, and palmitic acid diesters acylated by hydroxypalmitic acids. Odd chain n-alkanes (C_{23}–C_{31}) constitute the predominant hydrocarbons in beeswax with heptacosane (C_{27}), nonacosane (C_{29}), hentriacontane (C_{31}), pentacosane (C_{25}) and tricosane (C_{23}) are the major components. The most common alkenes in mellifra beeswax are odd chain alkenes (C_{27}–C_{39}) with a cis-double bond at position C_{10}. Free fatty alcohols with C_{33} (0.3%-1.8%) and C_{35} (0.3%) have also been identified in mellifra, cerana, and florea beeswax. Free fatty acids in beeswax are unbranched saturated molecules with even carbon numbers from C_{20} to C_{36}. Tetracosanoic acid (C_{24}) has been reported as the most abundant free fatty acid.

Zimnicka and Hacura [39] have examined the molecular structure of crude beeswax which originated from industrial and agricultural areas (Poland) by vibrational spectroscopy (FT-IR and Raman). They concluded that comb waxis constituted mainly from esters and hydrocarbons as major components together with small amounts of alcohol and amides. Other minor materials like trace of inorganic compounds of Si, P, and S were also detected in the sample from industrial area and it was attributed to pollution of bees environment.

Garnier et al. [40] have fractionated fresh and archaeological beeswax by aminopropyl cartridge anion–exchange column chromatography to neutral and acid compounds, and then neutral fractions were fractionated by flash chromatography on silica gel column chromatography. The fractions were inspected by using of HT–GC/MS, ESI–MS, SIM, and MRM. Their results have shown that homogenous long chain compounds are the main constituents of beeswax. Fresh beeswax contains hydrocarbons 24.6%, fatty acids 3.6%, n-alcohols 4.3%, monoesters 39.2%, hydroxymonoesters 9.5% and diesters 5.6%. The archaeological sample was shown to contain
fatty acids 5.8%, n-alcohols 1.7%, monoesters 67%, hydroxymonoesters 12.5%, trace of diesters, B-sitosterol 0.7%, and cholesterol 0.1%.

Asperger et al.[41] have identified beeswax by using thermally assisted hydrolysis and methylation gas chromatography mass spectroscopy (THM–GC/MS) and they found that beeswax contains straight-chain fatty acids of even carbon number exclusively. Minor amounts of unsaturated fatty acids (C18: 1 and C20: 1) and hydroxyl fatty acids (2-hydroxyl palmitic acid) were also detected. The presences of wax alcohol fractions which consist of minor amount of even numbered alkanols (mainly in range between C_{24}–C_{34} showing maximum at C_{30}) were also noticed.

Negri et al. [42] have investigated four samples of comb wax from different sites from Brazil by fractionation of the samples using silicagel column chromatography to hydrocarbons and monoesters compounds. The esters fractions were hydrolyzed first by using alcoholic potassium hydroxide and then derivatized prior to GC–MS analysis. The results showed that beeswax contains 15.1–23.5% aliphatic saturated and mono unsaturated hydrocarbons (C_{21} (1%), C_{22} (1%), C_{23}(1-3%), C_{24} (1-2%), C_{25} (3-13%), C_{26} (1-3%), C_{27} (12–30%), C_{28} (1-3%), C_{29} (1-16%), C_{30} (1–2%), C_{31} (17–19%), C_{31:1} (2%), C_{33} (3–5%), C_{33:1} (3-80%) and C_{35:1} (2-4%)), and 67.4–75.8% monoesters. The distribution of alcohols of monoesters was in the range C_{24}–C_{32} (C_{24} (15–60%), C_{26} (12–17%), C_{28} (10–18%), and C_{32} (2–16%)). Palmitic and oleic acids are the main compounds in monoesters (55–100%) and (14–33%) respectively. Other acids (C_{18:1} (4–11%), C_{20} (1%), C_{22} (2%), C_{24} (16%), C_{26} (4%) and C_{28} (3%)) have also reported.

Jan et al. [43] have extracted some organo-chlorine compounds in beeswax and honey by fractionating the samples using solvent extraction. Separated compounds were analyzed using high resolution gas chromatography. The results revealed the presence of hydrocarbons, esters, fatty acids and some organochlorine compounds. The presence of organochlorine compounds was attributed to the contamination of the area from which the sample was collected.

Namadaret al., Tollochet al., and Kimpe et al. [44–46] have examined hydrocarbons composition in beeswax samples using HT-GC/MS. They concluded that beeswax contains saturated odd and even hydrocarbons from n-C_{21} to n-C_{35}, with n-C_{25}, n- C_{27}, n- C_{29}, and n-C_{31} as dominate n-
alkanes. In addition, their results proved that beeswax contains n-olefins from n-C\textsubscript{29} to n-C\textsubscript{33} with n-C\textsubscript{31} and n-C\textsubscript{33} as dominate-alkenes.

Michael et al.[47] have isolated long chain aliphatic alcohols from beeswax using lipase catalyzed methanolysis in supercritical carbon dioxide. The inspection of the isolated compoundswas done using GC/MS. The results demonstrate that 47% of the beeswaxesters containing C\textsubscript{24}–C\textsubscript{34} alcohols combined primary with palmitic and oleic acids. The fatty alcohols from beeswax were isolated in the ratios of C\textsubscript{24}OH (90%), C\textsubscript{26}OH (13.9%), C\textsubscript{28}OH (18.3%), C\textsubscript{30}OH(36.9%) C\textsubscript{32}OH (20.8%); C\textsubscript{34}OH,1%, and abundance of fatty acid methyl esters found after methanalysis of beeswax were palmitic 51%, teracosanic acid 13%, hexadecenoic acid 12%, olic acid 5%. Hexacosanoic acid 5%, octacosanoic acid 5%, tricontanoic acid 4%, dotriacontanoic acid 3%, and tetratriacontanoic acid 3%.

Blum et al. [12] extracted volatiles oxygenated compound by steam distilled, and analyzed it by using GC–MS and conclude the major compounds identified were decanal 46%, 1-decanol 10%, nonanal 18%, octanal 6%, one heterocyclic aldehyde furfural 10% and benzaldehyde 10%. This variation in chemical composition of bees wax depends on type the genetics of bees [26,29,42,48], the age of the wax and the climatic circumstances of it product. And the beeswax contain from 284 components,74 major and 210 minor components [29,49].

### 2.4.2 Physical properties and structure of beeswax

The physical properties of beeswax have been reported by many researchers [24-26,29,30,32,39,48-51]. Endleinet al. [24] have studied the physical properties and structure of beeswax and they were found that when it freshly exudes it is white or colourless but will become coloured mainly by picking up and storing pollen and honey. Beeswax has relatively low boiling point (61–65 °C), moderately hard and a bit sticky, plastic and kneadable at body temperature, and non-crystalline.

In addition, beeswax is insoluble in water and resistant to many acids, but its soluble in most organic solvent such as ether, benzene, and chloroform [25,29,32,49]. Kuzeneet al., and Hossainet al. [30,32] investigated the physical properties of beeswax. Their findings showed that the melting point of the beeswax ranges between 62 to 65°C, relative density are 0.975 to 0.970
g/cm³, electrical resistance ranges between $5 \times 10^{12}$ to $20 \times 10^{12}$ohmm, and thermal conductivity coefficient is $2.5 \times 10^3$Jcm/S. Prava et al. [26] showed that the melting point of beeswax is more than 40°C and the value of viscosity is low above the melting point. In addition, they investigated the structure of beeswax using XRD. The X-ray diffraction of beeswax revealed that beeswax contains large number of crystalline and amorphous compounds.

Hossain et al.[32] have determined the some physical properties of beeswax and demonstrated that the beeswax is solid in normal temperature, it converts to brittle in temperature bellow 18°C, quickly becomes soft and pliable at round 35-40 °C, it melting point 62 – 65 °C, density equals 0.95 g/cm³, insoluble in water but soluble in alcohol, chloroform and ether and they are investigated of structure of beeswax by using scanning electron microscopy and were found beeswax contain many compounds of crystalline and semi crystalline.

Zimnicka and Hacura. [39] indicated the wax excreted by bees has liquid consistency and later it turned into solid state. And was studied the interesting part of the FTIRspectra of beeswax. The sample was heated to melting point temperature and quenched to room temperature from 1 to 10 minute after preparation there were changed in the spectral band of the CH$_2$ group observed in time (1, 4, 5, 10) it was the same phenomenon of forming a semi-crystal local structure of hydrocarbon (as reported for linear n- alkane in binary mixture in solid structure (Hacura).

Maia et al. [48] measured the physical properties of authentic and adulterated beeswax. The result showed the melting points of the classified authentic beeswax by cluster and principal compounds analysis were in the 63-67°C range, acidity value in the 14.4 – 23.0 mg KOH/g range and saponification value in the 65.5 – 124.2 mg KOH/g range. The adulterated bees wax sample the melting point, acid value and saponification value were 58.3–66.5°C, 8.2–17.6 mg KOH/g and 36.9–79.6 mg KOH respectively.

Aguilar et al. [50] have reported beeswax melting point in range 62 – 65 °C, specific gravity about 0.96, the acid value in range 17 - 24 mg KOH/g and saponification value 87 – 104 mg KOH/g.

Kameda et al. [51] investigated of molecular structure of crude of beeswax studied by solid – state C$^{13}$ – NMR and found the beeswax contains crystalline and semi crystalline material because it
was contain of humongous compound. The crystalline compound in beeswax present of 85%. Therefore beeswax is crystalline structure, because the beeswax was contain esters, hydrocarbons, fatty acids and other compound and all this compound contain long chain of methylene groups (long chain alkanes) and the n- alkanes are known to have various crystallographic forms such as Orthorhombic, triclinic, monoclinic and hexagonal forms. The form of crystal changes depend upon physical parameters such as temperature pressure and cooling rate.

2.4.3 Uses of beeswax

The uses of beeswax have been reported by many researchers [24,39,42,47,50,52-73]. Endlein et al. [24] mentioned that beeswax is used in considerable amount for lip stick and lip balm preparation to provide creamy textures, good adhesion to skin and well consumer accepted films. The oil gelling is also helpful to stabilize water in oil emulsions for skin and hair application, ointment for viscosity build up.

Zimnicka and Hacura. [39] inspected from studied of properties of beeswax, the beeswax was used for many applications such as wax ointment or medicine for curing respiratory illness it could be checked whether beeswax can also serve as environment pollution indicator.

Michael et al. [47] examined the biological effect of beeswax and found that the beeswax was used in pharmaceutical for lowering total cholesterol for used polycosanols. The polycosanols were used for lowering total cholesterol and the bad low density lipoprotein.

Aguilar et al., Castano et al., and Aleman et al. [50,52,53] showed that beeswax was used in food additives as a glazing agent in chocolate, snacks and coffee.

Bogdanov et al. [54] were beeswax uses in candles, wax foundation (rolled and poured wax foundation sheets), art (wax figures and status), sculptures (metal castings, modeling, jewellery and last wax casting), engraving (glass metal engraving), processed food (confectionery, bakery, packaging, coating of jetted sweets and liquor ice), pharmaceuticals (drugs, pills, capsules, salve and ointments), physiotherapy (compresses), natural therapy (ear plugs), cosmetics (creams, lotion, tip stick, mascara, eye shadows, deodorants, hair creams, depilatories and hair
conditioners), textiles (batik), handicrafts (eco design), musical instruments (flutes, didgeridoo, violins and drums), varnishes and polishes (paintings, art restoration, metal, wood and leather treatment) and industrial products (anti-corrosion rust inhibitor and lubricants).

Lucas et al., and Regert et al. [55, 56] reported that beeswax was widely used until the second half on the 19th century for a large variety of purposes in the fields of technology, art, medicine, and in religious rituals.

Bretle et al. [57] have concluded that beeswax is a useful tool to understand region-origin of food items and biological materials. Beeswax was used in various fields such as cosmetic, food, pharmaceutical, engineering and industry [58-61].

Beeswax was used for different purposes, such as a sealant, adhesive or a plasticizer in the production of adhesives, a fuel for illumination, a waterproofing agent, an insect repellent, ingredient in the production of medicinal ointment and lost wax [42, 62-73].
Chapter three

Materials and Methods

3.1 Materials

3.1.1 Beeswax samples

The beeswax samples were collected from two different regions of Sudan. Sample 1 was purchased from the local market in Aldamazin (Blue Nile State) and sample 2 was collected from wild bee colonies near Algeneina (Western Darfur State).

3.1.2 Chemicals

n-Hexane (Assay = 85%, density = 0.655–0.665 g cm$^{-3}$ at 20°C, Mwt= 86.16, AlphaChemika, India). Dichloromethane (Assay = 98%, density = 1.325 g cm$^{-3}$ at 20°C, boiling point = 39.8, Mwt= 86.16, SCOTT Science UIC, England). Diethyl ether (Assay = 98%, density = 0.713–0.717 g cm$^{-3}$ at 20°C, Mwt= 74, AlphaChemika, India). Hydrochloric acid (Assay = 35-38%, density = 1.189 g cm$^{-3}$ at 20°C, Mwt= 36.5, Alpha Chemika, India). Silica gel (60-120 mesh for column chromatography, TechnoPharmChem, India). Potassium hydroxide (Assay = 85%, density, Mwt= 56.11, SCOTT Science UIC, England). Activated Charcoal LR (Techno Pharm Chem, India). Potassium iodide (Assay = 99.8%, Mwt= 166, Alpha Chemika, India). Sodium thiosulphate (Assay = 99%, Mwt= 243.17, Alpha Chemika, India). Iodine mono chloride (Assay = 98%, Mwt= 162.36, Alpha Chemika, India). Acetic acid glacial (Assay = 99.5%, density = 1.047–1.052 g cm$^{-3}$ at 20°C, Mwt= 60.05, Alpha Chemika, India). Ethanol (Assay = 99.8%, density = 0.789 g cm$^{-3}$ at 20°C, Mwt= 46.07, Romil pure chemistry, United Kingdom). Chloroform (Assay = 99.8%, density = 1.474–1.480 g cm$^{-3}$ at 20°C, Mwt= 119.38, Alpha Chemika, India).

3.2 Treatment of the row beeswax
The beeswax was cleaned from the remaining honey by washing with hot water, then filtered while it was at 40°C and dried. The dried beeswax was dissolved in 50 mL n-hexane and charcoal was added to get rid of the coloured substances. The mixture was boiled at 70°C, filtered while it was hot, washed by using hot n-hexane, then evaporated and dried.

3.3 Physico-chemical parameters of beeswax

3.3.1 Acid value

0.255 g of the beeswax was dissolved in 20 mL chloroform. Then two drops of phenolphthalein were added and the solution was titrated against 0.05Methanolic potassium hydroxide with continuous shaking until the color changed to pink. A blank solution (20 mL of chloroform) was also titrated to correct solvent acidity. The acid value (in mg KOH/g) was calculated by the formula:

$$\text{Acid value} = 56.1 \cdot \frac{M \cdot (V - V^*)}{W}$$

Where V is the volume in mL of solution of ethanolic potassium hydroxide required by the sample; V* is volume in mL of solution of ethanolic potassium hydroxide required for the blank; M is the molarity of solution of ethanolic potassium hydroxide and W is mass in gram of the beeswax sample.

3.3.2 Peroxide value

0.255 g of the beeswax was dissolved in 25 mL of chloroform. 15 mL of glacial acetic acid and 5.0 ml of potassium iodide solution 10% were added. The mixture was gently shaken and kept in darkness for 5 min. then the solution was titrated against 0.1M sodium thiosulphate solution using starch as an indicator. A blank solution (was mixture from 25 mL of chloroform, 15 mL of glacial acetic acid and 5.0 mL of potassium iodide solution 10%) was titrated under the same conditions. The peroxide value (milli equivalents of oxygen /kg) was calculated by the formula:
Peroxidevalue = 1000 M\(\frac{V-V^°}{W}\)........................................................................(2)

Where V is the volume in mL of the sodium thiosulphate solution required by the sample; \(V^°\) is volume in mL of sodium thiosulphate solution required for the blank; M is the molarity of the sodium thiosulphate solution and W is mass in g of the beeswax sample.

### 3.3.3 Iodine value

0.313 g of the beeswax was dissolved in 20 mL of chloroform. 25.0 mL of solution of iodide mono chloride 2% was added. The mixture was softly shaken for 30 s and kept in darkness for 1h. After that 25 mL of aqueous solution of potassium iodide 10% was added, and the solution was titrated versus 0.1M solution of sodium thiosulphate using starch solution as indicator. A blank solution (was prepared by mixed of 20 mL of chloroform and 25.0 mL of solution of iodide mono chloride 2%. The mixture was softly shaken for 30 s and kept in darkness for 1h. After that 25 mL of aqueous solution of potassium iodide 10% was added) was also titrated under the same condition to correct the possible influence of the reagents. The iodine value (in g I /100 g of sample) was calculated by the formula:

Iodine value =1.269 \(\frac{V^°-V}{W}\)......................................................................................... (3)

Where V is the volume in mL of solution of sodium thiosulphate required by the sample; \(V^°\) is volume in mL of sodium thiosulphate solution required for the blank and w is mass in g of the beeswax sample.

### 3.3.4 Saponification value

0.293 g of the beeswax was dissolved in 25 mL of 0.5M ethanolic potassium hydroxide and refluxed at 65°C for two hours. The mixture was cooled and two drops of phenolphthalein were added and titrated against 0.5 M hydrochloric acid with continuous shaking until the end point. A blank (25 mL of 0.5 M ethanolic potassium hydroxide) assay was also conducted. The saponification value (in mg KOH/g) was calculated by the formula:
Saponification value = 56.1 M $\frac{V^o - V}{w}$

Where V is the volume in mL of hydrochloric acid required by the sample; $V^o$ is volume in mL hydrochloric acid required for the blank; M is the molarity of hydrochloric acid and w is mass in g of the beeswax sample.

### 3.4 Fractionation of beeswax by column chromatography

2.325 g of the beeswax sample was dissolved in 25 mL of hot dichloromethane and the solution was fractionated by passing through a silica gel column chromatography. 250 mL n-hexane was used as an eluent for separation of the hydrocarbons. After collecting the hydrocarbon fraction the ester fractions were eluted by using 500 mL di-chloromethane. The solvents were removed and the remaining components were dried and collected. The percentages of the fractions were calculated using the following equations:

Hydrocarbons% = (weight of the hydrocarbon fraction/weight of the sample) $\times 100$ ............... (5)

Esters% = (weight of the esters fraction/weight of the sample) $\times 100$ ..................................... (6)

### 3.5 Hydrolysis of esters compounds

0.2986 g of the esters fraction were dissolved in 50 mL ethanolic potassium hydroxide (1M) and refluxed at 65 °C for three hours. The mixture was cooled and transferred to a separatory funnel where 50 mL of distilled water and 50 mL of n-hexane were added. The mixture was shaken well and the organic layer was separated from the aqueous layer and collected. The previous process was repeated five times by using 50 mL n-hexane each time. The combined extracts (organic layer) were distilled by using simple distillation, evaporated, and dried. The percentage of the alcohols was calculated using the following equation:

Alcohols% = (weight of the alcohols fraction/weight of the sample) $\times 100$ ......................... (7)
The aqueous layer was acidified by using concentrated hydrochloric acid until pH of the solution was around 2. Then it was extracted by using 50 mL of n-hexane five times. The collected organic layer was evaporated and dried. The percentage of the acids fraction was calculated using the following equation:

\[
\text{Acids \%} = \left( \frac{\text{weight of the acid fraction}}{\text{weight of the sample}} \right) \times 100
\]  

(8)

### 3.6 Scanning electron microscopy (SEM)

Scanning electron microscopy is the best known and most widely-used of the surface analytical techniques [74]. The three most common modes of operation in SEM analysis are Back Scattered Electron imaging (BSE), Secondary Electron Imaging (SEI), and Energy-dispersive X-ray spectrometry (EDS) [75]. SEM, accompanied by X-ray analysis, is considered a relatively rapid, inexpensive, and basically non-destructive approach to surface analysis[76]. It is often used to survey surface analytical problems before proceeding to techniques that are more surface-sensitive and more specialized. In order to use the SEM and the microprobe facilities the samples have to be coated with a thin conducting layer of gold in order to prevent charging of the sample [77].

SEM was performed by using a JEOL 6400 (JEOL, Peabody, MA) scanning electron microscope to investigate the morphology of the beeswax samples. The sample was coated with gold and allowed to dry. The dried specimens were viewed using an accelerating voltage of 15 kV.

### 3.7 Characterization of beeswax and beeswax-fractions using FT-IR

Fourier transform-infrared spectroscopy (FT-IR) is an analytical technique used to identify organic (and in some cases inorganic) materials. Almost all organic molecules absorb radiations of various wavelengths in intermediate infrared region of the electromagnetic spectrum (\(\lambda = 1.5\) to 20 µm). Every compound show characteristic absorption bands in the infrared region of the spectrum. The absorption bands depending on change in dipole moment during the vibration of
the molecule or the functional group under study. Furthermore, a given bond may undergo several different type of vibratory motion (scissoring, rocking, wagging, and twisting). Hence, each bond gives rise to several bands. It has been observed that a particular vibrational band can be associated with specific group in a molecule, so most of the bands in a spectrum can be assigned due to the presence of certain specific chemical bonds [78-82].

A small amount of the sample was mixed thoroughly with potassium bromide and compressed into a thin transparent pellet using a hydraulic presser. The FT-IR spectra of the samples were obtained using a SHIMAZUIR-spectrometer in the wave-number range of 4000 to 400 cm\(^{-1}\).

### 3.8 Characterization of beeswax using GC/MS

Gas chromatography/mass spectrometry (GC/MS) is the marriage of two analytical methods into a versatile technique for the identification of complex volatile materials. Gas chromatography (GC) effectively separates the different constituents of the sample for subsequent analysis and identification by mass spectrometry (MS). The chromatographic separation relies on the interaction of the sample with a mobile phase and a stationary phase within the GC instrument column. The sample is carried through the column by the mobile phase, typically an inert gas. However, the sample is slowed in its travel through the column as the sample molecules repeatedly adsorb and desorb from the stationary phase in the column. The affinity of a particular molecule for the stationary phase determines the retention time of that constituent in the column. The molecules for each component of the sample will travel through the column at nearly the same rate and exit (elute) from the column within a narrow time band that is specific to that component. Thus, compounds with different retention times in the column are physically separated for presentation to a detector and analyzer. As a sample constituent elutes from the GC column, it enters the ionization chamber of the mass spectrometer where the molecules are ionized, typically by electron impact. When an electron impact with a sample molecule resulted in the loss of an electron from the molecule, a positive ion is formed. Some of the molecular ions are further fragmented into daughter ions and neutral fragments. The positive ions are then repelled out of the ionization chamber by a small positive charge within the chamber. Negative ions are also formed by the electron impact, but are not analyzed. The positive ions are separated
according to their mass by a mass analyzer. The magnetic poles separate the ions by their mass/charge ratio, successively focusing ions with increasing mass onto a detector for counting. The analyzer scans step-wise through a set range of mass values to evaluate the relative abundance of ions at each mass value [81-83].

10 mg of the sample were dissolved in 20 mL n-hexane. Chromatographic analysis was performed using QP2010 Plus gas chromatograph fitted with mass spectrometer detector and on column injection system. Separations were carried out on DB-5MS capillary column which is 30 m in length, 0.25 mm its internal diameter, and 0.25 μm its thickness of the film. The conditions for the analysis were set as follows:-

The oven temperature was set at 50 °C and held for 3 min and then increased at a rate of 10°C/min up to 180 °C and held for 3 min and then increased at a rate of 5 °C/min up to 325 °C and held for 15 min. The injector and detector temperature were 250°C and 325°C respectively. Helium was used as a carrier gas at a flow rate of 1.22 mL/min.

3.9 Characterization of hydrocarbons using GC/FID

Few milligrams of the sample were dissolved in 20 mL n-hexane. Chromatographic analysis was performed using Varian gas chromatograph fitted with flame ionization detector and on column injection system. Separations were carried out on PONA capillary column which is 50 m in length, 0.2 mm is the internal diameter and 0.5 μm is film thickness. The conditions for the analysis were set as follows:-

The oven temperature was set at 50°C and then increased at a rate of 10°C/min up to 100°C and further increased at a rate of 5°C/min up to 300°C. The injector and detector temperatures were 50°C and 300°C respectively. Helium was used as a carrier gas at a flow rate of 1.5 mL/min.
3.10 Thermo-gravimetric analysis (TGA)

Thermo-gravimetric analysis (TGA) is an analytical technique used to determine a material’s thermal stability and its fraction of volatile components by monitoring the weight change that occurs as a specimen is heated. The measurement is normally carried out in air or in an inert atmosphere, such as Helium or Argon, and the weight is recorded as a function of increasing temperature[84,85].

TGA analysis was used to study the thermal stabilities of the beeswax samples in a DuPont Thermal Analyzer (Model 990, Wilmington, DE). Samples with weights in the range of 5 to 10 mg were heated under a flowing nitrogen atmosphere (50 mL min\(^{-1}\)) from room temperature to 800 °C at a heating rate of 10 °C min\(^{-1}\), and the corresponding mass loss was recorded.
Chapter four

Results and Discussion

4.1 Physico-chemical parameters of beeswax samples

Table 4.1 shows the values of the physico-chemical parameters of beeswax samples 1 and 2. As can be seen from the table, the acid and the peroxide values of the two samples were found to be zero. For the peroxide value, this indicates that these samples did not undergo any oxidation reactions during the time of storage and processing, although one sample was collected from wild bee colonies (fresh sample from western Sudan) and the other which we did not know where and when it was collected (from Aldamazin local market). Furthermore, the acid value showed the absence of free fatty acids which indicate that the samples did not undergo any sort of hydrolysis reactions of the esters components (this is originally applied to fats and oils). It is important to mention that free fatty acids were reported by other researchers [34,38] as a main constituent of the beeswax. The iodine value which measures the presence of degree of unsaturation has demonstrated that both samples contain unsaturated constituents. In fats and oils, the saponification value measures the average molecular weight of the triacylglycerol in a sample. The smaller the saponification value the larger the average molecular weight of the triacylglycerol’s present.

Table 4.1: Represents the physico-chemical parameters of beeswax samples.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Sample 1</th>
<th>Sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acid value (mg KOH /g)</td>
<td>00.00</td>
<td>00.00</td>
</tr>
<tr>
<td>Peroxide value (meg O /kg)</td>
<td>00.00</td>
<td>00.00</td>
</tr>
<tr>
<td>Iodine adsorption value (g I/100g)</td>
<td>20.27</td>
<td>15.08</td>
</tr>
<tr>
<td>Saponification value (mg KOH /g)</td>
<td>47.87</td>
<td>75.23</td>
</tr>
</tbody>
</table>
Previous studies [46,47] have shown that both the acids and the alcohols which constitute the esters in beeswax are long chain-high molecular weight compounds. Noticeable difference in the saponification value between the two samples is quite clear which is difficult to be explained when other characterization techniques are considered and can only be attributed to experimental errors. Compared to previous studies [17] on physicochemical parameters of beeswax samples, considerable variations have be noticed in our samples especially in the case of acid and peroxide values.

4.2 Scanning electron microscopy of beeswax samples

Scanning electron microscopy was used to study the surface features of the beeswax samples. Figures 4.1 and 4.2 show SEM micrographs at 10µm magnification.

![SEM micrograph of beeswax sample 1](image)

**Figure 4.1: SEM micrograph of beeswax sample 1**

As can be seen from the micrographs, both samples have shown smooth surfaces with the presence of nodules-like shapes. Some differences in the micrographs between the two samples were clear which could be due to the way how these two samples were viewed or handled under
SEM. Properly SEM analysis should be taken for liquid nitrogen fractured surfaces (was not done in our samples) and not directly to the surface of the sample as received.

Figure 4.2: SEM micrograph of beeswax sample 2

4.3 Fourier transform-infrared (FT-IR) analyses of beeswax samples

FT-IR spectroscopic analysis was performed to examine the functional groups present in the beeswax (sample 1 and 2), fractions of beeswax, and components resulted from the alkaline hydrolysis of the esters in the range of 4000 cm\(^{-1}\) to 400 cm\(^{-1}\).

Table 4.2: characteristic infrared absorption frequencies of beeswax sample 1 and 2.

<table>
<thead>
<tr>
<th>Wave number (cm(^{-1}))</th>
<th>Functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>2918, 2849</td>
<td>stretching vibrations of saturated C–H</td>
</tr>
<tr>
<td>1736</td>
<td>C=O of esters</td>
</tr>
<tr>
<td>1463</td>
<td>C–H bending vibration</td>
</tr>
<tr>
<td>1175</td>
<td>C–O stretching vibration</td>
</tr>
<tr>
<td>725</td>
<td>C–C stretching vibration</td>
</tr>
</tbody>
</table>
However, the C=O of esters, C-H bending vibration, C-O stretching vibration and C-C stretching vibration in figure 4.4 have more intense absorption peaks than those of figure 4.3, which indicates a higher contents of the absorbing species in sample 2.

Figure 4.3   FT-IR spectrum of beeswax sample 1

It could be concluded that the beeswax samples 1&2 are heterogeneous mixtures of several compounds. It has been reported in the literature [25,37] that beeswax is composed of many compounds which include hydrocarbons, esters, free fatty acids as major components and minor amount of free fatty alcohols and other unidentified constituents.
Figure 4.4 FT-IR spectrum of beeswax sample 2

4.4 Gas chromatography/Mass spectrometry (GC/MS) of beeswax samples

GC/MS spectra of beeswax sample 1 and sample 2 are shown in figures 4.5 and 4.6 and the retention times, component, molecular formula, and percentage of each compound are presented in table 4.2 and 4.3. As can be seen from the tables, beeswax samples contain mixture of saturated and mono unsaturated odd and even numbered long chain hydrocarbons in the range between C\textsubscript{19} to C\textsubscript{53}. Additionally, the presence of the branched saturated hydrocarbons, long chain esters of palmitic acid, sulphonic acid and acid of flour (Nonadecyl pentafluoropropionate, Heptacosyl heptafluorobutyrate, and Docosylheptafluorobutyrate), long chain free alcohols and steroids have also been noticed. It could be observed that the long chain hydrocarbons constitute the highest percentage (73.78\%) of the total compounds present.
However, the results of the fractionation proved that the hydrocarbons form about 15.55% to 17.35% of the total components of the beeswax. This contradiction could be attributed to the fact that the highest temperature which the chromatograph reaches during the analysis was about 325°C. This temperature is not high enough to volatilize the components that have high molecular weights. Previous work by other researchers [38,42] have shown some esters with molecular weights reaches values784. It could also be seen that the even numbered hydrocarbons have higher percentages than odd numbered.

In addition, GC/MS of the beeswax sample 1 have shown the presence of minor amounts of mono-unsaturated aliphatic hydrocarbons (9-Tricosene and 17-Pentatriacontene). It is noticeable that the dotriacontane represents the highest percentage in hydrocarbons followed by the n-tetratriacontane, and n-hexatriacontane. The results also showed the presence of the even numbered hydrocarbons in rang between C_{10} to C_{54}. These compounds include 3,5-
dimethyloctane, 3,7-dimethyldecane, n-eicosane, n-tetracosane, n-octacosane, n-dotriacontane, n-tetratriacontane, n-hexatriacontane, n-tetracosane, n-tetrapientacontane. On the other hand, the odd numbered hydrocarbons were observed in range of C_{11}-C_{35}. 4,5-Dimethylnonane, 2,6,11-Trimethyldodecane, n-Nonadecane, n-Heneicosane, 9-Tricose, n-Nonacosane, 17-Pentatriacontene constitute these compounds.

Figure 4.6  GC/MS spectrum of beeswax sample 2

The esters compounds were observed in range of C_{17}-C_{36} and they are composed of n-hexadecanoic acid methyl ester, (Z)-14-tricosenyl formate, hexadecanoic acid, and eicosyl ester. Moreover, the detected long chain alcohols in beeswax are 1-heneicosanol, 1-heptacosanol, 1-triacontanol, and 1,30-triacontanediol. Finally, minor compounds of sulphate, flour and silicate esters were detected and may be due to contamination.
Table 4.3: Represents the compounds and their percentage in beeswax sample 1 by using GC/MS spectroscopy.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>compound</th>
<th>Molecular formula</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.169</td>
<td>4,5-Dimethylnonane</td>
<td>C_{11}H_{24}</td>
<td>0.07</td>
</tr>
<tr>
<td>9.952</td>
<td>3,5-Dimethylloctane</td>
<td>C_{10}H_{22}</td>
<td>0.07</td>
</tr>
<tr>
<td>12.647</td>
<td>3,7-Dimethyldecane</td>
<td>C_{12}H_{26}</td>
<td>0.07</td>
</tr>
<tr>
<td>15.536</td>
<td>2,6,11-Trimethyldodecane</td>
<td>C_{15}H_{32}</td>
<td>0.06</td>
</tr>
<tr>
<td>23.453</td>
<td>n-Hexadecanoic acid methyl ester</td>
<td>C_{17}H_{34}O_{2}</td>
<td>0.28</td>
</tr>
<tr>
<td>26.918</td>
<td>n-Nonadecane</td>
<td>C_{19}H_{40}</td>
<td>0.57</td>
</tr>
<tr>
<td>30.574</td>
<td>n-Eicosane</td>
<td>C_{20}H_{42}</td>
<td>3.86</td>
</tr>
<tr>
<td>32.268</td>
<td>n-Heneicosane</td>
<td>C_{30}H_{62}</td>
<td>0.27</td>
</tr>
<tr>
<td>33.291</td>
<td>Sulphurous acid, 2-propyl tridecyl ester</td>
<td>C_{18}H_{34}O_{3}S</td>
<td>0.09</td>
</tr>
<tr>
<td>33.478</td>
<td>9-Tricosene</td>
<td>C_{23}H_{46}</td>
<td>0.15</td>
</tr>
<tr>
<td>33.942</td>
<td>n-Tetracosane</td>
<td>C_{24}H_{50}</td>
<td>13.78</td>
</tr>
<tr>
<td>35.461</td>
<td>n-Nonacosane</td>
<td>C_{29}H_{60}</td>
<td>0.99</td>
</tr>
<tr>
<td>36.717</td>
<td>1-Heneicosanol</td>
<td>C_{31}H_{44}O</td>
<td>0.10</td>
</tr>
<tr>
<td>37.033</td>
<td>n-Dotriacontane</td>
<td>C_{32}H_{66}</td>
<td>24.78</td>
</tr>
<tr>
<td>37.275</td>
<td>1,1,1,5,7,7,7-Heptamethyl-3,3-bis(trimethylsiloxy)tetrasiloxane</td>
<td>C_{13}H_{40}O_{5}Si_{6}</td>
<td>0.04</td>
</tr>
<tr>
<td>37.421</td>
<td>n-Tettratriacontane</td>
<td>C_{34}H_{70}</td>
<td>1.11</td>
</tr>
<tr>
<td>39.482</td>
<td>1-Heptacosanol</td>
<td>C_{36}H_{74}</td>
<td>0.42</td>
</tr>
<tr>
<td>39.592</td>
<td>Nonadecyl pentafluoropropionate</td>
<td>C_{29}H_{39}F_{5}O_{2}</td>
<td>0.16</td>
</tr>
<tr>
<td>39.833</td>
<td>n-Hexatriacontane</td>
<td>C_{32}H_{74}</td>
<td>11.80</td>
</tr>
<tr>
<td>42.221</td>
<td>17-Pentatriacontene</td>
<td>C_{35}H_{70}</td>
<td>1.95</td>
</tr>
<tr>
<td>42.490</td>
<td>n-Tetartetracontane</td>
<td>C_{36}H_{90}</td>
<td>13.70</td>
</tr>
<tr>
<td>42.810</td>
<td>Tetrapentacontane</td>
<td>C_{38}H_{110}</td>
<td>0.32</td>
</tr>
<tr>
<td>43.451</td>
<td>Heneicosyl pentafluoropropionate</td>
<td>C_{36}H_{39}F_{5}O_{2}</td>
<td>0.19</td>
</tr>
<tr>
<td>44.402</td>
<td>(Z)-14-Tricosenyl formate</td>
<td>C_{32}H_{46}O_{2}</td>
<td>0.20</td>
</tr>
<tr>
<td>44.679</td>
<td>1-Triacontanol</td>
<td>C_{38}H_{62}O</td>
<td>10.88</td>
</tr>
<tr>
<td>45.215</td>
<td>4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6a,6b,7,7,8,8a,9,10,11,12a,14,14a,14b-octadecahydro-2H-picen-3-one</td>
<td>C_{30}H_{48}O</td>
<td>0.45</td>
</tr>
<tr>
<td>45.809</td>
<td>Lup-20(29)-en-3.beta.-ol</td>
<td>C_{30}H_{50}O</td>
<td>0.49</td>
</tr>
<tr>
<td>46.808</td>
<td>1,30-Triacontanediol</td>
<td>C_{38}H_{62}O</td>
<td>0.11</td>
</tr>
<tr>
<td>47.031</td>
<td>Docosyl heptafluorobutyrate</td>
<td>C_{36}H_{45}F_{7}O_{2}</td>
<td>3.33</td>
</tr>
<tr>
<td>47.175</td>
<td>5H-3,5a-Epoxydaphn[2,1-c]oxepin, dodecahydro-3,8,11a-tetramethyl-, [3S-(3.alpha.,5a.alpha.,7a.alpha.,11a.beta.,11b.alpha.,)]</td>
<td>C_{15}H_{30}O_{2}</td>
<td>0.14</td>
</tr>
<tr>
<td>58.677</td>
<td>Hexadecanoic acid, eicosyl ester</td>
<td>C_{36}H_{74}O_{2}</td>
<td>3.90</td>
</tr>
</tbody>
</table>

The GC/MS of sample 2 shows similar results as in the case of sample 1 although some differences were noticed. n-nonacosane represents the highest percentage in hydrocarbons.
followed by the n-tetracosane, n-tetracontane and n-hexatriacontane. It could be concluded that the slight variation between sample 1 and sample 2 due to different localities, treatment of the samples, the age of the wax, and the its climatic conditions.

Table 4.4: Represents the compounds and their percentages in sample 2 by using GC/MS spectroscopy.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>compound</th>
<th>molecular formula</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>9.183</td>
<td>4,5-Dimethylnonane</td>
<td>C₁₁H₂₂</td>
<td>0.07</td>
</tr>
<tr>
<td>9.967</td>
<td>3,5-Dimethyloctaneu</td>
<td>C₁₀H₂₂</td>
<td>0.06</td>
</tr>
<tr>
<td>12.659</td>
<td>3,7-Dimethyldecane</td>
<td>C₁₁H₂₆</td>
<td>0.06</td>
</tr>
<tr>
<td>15.548</td>
<td>2,6,11-Trimethyldodecane</td>
<td>C₁₈H₃₂</td>
<td>0.06</td>
</tr>
<tr>
<td>26.931</td>
<td>n-Nonadecane</td>
<td>C₁₉H₄₀</td>
<td>0.54</td>
</tr>
<tr>
<td>30.579</td>
<td>n-Eicosane</td>
<td>C₂₀H₄₂</td>
<td>3.4</td>
</tr>
<tr>
<td>32.280</td>
<td>n-Heneicosan</td>
<td>C₂₁H₄₄</td>
<td>0.22</td>
</tr>
<tr>
<td>33.489</td>
<td>cis-9-Tricosene</td>
<td>C₂₃H₄₆</td>
<td>0.35</td>
</tr>
<tr>
<td>33.934</td>
<td>n-Tetracosane</td>
<td>C₂₄H₅₀</td>
<td>12.57</td>
</tr>
<tr>
<td>34.425</td>
<td>n-Octacosane</td>
<td>C₂₅H₅₈</td>
<td>0.33</td>
</tr>
<tr>
<td>36.600</td>
<td>n-Tetraicosanol-1</td>
<td>C₂₆H₆₀</td>
<td>0.53</td>
</tr>
<tr>
<td>37.023</td>
<td>n-Nonacosane</td>
<td>C₂₉H₆₀</td>
<td>23.12</td>
</tr>
<tr>
<td>38.415</td>
<td>Dotriacontane</td>
<td>C₃₂H₆₆</td>
<td>0.43</td>
</tr>
<tr>
<td>39.283</td>
<td>Sulfurous acid, 2-ethylhexyl isohexyl ester</td>
<td>C₁₄H₃₀O₃S</td>
<td>0.07</td>
</tr>
<tr>
<td>39.828</td>
<td>n-Hexatriacontane</td>
<td>C₃₆H₇₄</td>
<td>10.97</td>
</tr>
<tr>
<td>40.211</td>
<td>1-Heptacosanol</td>
<td>C₂₇H₅₆O</td>
<td>1.2</td>
</tr>
<tr>
<td>41.146</td>
<td>Nonadecyl pentafluoropropionate</td>
<td>C₂₉H₅₈F₃O₂</td>
<td>0.37</td>
</tr>
<tr>
<td>41.943</td>
<td>2-cis-9-Octadecenyloxyethanol</td>
<td>C₃₀H₆₀O₂</td>
<td>0.09</td>
</tr>
<tr>
<td>42.226</td>
<td>Palmitic acid, octadecyl ester</td>
<td>C₃₀H₆₈O₂</td>
<td>3.01</td>
</tr>
<tr>
<td>42.480</td>
<td>n-Tetracontane</td>
<td>C₃₀H₆₂</td>
<td>11.75</td>
</tr>
<tr>
<td>42.818</td>
<td>Tritetracontane</td>
<td>C₃₀H₅₈</td>
<td>0.44</td>
</tr>
<tr>
<td>43.457</td>
<td>1-Octacosanol</td>
<td>C₂₉H₅₈O</td>
<td>0.25</td>
</tr>
<tr>
<td>44.233</td>
<td>Dodecyl palmitate</td>
<td>C₂₈H₅₆O₂</td>
<td>0.82</td>
</tr>
<tr>
<td>44.404</td>
<td>(Z)-14-Tricosenyl formate</td>
<td>C₃₀H₆₄O₂</td>
<td>1.85</td>
</tr>
<tr>
<td>44.674</td>
<td>1-Triacontanol</td>
<td>C₃₀H₆₂O</td>
<td>11.81</td>
</tr>
<tr>
<td>44.941</td>
<td>n-Tetracontane</td>
<td>C₃₀H₆₀</td>
<td>1.82</td>
</tr>
<tr>
<td>46.631</td>
<td>4,4,6a,6b,8a,11,11,14b-Octamethyl-1,4,4a,5,6,6a,6b,7,8,8a,9,10,11,12,12a,14,14a,14b-octadecahydro-2H-picen-3-one</td>
<td>C₃₀H₄₈O</td>
<td>0.11</td>
</tr>
<tr>
<td>46.743</td>
<td>Oleic acid, (Z)-9-octadecenyl ester</td>
<td>C₃₀H₆₈O₂</td>
<td>0.38</td>
</tr>
<tr>
<td>46.806</td>
<td>2-cis-9-Octadecenyloxyethanol</td>
<td>C₂₉H₅₆O₂</td>
<td>0.35</td>
</tr>
<tr>
<td>47.029</td>
<td>Heptacosyl heptadecyl ester</td>
<td>C₃₁H₅₅F₇O₂</td>
<td>2.69</td>
</tr>
<tr>
<td>47.182</td>
<td>Lup-20(29)-ene-3, 28-diol, (3.beta.)</td>
<td>C₃₀H₅₆O₂</td>
<td>0.42</td>
</tr>
<tr>
<td>54.967</td>
<td>Palmitic acid, tetradecyl ester</td>
<td>C₃₀H₆₀O₂</td>
<td>2.27</td>
</tr>
<tr>
<td>58.668</td>
<td>Palmitic acid, eicosyl ester</td>
<td>C₃₆H₇₂O₂</td>
<td>4.1</td>
</tr>
</tbody>
</table>
4.5 Thermogravimetric analysis of beeswax sample

Thermogravimetric analysis was carried out to study the thermal stabilities of samples 1 and 2. Figures 4.7 and 4.8 show the weight loss percentage of the two samples as a function of temperature. As can be seen from TGA curves, both samples were started to degrade appreciably above 300°C and when the temperature reaches 600 °C both samples were completely pyrolyzed. In general, the two samples have shown fairly similar decomposition pattern with few exceptions. The two major degradation steps in sample 1 occurred at 342 °C and 355 °C (approx.), with mass loss equals 54% and 71% (approx.) respectively. Three relatively small mass loss took place at 311 °C, 432 °C and 523 °C. Sample two gives almost similar values with slight variations. As it was determined previously these samples contain mixture of esters, hydrocarbons, and trace amounts of alcohol and other materials. It could be concluded that further studies which include separation of each component and study it is thermal stability is crucial to understand the thermal behaviour and the mechanism of degradation of these compounds.

Figure 4.7 TGA curve of beeswax sample 1
Figure 4.8  TGA curve of beeswax sample 2

4.6 Fractionation of beeswax samples by silica gel column chromatography

Table 4.5: Represents the percentages of the fractions of beeswax samples 1 and 2.

<table>
<thead>
<tr>
<th></th>
<th>Sample 1</th>
<th></th>
<th>Sample 2</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Fraction (1)</td>
<td>15.55%</td>
<td>Fraction (2)</td>
<td>49.9%</td>
<td></td>
</tr>
<tr>
<td>Fraction (1)</td>
<td>17.35%</td>
<td>Fraction (2)</td>
<td>47.0%</td>
<td></td>
</tr>
</tbody>
</table>

Fractionation of beeswax by silica gel column chromatography depends on the differences on polarities of the compounds to be fractionated, the solvents used, and the stationary phase[79]. Two fractions were collected by varying the composition of the solvent system used. Fraction 1 which was eluted by n-hexane is most probably pure hydrocarbons and fraction 2 which was eluted by dichloromethane is most probably esters. As can be seen from the table fraction 1
constitutes 15-17% of the whole wax whereas fraction 2 forms approximately 50% of the wax components.

4.6.1 FT-IR analysis of fraction 1

Fraction (1) was characterized by FT-IR spectroscopy and gas chromatography (GC/FID). Figure 4.9 shows the FT-IR spectrum of fraction 1.

![FT-IR spectrum of fraction 1 of sample 1](image)

**Figure 4.9** FT-IR spectrum of fraction 1 of sample 1

It could be observed that the spectrum shows two strong absorption peaks at about 2918 cm\(^{-1}\) and 2849 cm\(^{-1}\) which are attributed to symmetric and asymmetric stretching vibrations of saturated C-H bonds (sp\(^3\) hybridized). Additionally, the spectrum displays the presence of medium absorption peak at about 1463 cm\(^{-1}\) which could be due to C-H bending vibration. Finally, the absorption peak around 710-730 cm\(^{-1}\) could be attributed to rocking vibration of C-H. It could be concluded that fraction 1 is pure hydrocarbon.
4.6.2 Gas chromatography (GC/FID) analysis of fraction 1

GC/FID was performed to further characterize the components of fraction 1. Table 4.5 represents the retention time, components and their percentages. As can be seen from the table, fifteen hydrocarbons were present which they ranged between C19 and C33. Both saturated long chain odd and even numbered hydrocarbons were detected.

Table 4.6: The summary of the GC/FID chromatography for the n-hexane fraction (fraction 1) of sample 1.

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Compound</th>
<th>Number of carbon atoms</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.174</td>
<td>Nonadecane</td>
<td>n-C19</td>
<td>0.11</td>
</tr>
<tr>
<td>22.689</td>
<td>Eicosane</td>
<td>n-C20</td>
<td>0.07</td>
</tr>
<tr>
<td>23.31</td>
<td>Henecosane</td>
<td>n-C21</td>
<td>0.35</td>
</tr>
<tr>
<td>23.991</td>
<td>Docosane</td>
<td>n-C22</td>
<td>0.11</td>
</tr>
<tr>
<td>24.76</td>
<td>Tricosane</td>
<td>n-C23</td>
<td>2.58</td>
</tr>
<tr>
<td>25.641</td>
<td>Tetracosane</td>
<td>n-C24</td>
<td>0.30</td>
</tr>
<tr>
<td>26.692</td>
<td>Pentacosane</td>
<td>n-C25</td>
<td>15.10</td>
</tr>
<tr>
<td>27.899</td>
<td>Hexacosane</td>
<td>n-C26</td>
<td>0.93</td>
</tr>
<tr>
<td>29.421</td>
<td>Heptacosane</td>
<td>n-C27</td>
<td>37.39</td>
</tr>
<tr>
<td>31.12</td>
<td>Octacosane</td>
<td>n-C28</td>
<td>0.57</td>
</tr>
<tr>
<td>33.294</td>
<td>Nonacosane</td>
<td>n-C29</td>
<td>14.5</td>
</tr>
<tr>
<td>35.823</td>
<td>Triacontane</td>
<td>n-C30</td>
<td>0.67</td>
</tr>
<tr>
<td>39.046</td>
<td>Hentriaccontane</td>
<td>n-C31</td>
<td>21.19</td>
</tr>
<tr>
<td>42.762</td>
<td>Dotricontane</td>
<td>n-C32</td>
<td>0.23</td>
</tr>
<tr>
<td>47.412</td>
<td>Tritricontane</td>
<td>n-C33</td>
<td>4.5</td>
</tr>
</tbody>
</table>

The most abundant compounds in hydrocarbons are heptacosane 37 % followed by hentriacontane 21.19 %, pentacosane 15.1 % and nonacosane 14.5 %. The odd numbered hydrocarbons have the highest percentages compared to the even numbered hydrocarbons.
4.7 FT-IR analysis of fraction 2

Figure 4.10 shows the FT-IR spectrum of fraction 2. It could be seen that the spectrum represents characteristic symmetric and asymmetric C-H stretching vibrations at 2918 cm$^{-1}$ and 2849 cm$^{-1}$, C-H bending vibration at 1472 cm$^{-1}$, strong carbonyl (C=O) stretching vibration of esters at 1736 cm$^{-1}$, and C-O stretching vibration at 1175 cm$^{-1}$. It could be concluded that fraction 2 is ester.

![FT-IR spectrum of fraction 2 of sample 1](image)

Figure 4.10 FT-IR spectrum of fraction 2 of sample 1

4.8 Hydrolysis and Characterization of the alkaline components of fraction 2

Fraction 2 which is most probably ester was further hydrolyzed in alkaline medium and the resultant compounds were separated by solvent extraction and characterized by FT-IR spectroscopy. Table 4.6 displays the percentage of each component of the ester. In general, both samples have shown almost equal percentages of the acids (56%) and the alcohols (40%) which form the components of the esters.
Table 4.7: Shows percentages of compounds were resulted from hydrolysis of ester.

<table>
<thead>
<tr>
<th>Ester fractions of sample 1</th>
<th>Ester fractions of sample 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alcohol</td>
<td>Acid</td>
</tr>
<tr>
<td>56.4%</td>
<td>41.31%</td>
</tr>
</tbody>
</table>

The separated acids and alcohols were further characterized by FT-IR. Figure 4.11 shows a number of characteristics absorption peaks. The intense and broad band at 3330 cm\(^{-1}\) is due to stretching vibration of -OH group whereas the two peaks around 2918 cm\(^{-1}\) and 2849 cm\(^{-1}\) are attributed to symmetric and asymmetric stretching vibrations of C-H bond. Furthermore, the peak at 1474 cm\(^{-1}\) is due to bending vibration of C-H bond, and at 1061 cm\(^{-1}\) is due to stretching vibration of C-O. Finally, the peak at 719 cm\(^{-1}\) is due to C-H rocking vibration. Obviously, this spectrum represents alcohol fraction.

Figure 4.11 FT-IR spectrum of alcohols of sample 1
Figure 4.12: FT-IR spectrum of acids of sample 1

Figure 4.12 shows the FT-IR spectrum of the acid fraction. As can be noticed, the intense broad band around 3474 cm\(^{-1}\) is attributed to O-H stretching vibration. The carbonyl group of the acids gives strong sharp peak at 1703 cm\(^{-1}\). The characteristic two peaks at 2974 cm\(^{-1}\) and 2934 cm\(^{-1}\) are due to symmetric and asymmetric stretching vibration of C-H whereas the bending vibration of this functional group appeared at 1468 cm\(^{-1}\) and 1366 cm\(^{-1}\) respectively. In addition, two intense peaks present around 1217 cm\(^{-1}\) and 1180 cm\(^{-1}\) are due to C-O stretching vibration. Finally, the peak at 1148 cm\(^{-1}\) could be assigned to C-C stretching vibration whereas the peaks at 951 cm\(^{-1}\) and 912 cm\(^{-1}\) are due to C-O bending vibration coupled with OH out of plane.
Beeswax is one of most important naturally occurring waxy materials. It has found applications in many fields such as medicinal, cosmetics, and food additives. The properties of beeswax depend on its origin, age, and climatic conditions.

Purified two samples of beeswax were collected from different region in Sudan and their column chromatography fractions have been investigated in this study. FT-IR, GC/MS, GC/FID have demonstrated that these two samples are heterogeneous in nature. The two major components were found to be the esters and the hydrocarbons. Minor amounts of free fatty acids and alcohol were also detected by GC/MS. Surprisingly, both the physicochemical parameters and the FT-IR analyses have shown the absence of these compounds. This may be interpreted by their low concentrations or abundance in these samples.

Although the results of GC/MS have shown that the hydrocarbons form the major constituents in the two samples but this could probably due to the lack of detection of long chain ester compounds because of their low volatility and relatively low temperature (max. 380 °C) during GC analysis. Better resolution and detection could be achieved with high temperature GC. For the same reason it was not possible to detect the acids and alcohols fractions, which were eluted from hydrolysis of ester fraction in alkaline solution with GC/MS and only poorly resolved spectra were obtained.

It was obvious from this work that the maximum temperature for safe uses of beeswax in different applications is about 290 °C and any temperature above 320 °C results in considerable degradation of the wax. For better understanding of degradation mechanism and kinetics, separation and characterization of individual components is substantial.
Applications of other techniques such as polarized optical microscopy, differential scanning calorimetry, and NMR are advisable and will produce valuable information about the structure and thermal behaviour of these samples.
Reference


DOI: 10.1051/apido:2007033

DOI: 10.1007/bf02609202

DOI: 10.1016/s0021-9673(02)00825-7


DOI: 10.1016/j.foodchem.2012.09.003

DOI: 10.1007/BF02027565

(E901) as a glazing agent and as carrier for four flavors. The European Food Safety Authority Journal 2007; 615: pp. 1-28.


   DOI: 10.1016/s0965-2299(03)00120-1

   DOI: 10.1111/j.1468-0092.1987.tb00138.x


   DOI: 10.1111/j.1475-4754.1995.tb00730.x

   DOI: 10.1016/s0305-4403(95)80156-1


   DOI: 10.1002/jssc.200301608


DOI: 10.1016/s0378-3820(02)00007-3

DOI: 10.1016/j.jhazmat.2005.02.003

DOI: 10.1016/S0166-526X(06)47001-X