University of Gezira

Physicochemical Properties of Sudanese Crude Beeswax

By:

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Physicochemical Properties of Sudanese Crude Beeswax

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Department of Applied Chemistry and Chemical Technology
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January, 2016
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Date of Examination: January/ 2016
DEDICATION

I would like to dedicate this work to:

My small family: beloved husband & dear son.

Big family: great Dad & Mum, sweet brothers & sister.

To all my friends & anyone who contributed to this work.
Acknowledgement

I would like to thank Dr. Mubarak El Siddig for his great help in all steps of this research and valuable guidance. Deep thanks go to my husband Dr. Adil Klil for his assistance and standing beside me always. To my beloved family & great friends specially Suzan. Also my thanks to the staff of chemistry laboratory, Ahafad University.
Physicochemical Properties of Sudanese Crude Beeswax
Mervat Saeed Ajeeb Sideek

Abstract

Beeswax is the oldest natural animal wax used by Man. It is the most important among natural waxes because of its unique properties and useful applications in medicine, cosmetics, food, engineering and industry. It is a very complex material made of saturated and unsaturated n-Alkanes, long-chain wax esters, fatty acids, diesters, hydroxyesters, and other minor constituents, such as flavonoids. The objective of this work was to study the physicochemical properties of a sample of crude beeswax obtained from the local market. The physicochemical properties of the sample determined according to Kenyan Standards were as follows: melting point 64°C-65°C; acid value 17.29; saponification value 96.7; ester value 79.41; ester /acid ratio 4.59; unsaponifiable matter 49%. All these values lie within the range reported in literature for crude beeswax except for the ester /acid ratio which was slightly higher than the range (3.3 - 4.3) reported in literature, and the unsaponifiable matter which was slightly lower than the range (50-60) reported in literature. Analysis of the unsaponifiable matter by GC/MS showed the presence of (s)-2,3-dihydro-4-(1methyl propyl)-furan; 5-methyl,4-heptene-3-one; oxalic acid, allyl tridecyl ester as major components constituting 60.27, 32.65 and 7.98%, respectively. Analysis of the saponifiable matter by GC/MS showed the presence of Palmitic acid; Petroselanic acid as major components constituting 86.62 and 7.94%, respectively. Lignoceric acid, stearic acid and linoleic acid constituted 2.76, 1.07 and 0.87%, respectively. The presence of these acids in crude beeswax has been reported in literature. The results obtained in this study are in conformity with those reported for crude beeswax. It is recommended that part of the crude beeswax produced locally be upgraded by bleaching to improve its colour and quality for use in the cosmetic and pharmaceutical formulations.
الخواص الفيزيائية والكيميائية لعينة خام شمع النحل السوداني

ميرفت سعيد عجيب صديق

ملخص الدراسة

يعتبر شمع النحل من أقدم وأهم أنواع الشموع الطبيعية الحيوانية المستخدمة بواسطة الإنسان نسبة لخواصه الفريدة المستفاد منها في المجالات الطبية والتجميلية والغذائية والهندسية والصناعية. وهو من المواد ذات التركيب المعقد مثل الألكانات المشبعة وغير المشبعة والاسترات الشمعية طويلة السلسلة والأحماض الدهنية والاسترات الثنائية و الاسترات الهيدروكسيدية ومكونات أخرى بكميات قليلة مثل الفلافونويدات. كان الهدف من هذا العمل دراسة الخواص الفيزيائية والكيميائية لعينة خام شمع النحل أخذت من السوق المحلي. تم تحديد الخواص الفيزيائية والكيميائية وفقًا للمواصفات الكينية وكانت النتائج كما يلي:

نقطة الانصهار (64-65) درجة مئوية، قيمة الحمض 17.29، قيمة التصبغ 96.7, قيمة الاستر 79.4، معدل الاستر للحمض 4.50، نسبة المواد الغير متصبنة 49% وقد جاءت هذه النتائج مطابقة للنتائج المراجع باستثناء نسبة الاستر للحمض التي كانت أعلى قليلاً من تلك المنشورة بالمراجع (3.3-4.3). وكذلك المواد غير المتصبنة والتي كانت أقل قليلاً من تلك المنشورة بالمراجع (50-60). أوضح تحليل المواد غير المتصبنة بطريقة GC / MS وجود المواد الآتية: (8) -3.2 داي هابيدرو-4 (1 ميثيل بروبيل) - فيوران؛ 5 - ميثيل 4-هيدين-3- أون ؛ حمض أوكساليك، 6 - أكسيديساسيل أستر كمواد رئيسية بالنسبة التالية: 60.27، 23.65، 7.97% على التوالي. أما المواد المتصبنة فقد كانت كما يلي: حمض البالتمتيك ؛ حمض بينرو سيلينيك بنسبة التالية: 86.62% على التوالي كمواد رئيسية وحمض لينوناليك بنسبة التالية: 26.76، 10.74، 0.74% على التوالي.

وجود هذه الأحماض في شمع النحل الخام موضوع الدراسة تتفق مع ما ورد في المراجع. وقد كانت النتائج المتحصل عليها في هذه الدراسة أيضًا متفقة مع ما ورد بالراجع لشمع النحل الخام. أوصت الدراسة بإجراء عملية التبييض لجزء من شمع النحل الخام المنتج لتحسين لونه و خواصه لغرض الاستعمال في المستحضرات التجميلية والتركيب الصيدلانية.
contents

Dedication i
Acknowledgement ii
Abstract (english ) iii
Abstract (arabic ) iv
Contents v
List of Tables vii
List of Figures viii
List of Appendices' ix

Chapter one: Introduction
Introduction 1
1.2-Aim and Objective 3

Chapter Two: Literature Review
2.1 Composition of beeswax 4
2.2 Adulteration of beeswax 4
2.3 Classification of beeswax 7
2.3.1 yellow beeswax 7
2.3.2 white beeswax 7
2.4 Physical properties of beeswax 8
2.5 Manufacturing of beeswax 8
2.5.1 Crude beeswax 8
2.5.2 Purified and Bleached 8
2.5.2.1 Acids 8
2.5.2.2 Hydrogen peroxide solution
2.5.2.3 Sun bleaching
2.5.2.4 Potassium permanganate
2.6 Uses

Chapter Three: Material and Methods

3.1. Material
3.2. Determination of the physicochemical properties of the crude beeswax

Chapter Four: Results and Discussion

4.1. Calculation of acid value
4.2. Calculation of Saponification value
4.3. Calculation of ester value
4.4. Calculation of ester/acid ratio
4.5. Determination unsaponifiable matter
4.5.1 Composition of the Unsaponifiable Matter
4.5.2 Composition of saponifiable matter 22

Conclusion and Recommendation 25

References 26
List of Tables

Table (1) : General composition of beeswax 5

Table(2): Comparison of the properties of Sudanese crude bees-wax with Switzerland and Kenya standards 18

Table (3) : Result of analysis of the unsaponifiable matter by GC/MS 19

Table (4): Analysis of the saponifiable matter obtained by GC/MS Process 22
List of Figures

Figure (1) : Crude beeswax  11

Figure (2) : GC/MS Instrument  14
<table>
<thead>
<tr>
<th>Appendix</th>
<th>Description</th>
<th>Page</th>
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<td>(1)</td>
<td>5-methyl,4-heptene-3-one</td>
<td>30</td>
</tr>
<tr>
<td>(2)</td>
<td>(s)-2,3-dihydro-4-(1methyl propyl)-furan</td>
<td>31</td>
</tr>
<tr>
<td>(3)</td>
<td>Dibenzyl ether</td>
<td>32</td>
</tr>
<tr>
<td>(4)</td>
<td>4-heptanol, 4-ethyl-2,6-dimethyl</td>
<td>33</td>
</tr>
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<td>(5)</td>
<td>Oxalic acid, allyl tridecyl ester</td>
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<td>(6)</td>
<td>Butylated hydroxytoluene Palmitic acid</td>
<td>35</td>
</tr>
<tr>
<td>(7)</td>
<td>Palmitic acid</td>
<td>36</td>
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<td>(8)</td>
<td>Linoleic acid</td>
<td>37</td>
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<td>(9)</td>
<td>Methyl Petroselinate</td>
<td>38</td>
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<td>(10)</td>
<td>Stearic acid</td>
<td>39</td>
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<tr>
<td>(11)</td>
<td>Lignoceric acid</td>
<td>40</td>
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</table>
Chapter One

Introduction
Waxes are among the oldest worked materials used by humans. Their value as versatile construction materials (‘‘man’s first plastic’’) was discovered very early. Today, waxes are used mostly as additives and active substances. The use of waxes is expected to increase in the future because of their generally favorable toxicological and ecological properties.

The historic prototype of all waxes is beeswax. Since it could be obtained with relatively little effort, it was popular in antiquity, and even now the term wax is occasionally used in everyday speech as a synonym for beeswax. However, the scientific and commercial definition of wax covers a much wider area.

Beeswax is the oldest natural animal wax used by Man. It is the most important among natural waxes because of its unique properties and useful applications in medicine, cosmetics, food, engineering and industry (Negri et al. 2000). From a chemical standpoint, beeswax is a very complex material made of saturated and unsaturated $n$- Alkanes, long-chain wax esters, fatty acids, diesters, hydroxyesters, and other minor constituents, such as flavonoids (Kolattukudy, 1976).

Beeswax is an end product of the metabolism of a honeybee class (Apis mellifera, L. Fam. Apidae), which belongs to the Apis genus. This species of honeybee is found worldwide and domesticated to a high degree (Büll, 1958–1970).

Bees need wax as construction material for their combs. They produce it in their wax glands, which are fully developed in 12 to 18 days old workers (Hepburn, 1986). Crude beeswax varies in color from deep brown to light yellow. The color depends upon the type of flower from which the bees have gathered pollen, the amount of natural contaminants such as propolis, and the age and treatment of
the combs. Generally, the light yellow crudes are of better quality because they bleach more readily than the darker crudes (Bennett, 1963).

Two types of beeswax are marketed: yellow beeswax and white beeswax. Yellow beeswax is a yellow or light-brown solid that is somewhat brittle when cold and presents a characteristic odour of honey. White beeswax is a white or yellowish white solid having a characteristic, but faint, odour of honey (Kuznesof, 2005).

Industrial beeswax production began in the 19th century. The good quality of beeswax depends greatly on the production methods. There are two wax extraction methods: melting and chemical extraction. Melting is the most frequently used procedure. Wax can be melted by boiling water, by steam, or by electrical or solar power (Temnov, 1967). Beeswax, mainly the yellow variety, is one of the raw materials used in the production of household candles, expensive decorative candles, and candles for religious purposes. Most bleached and purified beeswax is used in cosmetics and pharmaceuticals (to regulate the consistency of lipsticks, creams, ointments, and suppositories) and in the food sector, as a release agent and a polish in confectionery production (Kahl et al., 1994), as a component in dietary food supplements, glazings and coatings, chewing gum, and as a carrier for food additives (including flavours and colours) (Kuznesof, 2005). At present more than 15 000 t of beeswax are available on the world market (Kahl et al., 1994). The main raw materials for wax formation are carbohydrates, i.e. the honey sugars fructose, glucose and sucrose. The ratio of sugar to wax can vary from 3 to 30:1, a ratio of around 20:1 being typical for central Europe. The stronger the colony, the smaller the ratio, the more economical the wax production for the colony. One Lang troth frame, containing only 100 g of wax can hold 2-4 kg of honey. (Weiss, 1965).
1.2-Aim and Objective

1. To study the physicochemical properties of a sample of crude beeswax.

2. To compare the composition of the sample with literature.
Chapter Two

Literature Review
2- Literature Review

2.1- Composition of beeswax:

Beeswax is an extremely complex material containing over 300 different substances. It consists mainly of esters of higher fatty acids and alcohols. Apart from esters, beeswax contains small quantities of hydrocarbons, acids and other substances. In addition, approx. 50 aroma components have been identified (Ferber et al., 1977).

Crude beeswax contains a large number of minor components (e.g. terpenoids and flavonoids), most of which appear to be plant-derived (Puleo, 1991), the colour of the wax will depend to some extent on the type of flora visited by the bees. Volatile products have also been detected at low levels (Ferber et al., 1977). Oxygenated compounds produced by the bees are also present, amongst which decanal (ca. 50% of the oxygenated volatiles), 1-decanol, nonanal, octanal, furfural, and benzaldehyde are responsible for the bouquet of the wax (Blum et al., 1988).

Wax esters are chemically diverse, and most are formed from primary or secondary alcohols. The structure of beeswax is crystalline. The crystallisation of beeswax depends on the storage (Bennett, 1963).

2.2- Adulteration of beeswax:

Beeswax is sometimes adulterated with various materials. Stearic acid, Japan wax, hard paraffin and tallow have all been reported as adulterants. The purity of beeswax is tested by the ratio of the ester value to the acid value. Even this is not reliable if taken by itself. Wax like mixtures can be made which, although free from beeswax, have a normal ratio number, as for example,
a mixture obtained by melting 37.5 parts of Japan wax, 6.5 parts of stearic acid, and 6.5 parts of paraffin or ceresin (Bennett, 1963).

The following table shows general composition of beeswax (Tulloch, 1980):

Table (1): General composition of beeswax:

<table>
<thead>
<tr>
<th>Number of components in fraction</th>
<th>Component</th>
<th>Quantity %</th>
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<tbody>
<tr>
<td>Monoesters</td>
<td>35</td>
<td></td>
</tr>
<tr>
<td>Diesters</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Triesters</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Hydroxy monoesters</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Hydroxy polyesters</td>
<td>8</td>
<td></td>
</tr>
<tr>
<td>Acid esters</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Acid polyesters</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Hydrocarbons</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td>Free acids</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Alcohols</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>others</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>100</td>
<td></td>
</tr>
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</table>

The ratio of ester values to acids, a character used by the various pharmacopoeias to describe pure beeswax is changed significantly by prolonged or excessive heating. At 100 °C for 24 hours the ratio of ester to acid is changed beyond the limits set for pure beeswax. Longer heating or higher temperatures lead to greater degradation and loss of esters (Tulloch, 1980). These changes also influence the physical characteristics of the wax. Thus, excessive heating during
rendering or further processing changes the wax structurally and alters the beneficial characteristics of many substances of which wax is composed, there are also some proteins, which are added by the bees (Kurstjens et al., 1990).

The mass spectra of volatile compounds of beeswax(tetracosanic acid, triacontanol, triacontanyl, tetracosanyl 15-hydroxypalmitate, fatty acid, alcohol, monoester, and hydroxymonoester) have been described in literature (Garnier et al., 2002). The presence of a homologous series of saturated fatty acids (C16:0, C18:0, and C22:0-C34:0) with even carbon numbers of which tetracosanoic acid (C24:0) is the main constituent has also been reported by the same authors, which is in agreement with other investigations. The major monoesters of beeswax contain an even number of carbon atoms and are made of even-numbered n-alcohols (C24 to C34) esterified by palmitic acid.

The neutral components have been fractionated by flash chromatography on a silica column into three different fractions:

(i) hydrocarbons, (ii) monoesters, and (iii) coeluted n-alcohols and hydroxymonoesters.

Hydrocarbons, Alkanes and alkenes provide a typical pattern for the recognition of beeswax, extensively described by Stransky and Streibl (1971), consisting of n-alkanes (C23-C33) and n-alkenes (C27-C35) identified by their retention times by HTGC analysis when compared to those of commercial standards. The alkane / alkene ratio tends to decrease when the carbon length increases. Isolation of hydrocarbons from polar compounds, such as fatty acids and alcohols, avoids co elution phenomena and allows identification of monounsaturated alkenes with 27, 29, 31, 33, and 35 carbon atoms, present at a very low level (Garnier et al., 2002).
2.3-Classification of beeswax:

2.3.1-yellow beeswax: (synonyms: Cera Flava ;Cera Flav)

The colour of the freshly produced beeswax is white, later it turns to yellow. Yellow beeswax is a secretion formed by the hive bee, Apis mellifera L., and possibly other species of Apis ,and is used by insects to form the cells of the honey comb. After extraction, the wax is melted with water, separated and strained. The typical yellow colour originates from propels and pollen colorants. However, depending on the relative amounts of different pollens and propels pigments, wax colour can vary.

Esters comprise about 70% of yellow wax, the chief of which is myricyl palmitate, together with free wax acids, hydrocarbons, lactones, cholesterol ester and pollen pigment. Yellow beeswax is insoluble in water, slightly soluble in alcohol, soluble in chloroform, in warm ether and in fixed and volatile oils. It is used in preparation of ointments and in control of hemorrhage in bone in cranial surgery.

2.3.2-White beeswax: (Synonyms: Cera Alba;Cera Alb):

White beeswax is obtained by bleaching yellow beeswax. The bleaching may be effected by exposure to the wax in thin layers of the action of air, sunlight and moisture, or by treatment with chemicals such as potassium dichromate and sulphuric acid.

It is a yellowish-white solid with a slight characteristic odor; in thin layers it is translucent and has melting point of 62°-65°C. It is insoluble in water; slightly soluble in alcohol; soluble in chloroform in warm ether, and in fixed and volatile oils. It complies with the requirements of the British Pharmacopoeia.
2.4-Physical properties of beeswax:

Pure beeswax has a specific gravity of 0.95. It melts at 64.4°C, and hardens to 63.4°C (Vansell and Bisson, 1940). Beeswax does not dissolve in water, very slightly soluble in cold alcohol and completely dissolves in volatile oils, chloroform, ether, benzene at 30°C, and carbon disulfide at 30°C. During storage at low temperatures beeswax often develops a powdered substance on its surface, which is called the flour (Slijepac, 2013).

2.5-Manufacturing of beeswax:

2.5.1-crude beeswax:

Crude beeswax is manufactured by different methods, but the simplest method is the Hot water extraction using forced immersion, whereby the combs are placed in a tightly tied jute sack. The sacks are placed in a recipient with water and boiled. As wax is lighter than water, it filters through the jute and rise to the surface. After all combs have all melted, the pot is let cool down. The wax solidifies as it cools, forming a block on the water surface. The waste left in the sack is thrown out.

2.5.2 Purified and Bleached:

Purified and bleached beeswax can be obtained by one of the following methods:

2.5.2.1 Acids

Acids will bind a part of the iron which is responsible for wax darkening. Also they help breaking of emulsions and help the settling of impurities. Thus 2-3
g concentrated citric acid or oxalic acid, or 1 ml concentrated sulfuric acid are added to 1 l of water per kg wax.

2.5.2.2 **Hydrogen peroxide solution**

Concentrated hydrogen peroxide solution (about 35 % in basic milieu) is added to hot wax (100°C). It is essential that the peroxide is used up in the bleaching process. Excess peroxide could cause problems in the manufacture of creams and ointments.

2.5.2.3 **Sun bleaching**

Bleaching in a solar extractor will lighten the colour of the wax. In order to achieve bleaching, the wax should be exposed to the sun for several days.

2.5.2.4 **Potassium permanganate**

Wax is heated at about 90°C for 30 minutes in 0.01 % potassium permanganate in slightly acidic milieu, and the solution is exchanged with water (Bogdanov, 2009).

2.6-Uses:

Beeswax has many and varied uses. Purified and bleached beeswax is used in the production of food, cosmetics, and pharmaceuticals. The three main types of beeswax products are yellow, white, and beeswax absolute. Yellow beeswax is the crude product obtained from the honeycomb, white beeswax is bleached yellow beeswax, and beeswax absolute is yellow beeswax treated with alcohol (Anon, 2009).

In food preparation, it is used as a coating for cheese; by sealing out the air, protection is given against spoilage (mold growth). Beeswax may also be used as a food additive E901, in small quantities acting as a (glazing agent), which serves to prevent water loss, or used to provide surface protection for some fruits.
Beeswax is also a common ingredient of natural chewing gum (Frosch, et al., 2003). In foods and beverages, white beeswax and beeswax absolute are used as stiffening agents (Anon, 2005-2014).

Use of beeswax in skin care and cosmetics has been increasing. A German study found beeswax to be superior to similar barrier creams (usually mineral oil-based creams such as petroleum jelly), when used according to its protocol (Frosch et al., 2003). Beeswax is used in lip balm, lip gloss, hand creams, and moisturizers; and in cosmetics such as eye shadow, blush, and eye liner. Beeswax is an important ingredient in moustache wax and hair pomades, which make hair look sleek and shiny. Candle-making has long involved the use of beeswax, which is highly flammable, and this material traditionally was prescribed (in large part), for the making of the Paschal candle or "Easter candle" (Anon, 1913). Beeswax is an ingredient in surgical bone wax, which is used during surgery to control bleeding from bone surfaces; shoe polish and furniture polish can both use beeswax as a component, dissolved in turpentine or sometimes blended with linseed oil or tung oil. White beeswax is used in preparation of ointments and occasionally to adjust the melting point of suppositories.

As medicine, beeswax is used for lowering cholesterol and for relieving pain. It is also used for swelling (inflammation), ulcers, diarrhea, and hiccups. Beeswax has mild anti-swelling (anti-inflammatory) effects. There is also some evidence that it might help protect the stomach.

In manufacturing, yellow and white beeswax are used as thickeners, emulsifiers, and as stiffening agents in cosmetics. Beeswax absolute is used as a fragrance in soaps and perfumes. White beeswax and beeswax absolute are also used to polish pills (Anon, 2005-2014).
Chapter Three

Material and Methods
3.Material and Methods:

3.1.Material:

The crude beeswax sample was obtained from the Sudanese Center for Honey at Khartoum. The wax was packed in thin polythene in the form of rods weighing about 70 g, as shown in the following figure:

![Crude beeswax](image)

Figure (1): Crude beeswax

3.2. Determination of the physicochemical properties of the crude beeswax

The physicochemical properties of the crude beeswax were determined according to the Kenyan Standard CD/05-1279: 2013, ICS 67.120 (Specification for Natural Beeswax).

3.2.1. Determination of melting point:

About 1 g of the crude beeswax was melted in test tube by heating on water bath. The bulb of a thermometer was dipped into the molten wax, so as to get the bulb thinly coated with wax, and the thermometer was taken out. The thermometer was inserted into another test tube through a bored cork and the test
tube placed in the water bath. The temperature of the water was raised gradually, at the rate of 1 °C in 3 minutes. The temperature at which a transparent drop formed on the end of the thermometer bulb was recorded as the melting point of the wax.

**3.2.2. Determination of acid value:**

The acid value is the number of milligrams of potassium hydroxide needed to neutralize the free fatty acids present in one gram of fat or oil.

3 g of the crude beeswax were placed in a 200 ml flask. Then, 25 ml of neutralized, dehydrated alcohol were added and the wax warmed until it melted. The mixture was shaken and few drops of phenolphthalein indicator solution were added. The warm mixture was titrated with 0.5 N alcoholic potassium hydroxide solution until a permanent, faint pink colour was obtained.

The acid value was calculated according to the following formula:

\[
\text{Acid value} = 56.1 \times V \times N / M
\]

where,

\( V \) = volume in mL of standard potassium hydroxide solution used,
\( N \) = normality of standard potassium hydroxide solution,
\( M \) = mass in g of the wax taken for the test.

**3.2.3. Determination of saponification value:**

The saponification value is the number of milligram equivalents of 0.5 N potassium hydroxide required to saponify one gram of oil or fat.

2 g of the wax were weighed in a tared conical flask. Then, 25 mL of methyl ethyl ketone were added, followed by 25 ml of 0.5 N alcoholic potassium hydroxide solution. Few pieces of anti-bumping granules were added and the flask connected to an air condenser and the mixture refluxed on an electric heater for 2 hours.
After the flask and condenser have cooled down, the inside of the condenser was washed with about 10 ml of rectified spirit. A few drops of phenolphthalein indicator solution were added and the mixture titrated with standard 0.5 N hydrochloric acid. A blank test was carried out determined at the same time.

**Saponification value** = \( 56.1x (B – S)x N / M \)

Where:
- \( B \) = volume in ml of the standard hydrochloric acid required for the blank test.
- \( S \) = volume in ml of standard hydrochloric acid required for saponification of the wax.
- \( N \) = normality of standard hydrochloric acid.
- \( M \) = mass in g of the wax taken for the test.

### 3.2.4. Determination of ester value:

The number of milligrams of potassium hydroxide required to saponify the fatty acid esters in one gram of a fat, wax, oil etc, equal to the saponification value minus the acid value.

### 3.2.5. Calculation of ester /acid value:

The ester/acid ratio was calculated by dividing the ester value by the acid value.
3.2.6. Determination of unsaponifiable matter:

The mixture resulting from saponification of the wax was transferred to a separating funnel. The saponification flask was washed with 20 ml distilled water and the washing transferred to the separating funnel. Then 30 ml of distilled water were added to the separating funnel, and 3 x 15 ml the ether added to the mixture extract. The ether extract was transferred to another clean separating funnel and washed with 3 x 15 ml distilled water to remove KOH. The ether layer was transferred to 100 ml conical flask and dried with anhydrous Na$_2$SO$_4$. Finally, the ether was evaporated and the resulting unsaponifiable matter was weighed and the percentage calculated. A sample was analyzed by GC-MS. Using the following conditions:

![GC/MS Instrument]

Figure (2): GC/MS Instrument
Instrument:

GC: varian CB – 3800
MS: varian 4000
Column: BD / 5 (30 m × 0.25 mm)
Carrier gas: helium (flow rate 1 ml / min)
Temperature program:
- 80°C for 5 min.
- 280°C (5°C / min) hold for 10 min.
- 300°C (10°C / min) hold for 5 min.

3.2.7. Analysis of the saponifiable matter:

After removal of the unsaponifiable matter, the lower layer was acidified with HCl, and extracted with 3 x 15 ml of ether to recover the acids. The ether extracts were washed with 3 x 15 ml distilled water in another clean separating funnel until neutral to litmus paper. The ether extracts were combined in a 150 ml conical flask and dried with anhydrous Na₂SO₄. The saponifiable matter was analyzed by GC-MS using the same conditions above.
Chapter Four

Results and Discussion
4- Results and Discussion

4.1. Calculation of acid value:
Average volume of potassium hydroxide:

\[
\frac{(1.8+1.9)}{2} = 1.85 \text{ ml}
\]

Acid value \(= 56.1 \times (V) \times (N) / M\)

\[= 56.1 \times (1.85) \times (0.5) / 3\]

\[= 17.29\]

This result lies within the range \((17 - 24)\) reported in literature (Kenya Standards 2013).

4.2. Calculation of Saponification value:
Amount of 0.5 N HCl required for titration of the blank \(= 19.0 \text{ ml}\)
Amount of 0.5 N HCl required for titration of the excess of KOH \(= 12.1 \text{ ml}\)

Saponification value \(= 56.1 \times (B - S) \times N / M\)

\[= 56.1 \times (19.0 - 12.1) \times 0.5 / 2 = 96.7\]

This result lies within the range \((88 - 102)\) reported in literature (Kenya Standards 2013).

4.3. Calculation of ester value:
The ester value is equal to the difference between the saponification value and the acid value.

\[96.7 - 17.29 = 79.41\]
This result is slightly higher than the range (70 - 79) reported in literature (Kenya Standards 2013).

4.4. Calculation of ester /acid ratio:

ester /acid ratio: Ester value / Acid value.

\[
79.41 \div 17.29 = 4.5
\]

This result is slightly higher than the range (3.3 - 4.3) reported in literature for European beeswax (EP 2008, Kenya standards 2013), but significantly lower than the range (8-9) reported for the Asian beeswax.

The ratio of ester values to acids, a parameter determined in the pharmacopoeia gives information whether pure natural beeswax is changed significantly by prolonged or excessive heating. When heating wax at 100°C for 24 hours the ratio of ester to acid is changed beyond the limits set for pure beeswax. Longer heating or higher temperatures lead to greater degradation and loss of esters (Tulloch, 1980).

4.5. Determination of unsaponifiable matter:

The weight percentage of Unsaponifiable Matter was: 49%

This result is slightly lower than the range (50-56%) reported in literature
Table (2): shows the comparison of the properties of Sudanese crude beeswax with other standards.

<table>
<thead>
<tr>
<th>Test</th>
<th>Sudanese crude beeswax</th>
<th>Literature</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>KS</td>
<td>CFAS</td>
</tr>
<tr>
<td>Melting point</td>
<td>64ºC-65ºC</td>
<td>62º-65ºC</td>
</tr>
<tr>
<td>Acid value</td>
<td>17.29</td>
<td>17-24</td>
</tr>
<tr>
<td>Saponification value</td>
<td>96.7</td>
<td>88-102</td>
</tr>
<tr>
<td>Ester value</td>
<td>79.41</td>
<td>70-79</td>
</tr>
<tr>
<td>Ester/acid ratio</td>
<td>4.59</td>
<td>3.0-4.3</td>
</tr>
<tr>
<td>Unsaponifiable percentage</td>
<td>49%</td>
<td>50-60%</td>
</tr>
</tbody>
</table>

Key:

4.5.1- Composition of the Unsaponifiable Matter:

Table (3): analyses of the unsaponifiable matter by GC/MS:

<table>
<thead>
<tr>
<th>No</th>
<th>Compounds</th>
<th>RT min</th>
<th>M W</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5-methyl,4-heptene-3-one</td>
<td>14.71</td>
<td>126</td>
<td>23.65</td>
</tr>
<tr>
<td>2</td>
<td>(s)-2,3-dihydro-4-(1methyl propyl)-furan</td>
<td>16.22</td>
<td>126</td>
<td>60.27</td>
</tr>
<tr>
<td>3</td>
<td>Dibenzyl ether</td>
<td>19.41</td>
<td>198</td>
<td>2.320</td>
</tr>
<tr>
<td>4</td>
<td>4-heptanol, 4-ethyl-2,6-dimethyl-</td>
<td>23.31</td>
<td>172</td>
<td>1.856</td>
</tr>
<tr>
<td>5</td>
<td>Oxalic acid, allyl tridecyl ester</td>
<td>68.53</td>
<td>312</td>
<td>7.978</td>
</tr>
</tbody>
</table>

The GC/MS analysis of the unsaponifiable matter showed the presence of compounds, of which 5-methyl, 4-heptene-3-one (MW 126) was the first one with retention time 14.71 min, and a percentage of 23.65% by weight of the total compounds. The base peak m/e (97) (Appendix 1) is due to the loss of an ethyl group (CH₃CH₂-) as shown in the following structure.

![Structure of 5-methyl, 4-heptene-3-one](attachment:image)
The second compound was (S)-2,3-dihydro-4-(1-methyl propyl) – furan (MW 126) with retention time 16.22 min and percentage of 60.27 %, i.e. the major component in the unsaponifiable matter. The base peak m/e (97) (Appendix 2) is due to the loss of an ethyl group (CH$_3$ CH$_2$ - ) as shown in the following formula.

![Formula for (S)-2,3-dihydro-4-(1-methyl propyl) – furan]

The third compound was dibenzyl ether (MW198) with retention time 19.41 mins and a percentage of 2.320 %. The base peak m/e (91) (Appendix 3) is due to the benzyl fragment (C$_6$H$_5$CH$_2$ - ) as shown in the following formula.

![Formula for Dibenzyl ether]

The appearance of the characteristic peaks at m/e 77 (C$_6$H$_5$ +), 78 (C$_6$H$_6$ +), (C$_6$H$_7$ +) is due to α-cleavage and hydrogen rearrangements of monoalkylbenzenes (Silverstein et al, 1980 ).
The fourth compound was 4-ethyl-2,6-dimethyl-4-heptanol (MW172) with retention time 23.31 mins and a percentage of 1.856%. The base peak m/e (57) (Appendix 4) is due to the fragment \([(CH_3)_2 CH CH_2 –]\)\(^+\) as shown in the following formula.

![4-ethyl-2,6-dimethyl-4-heptanol](image)

The fifth compound was Oxalic acid, allyl tridecyl ester (MW312) with retention time 68.53 min. and a percentage of 7.978%. The base peak at m/e (41) (Appendix 5) is due to the fragment \((CH_2=CH CH_2 -)\)\(^+\) as shown in the following formula.

![Oxalic acid, allyl tridecyl ester](image)
4.5.2 - composition of saponifiable matter:

table (4): Analysis of the saponifiable matter obtained by GC/MS:

<table>
<thead>
<tr>
<th>NO</th>
<th>Compounds</th>
<th>RT min</th>
<th>MW</th>
<th>Area %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Butylated hydroxytoluene</td>
<td>20.50</td>
<td>220</td>
<td>0.7402</td>
</tr>
<tr>
<td>2</td>
<td>palmitic acid</td>
<td>29.64</td>
<td>256</td>
<td>86.62</td>
</tr>
<tr>
<td>3</td>
<td>Linoleic acid</td>
<td>32.82</td>
<td>280</td>
<td>0.8715</td>
</tr>
<tr>
<td>4</td>
<td>methyl Petroselinat</td>
<td>32.92</td>
<td>282</td>
<td>7.940</td>
</tr>
<tr>
<td>5</td>
<td>Stearic acid</td>
<td>33.39</td>
<td>284</td>
<td>1.071</td>
</tr>
<tr>
<td>6</td>
<td>Lignoceric acid</td>
<td>42.89</td>
<td>368</td>
<td>2.762</td>
</tr>
</tbody>
</table>

The presence of Butylated hydroxytoluene (RT 20.50 min, MW 220) in the saponifiable matter is due to the presence of the phenolic group in the molecule, which reacts with NaOH. The base peak at m/e (205) (Appendix 6) is due to loss of a methyl group (CH₃) from the molecule (M-CH₃).
The peaks at m/e 91 and 65 are due to the following rearrangement and elimination:

The major component was found palmitic acid (Hexadecanoic acid) (RT 29.64 min, MW 256) with a percentage of 86% of the saponifiable matter.

The presence of the palmitic and stearic acids in beeswax has been reported by (Stransky et al, 2000).

The base peak at m/e (43) of the palmitic acid (Appendix 7) is due to loss of the alkyl group (CH$_3$CH$_2$CH$_2$-)$^+$.  

palmitic acid, methyl ester
The second major component of percentage 7.9 % was Petroselinic acid (RT 32.92 min, MW 282).

The presence of the lignoceric acid (tetracosanoic acid) (RT 42.89 min, MW 368) in beeswax has also been reported in literature (Stransky and Streibl, 197, Aicholz and Lorbeer, 2000).
Conclusion and Recommendation

Conclusion:

A sample of crude beeswax was obtained from the local market and analyzed for its physicochemical properties. The results were as follows: melting point 64°-65°C; acid value 17.29; saponification value 96.7; ester value 79.41; ester/acid ratio 4.; unsaponifiable matter 49%. The results were in agreement with those reported for crude beeswax in literature and pharmacopoeias.

The GC/MS analysis of the unsaponifiable matter showed the presence of (s)-2,3-dihydro-4-(1methyl propyl)-furan; 5-methyl,4-heptene-3-one; oxalic acid, allyl tridecyl ester; 4-heptanol, 4-ethyl-2,6-dimethyl; dibenzyl ether. The GC/MS analysis of the saponifiable matter showed the presence of Palmitic acid; Petroselinic acid, Lignoceric acid, stearic acid and linoleic acid, which have been reported to be present in crude beeswax.

Recommendation:

It is recommended that part of the crude beeswax produced locally be upgraded by bleaching to improve its colour and quality for use in the cosmetic and pharmaceutical formulations.
References

26


20. Slijepac; D. 2013. Safety of beeswax productions. IV International Symposium "Agrosym".


Appendices
Appendix (1): 5-methyl,4-heptene-3-one
Appendix (2): (s)-2,3-dihydro-4-(1methyl propyl)-furan
Appendix (3) : Dibenzyl ether
Appendix (4): heptanol, 4-ethyl-2,6-dimethyl-
Appendix (5): Oxalic acid, allyl tridecyl ester
Appendix (6): Butylated hydroxytoluene
Appendix (7): palmitic acid
Appendix (8) : Linoleic acid
Appendix (9) : methyl Petroselinat
Appendix (10) : Stearic acid
Appendix (11) : Lignoceric acid