INSTITUTO POLITÉCNICO DE BRAGANÇA — Escola Superior Agrária

UNIVERSITY of SAIDA Dr MOULAY TAHAR

Faculty of Science
Department of Biology

Laboratory of Biotoxicology, Pharmacognosy and Biological Valorization of Plants (LBPVBP)
Centro de Investigação de Montanha (CIMO) IPB

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Quality evaluation of Algerian honeys: Eucalyptus, Jujube, Spurge and Multifloral

Submitted by: KAID SELOUA

Before the jury:

Mr. Kahloula Khaled	Professor	University Of Saida	President
Mr. Ziani Kaddour	Associate Professor « A »	University Of Saida	Supervisor1
Mr. Miguel José R.V.B	Professor	Polytechnic Institute of Bragança	Supervisor2
Mme. Hachem Yasmina	Associate Professor « B »	University Of Saida	Examiner

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ملخص

العسل منتج طبيعي يستهلك على نطاق واسع. وليس فقط مرغوبا فيه لمداقه وقيمته الغذائية. ولكن أيضا لفوائده الصحية مند العصور القديمة كان استهلاك العسل بشكل عام مرتبط بالخصائص الطبية حيث استخدم العسل تقليديا في التام الجروح والحروق ولعلاج نزلات البرد والتهابات الحلق. على مدى العقد الماضي تم تنفيد استراتيجية انمائية للزراعة في الجزائر في هدا السياق تم ايلاء اهتماما خاصا لتربية النحل وخاصة انتاج العسل مع اكثر من 20000مربي نحل و 1.2 مليون خلية تم تسجيلها مند عام 2010 .يعتبر تقييم الجودة ووضع معايير فيزيائية كيميائية للعسل الجزائري خطوة مهمة في تثمين قطاع النحالين من اجل المساهمة في معرفة خصائص العسل الجزائري احادي الازهار ومتعدد الازهار. سيتم اجراء توصيف شامل في العسل مع اصل محدد مثل عسل اللبينة والسدر والاوكاليبتس والعسل متعدد الازهار عن طريق التحليل الفيزيوكيميائي والميليسوبالينواوجي (اللون الرطوبة درجة الحموضة التوصيل الكهربائي مؤشر دياستاز البرولين هيدروكسي ميثيل فيرفورال والمحتوى المعدني)وكدلك تقييم ملامح السكر والفينول وكدا نشاط مضاد للأكسدة (تقليل الطاقة سيتم ايضا تقييم نشاط ازالة الجدور الحرة) والنشاط المضاد للورم .اخيرا سيكون البحث عن وجود المخلفات مثل المضادات الحيوية التيتراسيكلين والسولفاناميد من خلال نظام فحص مستقبلات التحليل المتعدد.

كلمات مفتاحية : العسلة, نحلة ابيس ميلفيرا, تربية النحل, متعدد الازهار, احادي الازهار, الخصائص الفيزيوكيميائية.

Abstract

Honey is a widely consumed natural product, not only desirable for its taste and nutritional value, but also for its health benefits. Since ancient times, honey consumption is generally associated with medicinal properties, being traditionally used for healing wounds and burns and for the treatment of colds and sore throats. Over the last decade, a development strategy for agriculture in Algeria was implemented and, in this context, special attention was given to beekeeping, and especially to honey production, with more than 20 000 beekeepers and 1.2 million colonies been registered since 2010. The quality assessment and the establishment of physicochemical parameters for the Algerian honey is an important step in the valorization of the beekeeper sector, turning honeys with specific floral origins in add-value products, commercially more desirables. In order to contribute to the knowledge of the properties of monofloral and multifloral honeys from Algeria in more detail, an extensive characterization will be performed in honeys with a specific origin such as *Ziziphus lotus*, *Euphorbia bupleuroides*, *Eucalyptus globulus* and multifloral honeys. For that, Melissopalynological and physicochemical analysis (color, moisture, pH, acidity, electrical conductivity, diastase index, proline, 5- hydroxymethylfurfural and mineral content) will be performed, as well as the evaluation of the sugar and phenolic profiles. Nutritional composition, antioxidant activity (reducing power, DPPH free radical scavenging activity and anti-tumor activity) will also be evaluated. Finally, the presence of residues, such the antibiotics tetracyclines and sulphonamides, will be evaluated through the multi-analyte receptor assay system Charm II.

Keywords: Honey, *Apis mellifera*, beekeeping, multifloral, monofloral, physicochemical parameters.

Resumo

O mel é um produto natural amplamente consumido, não apenas desejável pelo seu sabor e valor nutricional, mas também pelos seus benefícios relacionados com a saúde. Desde os tempos antigos, o consumo de mel está, geralmente, associado às suas propriedades medicinais, sendo tradicionalmente usado no tratamento de feridas e queimaduras e para o tratamento de constipações e dores de garganta. Devido a características associadas, essencialmente ou exclusivamente, com a origem geográfica e botânica específica, o mel pode ser classificado como um produto premium geralmente reconhecido como um produto de alta qualidade e valor. Na última década, foi implementada uma estratégia de desenvolvimento agrícola na Argélia e, nesse contexto, foi dada atenção especial à apicultura, principalmente, à produção de mel, com mais de 20.000 apicultores e 1,2 milhão de colónias registadas desde 2010. A avaliação da qualidade o estabelecimento dos parâmetros físico-químicos para o mel argelino são um passo importante na valorização do setor apícola, transformando méis com origens florais específicas em produtos de valor adicionado, comercialmente mais desejáveis. Com o objetivo de contribuir para o conhecimento das propriedades dos méis monoflorais e multiflorais da Argélia em mais detalhe, será realizada uma extensa caracterização de méis com uma origem botânica específica, tal como de *Ziziphus lotus*, *Euphorbia bupleuroide*s, *Eucalyptus globulus* e méis multiflorais. Para isso, serão realizadas análises melissopalinológicas e físico-químicas (cor, humidade, pH, acidez, condutividade elétrica, índice diastático, prolina, 5-hidroximetilfurfural e o conteúdo em minerais), bem como a avaliação do perfil em açúcares e compostos fenólicos. Também será identificada a composição nutricional e a atividade antioxidante (poder redutor, poder bloqueador de radicais livres e atividade anti-tumoral). Finalmente, a presença de resíduos, como os antibióticos tetraciclinas e sulfonamidas, serão avaliados através do sistema de ensaio Charm II.

<u>Palavras-chave :</u> Mel, *Apis mellifera*, Apicultura, multifloral, monofloral, parâmetros físico-químicos.

Résumé

Le miel est un produit naturel largement consommé, non seulement souhaitable pour son goût et sa valeur nutritionnelle, mais aussi pour ses bienfaits pour la santé. Depuis l'Antiquité, la consommation de miel est généralement associé à des propriétés médicinales, étant traditionnellement utilisé pour guérir les plaies et les brûlures et pour le traitement du rhume et des maux de gorge. Au cours de la dernière décennie, une stratégie de développement de l'agriculture en Algérie a été mise en œuvre et dans ce contexte, une attention particulière a été accordée à l'apiculture, et notamment à la production de miel, avec plus de 20 000 apiculteurs et 1,2 million de colonies enregistrées depuis 2010. L'évaluation de la qualité et l'établissement de paramètres physico-chimiques pour le miel Algérien est une étape importante dans la valorisation de la filière apicole, en tournant les miels avec origines florales spécifiques dans les produits à valeur ajoutée, commercialement plus souhaitables. Afin de contribuer à la connaissance des propriétés des miels monofloraux et multifloraux d'Algérie, une caractérisation approfondie sera effectuée dans les miels avec une origine spécifique comme *Ziziphus lotus*, *Euphorbia bupleuroides*, *Eucalyptus globulus* et miels multifloraux. Pour cela, des analyses mélissopalinologiques et physico-chimiques (couleur, humidité, pH, acidité, conductivité électrique, indice de diastase, proline, la teneur en hydroxymethylfurfural et en minéraux) sera effectuée, ainsi que l'évaluation des profils de sucre et de composés phénoliques. Composition nutritionnelle, activité antioxydante (pouvoir réducteur, L'activité de piégeage des radicaux libres DPPH) et l'activité anti-tumorale. Finalement, la présence de résidus, tels que les antibiotiques tétracyclines et sulfamides, sera évalués par le système de dosage des récepteurs multi-analytes Charm II.

Mots clé : le miel, Apis mellifera, Apiculture, multifleurs, monofleurs, paramètres physicochimique

Index

Index of figures

Index of tables

Abbreviations List

B2: thromboxane.

BC: Before Christ.

COX-2: cyclooxygenase-2.

DAD: Diode-array detection.

DPPH: 2, 2-diphenyl-1-picrylhydrazyl).

EC: Electrical conductivity. **ESI:** Electrospray ionization. **Eu:** European Union

HPLC-RI: High Performance Liquid Chromatography with refractive index.

HMF: Hydroxymethylfurfural.

INOS: Inducible nitric oxide synthase.

MS: Mass spectrometry.

Pfund: Honey color scale. **PGE2:** Prostaglandin E2, **PGF2α**: Prostaglandin F2a. **Ph:** Potential for hydrogen. **Ppm**: parts per million.

RJ: Royal jelly.

SRB: Sulforhodamine B

UPLC: Ultra-pressure liquid chromatography.

UV-VIS: Ultraviolet–visible

Introduction

Arid and semi-arid zones represent nearly two-thirds of Algerian area. The immensity of these territories and the absence of systematic studies of steppic bee flora, make honeys from these regions poorly studied and poorly understood.

Algerian beekeepers who have constantly attempted to rescue and guarantee the common characteristics of honey hope to discover different markets from local ones. For that, an extensive study of the Algerian honey is needed, having in mind the establishment of quality and authenticity guidelines and regulations. The aim of the whole study is to evaluate the quality of Algerian honey and verify its compliance with the established standards of Codex. For that, ten samples with different botanical and geographical origin (*Euphorbia jujube, Eucalyptus multifloral*) will analyzed regarding the following physicochemical parameters; Melissopalynological analysis, color, moisture, acidity, pH, ash content, electrical conductivity, diastase index, proline, 5- hydroxymethylfurfural (5-HMF), and mineral content and total phenolic compounds content. Nutritional composition, antioxidant activity (reducing power, DPPH free radical scavenging activity and) anti-tumor activity will also be evaluated. Finally, the presence of antibiotics, recurrent residues in honey, such as tetracyclines and sulphonamides will be also screened.

In this part of my work I will first focus on bibliographic data that have a direct link with this subject; the honey bee, bee products, honey generalities than the methodology of my experimental work.

1- Honey bees and bee products

1.1-Apis mellifera

Apis mellifera naturally occurs in Europe, the Middle East, and Africa. This species has been subdivided into at least 20 recognized subspecies (Mortensen, Schmehl and Ellis, 2013).Like all Hymenopterans, honey bees have haplo-diploid sex determination; Unfertilized eggs develop into drones (males), and fertilized eggs develop into females. Female larvae, which taken care with a standard food regimen of pollen, nectar, and brood nourishment become grown-up worker bees. Female larvae fed with a rich food regimen of royal jelly, pollen, and nectar becomes queens (Mortensen, Schmehl and Ellis, 2013**).** Worker honey bees are non-reproductive females. They are the smallest in physical size of the three ranks and their bodies are designed specifically for pollen and nectar collection **(Fig.1.A).** Queen honey bee **(Fig.1.B)** is the only reproductive female in the colony under normal situations. Her head and thorax are comparative in size to that of the worker, while the abdomen is more extended and plumper**.** Drones are the male cast of honey bees. Drone's head and thorax are bigger than those of the females, (**Fig.1.C**) (Mortensen, Schmehl and Ellis, 2013).

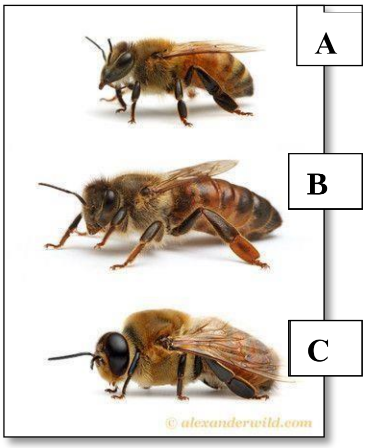


Figure 1.(*A) Worker European honey bee, Apis mellifera Linnaeus. (B) A Queen. (C) Drone (male) European honey bee, Apis mellifera. Photograph by Alexander wild https://www.alexanderwild.com/Insects/Stories/Honey-Bees/i-3DtbsJ.*

1.2-Bee Product

1.2.1-Beeswax

Beeswax is an extremely inert common material that is secreted by worker bees from the wax glands (Avshalom, Yaacov, 1996). Bees use beeswax to grow their young and to construct honeycomb cells where pollen and honey are stored. When secreted by bees, beeswax is white, but in the honey combs rapidly obscures due to the contact with the bees and also the pollen and honey (Avshalom, Yaacov, 1996).

1.2.2-Propolis

The word Propolis comes from the Greek «pro» = in front, «polis» = city, and means a substance with a protecting role for the bee colony (Bogdanov, 2014). Bees gathered resinous exudates from leaf buds, shoots and petioles of leaves from different plants with their mandibles, which once introduced into the hive, are mixed with wax and salivary secretions, in order to produce Propolis, which is used as a building and defence material within the hive. Propolis has a very complex composition and depends on the plant origin (Bankova and De Castro, 2000). The main chemical classes and most bioactive compounds found in Propolis are the phenolic compounds, which are responsible for most of the bioactivities (Bankova and De Castro, 2000).

1.2.3-Royal jelly

Royal jelly is a bee product secreted by the hypopharyngeal and mandibular glands of the nurse working bees (Zahran et al., 2016). Between the 6^{th} and 12^{th} day of their life cycle. RJ is a white-yellow colloid with a pH between 3.6–4.2, with a variable composition which depends on the metabolic and physiologic condition of worker bees, bee specie and on the seasonal and local conditions (Scorselli, Donadio, 2005).

1.2.4-Bee pollen and bee bread

Pollen grains are microscopic structures, male gametes located in the anthers of stamens, indispensable for the fertilization of the female sexual organ of the flower (Krell, 1996). Pollen is extremely important for the hive, it is the main source of food for the larvae providing them with important nutrients for their development such as proteins, and carbohydrates, lipids, vitamins and minerals (Luz et al.,2010).

1.2.5-Bee venom

Bee Venom (BV) is an odorless and transparent liquid is produced by female worker bees containing a hydrolytic mixture of proteins with acid pH (4.5 to 5.5) that bees often use as a defense tool against predators. One drop of BV consists of 88% of water and only 0.1 µg of dry venom (Bellik, 2015).

1.2.6-Honey

The *Codex Alimentarius* defined honey as a natural sweet substance, produced by honeybees from the nectar of plants, secretions of living parts of plants, or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature (Codex Alimentarius, 2001). The Definition of honey under European Union (EU) legislation is very similar, with the difference that it stipulates the bee species as being *Apis mellifera* (Directive 2001/110/EC).

2-Honey Categories Concerning Origin

2.1-Nectar honey

This type of honey is produced by bees after they harvest the nectar of the plants. Nectar is a sugar-rich liquid produced by plants in glands called nectaries, and mainly exist to encourage pollination by insects and other animals. About 95% of the dry substance are sugars, the rest are amino acids (0.05 %), minerals (0.02-0.45 %) and restricted amounts of organic acids, nutrients, and vitamins blends (Bogdanov, 2014). According to their botanical origin, nectar honeys can be classified as monofloral honeys, if they are produced from a single family or plant species, or as multifloral honeys when there is no floral species that stands out. This assessment is often carried out through an analysis of pollen grains that are present in honey, considering that when collecting nectar in the flower, bees transport pollen grains that they will inadvertently introduce into honey (Bear, 2009).

2.2-Honeydew honey

Honeydew honey is formed from secretions of living parts of plants, or they may have an animal origin, formed from the excretions of sucking insects (Hemiptera, mostly aphids) (Terrab et al., 2003).These insects break the plant cell and ingest the sap. The excess is excreted as droplets of honeydew, which are gathered by the bees (Bogdanov, 2014). Honeydew is a solution with varying sugar concentration (5-60 %), containing mainly sucrose, besides higher sugars (oligosaccharides). There are also smaller amounts of amino acids, proteins, minerals, acids and vitamins. Also, honeydew contains cells of algae and fungi (Bogdanov, 2014).

3-Chemical Composition of honey

Honey is composed mainly by sugars. Glucose and fructose and in a less amount water and other components like minerals, vitamins, proteins and amino acids, **Table 1.**

Table 1. Honey composition after (Bogdanov, 2009) values in g/100g

	Nectar honey g/100g		**Honey dew honey g/100g**	
	Average	Min-Max	Average	Min-Max
Water content	17.2	15-20	16.3	15-20
Fructose	38.2	30-45	31.8	28-40
Glucose	31.3	24-40	26.1	19-32
Sucrose	0.7	0.1	0.5	0.1-4.7
Other disaccharides	5.0	4.8	4.0	16
Melezitose	<0.1	28	4.0	0.3-22.0
Erlose	0.8	-	1.0	0.16
Other oligosaccharides	3.6	0.56	13.1	0.1-0.6
Total sugars	79.7	0.5-1	80.5	-
Minerals	0.2	0.1-0.5	0.9	0.6-2
Amino acids proteins	0.3	0.2-0.4	0.6	0.4-0.7
Acids	0.5	0.2-0.8	1.1	0.8-1.5
pH	3.9	3.5-4.5	5.2	4.5-6.5

3.1-Sugars

Sugars are the main constituents of honey, comprising about 95 % of honey dry weight (Bogdanov, 2014). The monosaccharides glucose and fructose are the main sugars found in honey, which are the building blocks of the more complex honey sugars and are the resulting products of the disaccharide sucrose hydrolysis (White, 1980). The main oligosaccharides in nectar honeys are disaccharides: sucrose, maltose, turanose, erlose. Honeydew honey also contain the trisaccharides melezitose and raffinose. Trace amounts of tetra and pentasaccharides have also been isolated, including isomaltotetraose and isomaltopentaose (Bogdanov, 2014).

3.2-Water content

Water is the second largest constituent of honeys, and its content is also related to the maturity of this product. Moisture content of honey can be influenced by floral and geographical origin, climatic factors, season of the year, processing and storage conditions, as well as the degree of maturity achieved in the hive (Gallina et al., 2010).

The moisture content has significant impact on the physical properties of honey, such as, viscosity and crystallization, but also taste, color, flavor, solubility, conservation and specific gravity and the shelf life of the product. According to the Codex Alimentarius Committee on Sugars, the moisture content in honey should not exceed 20 g 100 g^{-1} (Codex Alimentarius, 2001). If the moisture content is high the honey is more likely to ferment due to the presence of yeasts and osmophilic microorganisms. Since honey is hygroscopic, the moisture in honey can also increase during the processing operations of the product, as well as the inadequate storage conditions (White, 1980).

3.3-Proteins and amino acids

Proteins and amino acids in honey are originated from both bees (salivary glands), and plants (nectar, honeydew and mainly pollen). About 20 different nonenzymatic proteins have been identified in honey (De-Melo et al., 2018). The quantity of proteins can vary from 0.1 to 0.7%, **Table 1**. Overheated or long-time stored honeys show a reduction or absence of protein content (De-Melo et al., 2018). Around 26 amino acids have been detected in honey, such as proline, glutamic acid, alanine, phenylalanine, tyrosine, leucine, among others. (Cotte and Giroud, 2004). The most abundant amino acid found in honey is proline, ranging from 50 to 85% of the total. The proline content in honeys should be higher than 200 mg/kg. When the values of this amino acid are significantly lower than 180 mg/kg, the minimum value that has been agreed for genuine honey, it indicates sugar adulteration. Proline can be seen as quality criteria for honey ripness (Von-der, Dustmann, 1991).

3.4-Enzymes

The degrees of enzymes present in honey are sometimes used as an indicator for honey quality and freshness and overheating. Enzymes in honey are originates from the honey bees or from the plant visited by the bees. Diastase (amylase) digests starch to maltose and is relatively stable to heat and storage and invertase (saccharase, glucosidase), catalyses mainly the conversion of sucrose to glucose and fructose, but also many other sugar conversions (Raude, 1994). Also, glucose oxidase and catalase regulate the production of H_2O_2, one of the honey antibacterial factors (Bogdanov, 2014). The enzyme content also depends on temperature; honey botanical origin, nectar abundance flow, state and strength of the colony, seasonal activity of the bee, bee specie, diet, age and physiological stage of the bee (De-Melo et al., 2018).

Diastase activity is a physicochemical parameter usually investigated as marker of honey freshness (Fechner et al., 2016; Flores et al., 2015). It can be expressed in Schade, Göthe or diastase units and honey generally should present diastase activity of at least 8 Schade units, which is the minimum value accepted by regulatory organizations (Codex Alimentarius Commission, 2001). Similar to 5-HMF, the diastatic activity can be used as an indicator of aging and increase temperature because the diastatic activity may be reduced during storage or when the product is subjected to heating above 60 ^{o}C (Fechner et al., 2016; Flores et al., 2015).

3.5-Organic acids

Honey contains organic acids, in equilibrium with the corresponding lactone, representing less than 0.5% of total solids. They are important for honey taste, aroma, color, acidity and honey preservation, making it difficult for microorganisms to grow (Bogdanov, 2014). Organic acids in honey have different sources, while some acids can come directly from nectar or honeydew, the majority, are produced from sugars by the action of enzymes secreted by bees during ripeness and storage (De-Melo et al., 2018). Gluconic acid is the main honey organic acid, representing the 70–90% of the total (Bogdanov, 2014). It comes from glucose by the action of glucose oxidase. In addition to gluconic acid, more than 30 different non-aromatic organic acids were found in honey. Legally, organic acids should not exceed 50 meq/kg. For honey intended for industry, the tolerated limit is of 80 milliequivalents (Lequet, 2010).

3.6-Vitamins

Honey has small amounts of vitamins, which come mainly from the pollen grains in suspension (Matzke and Bogdanov, 2003). Vitamins found in honey include thiamine (B1), riboflavin (B2), nicotinic acid (B3), pantothenic acid (B5), pyridoxine (B6), biotin (B8 or H) and folic acid (B9) and also vitamin C. Those vitamins present in honey are preserved due to the low pH of honey. The commercial filtration of honey may cause a reduction in vitamin content due to the almost complete removal of pollen. Also, the loss of vitamins in honey can happen due to the oxidation of ascorbic acid by the hydrogen peroxide produced by glucose oxidase (Ciulu et al., 2011).

3.7-Mineral content

Mineral composition in honey is generally low, ranging between 0.02 and 0.3% in nectar honeys, while in honeydew honeys can reach 1% of the total (Felsner et al., 2004). Its content can vary with the soil and climatic conditions, as well as the chemical composition of the different nectars originated from the different botanical sources. Also, the harvesting, the beekeeping techniques. The main minerals found in honeys are potassium, sodium, calcium and magnesium and in lesser amounts iron, copper, manganese, chlorine. In minor quantities, as trace elements, are found boron, phosphorus, sulfur, silicon, bare and nickel, among others (Doner, 2003). Generally, dark honeys contain more minerals than the light ones, being higher in honeydew honeys (Bear, 2009). The mineral content is correlated with the ash percentage and the electrical conductivity (Da Silva et al., 2016).

3.8-Volatiles compounds

Researchers began the study of honey aromatic substance in the mid of 1960. Honey volatiles are the substances responsible for the honey fragrance. Most of them are derived from plants, but also some are included by the honey bees. Until now around 600 compounds have been identified in the volatile fraction of honey, and some are used as markers of monofloral honeys, such as 3,9-epoxy-1-*p*-mentadieno, t-8-*p*-menthan-oxide-1,2-diol and cis- rose, which have been proposed as markers of lemon honey; diketones, sulfur compounds and alkanes are characteristic of eucalyptus honey, while hexane and heptanal are the main compounds in the aroma of lavender honeys (Castro-Vázquez et al., 2007). Other volatile from different chemical families are present in honey at very low concentrations, such as monoterpenes, C13-norisoprenoid, sesquiterpenes, benzene derivatives and, to a lower content, superior alcohols, esters, fatty acids, ketones, terpenes and aldehydes (Pontes et al., 2007).

3.9-Phenolic compounds

Phenolic compounds are plant-derived secondary metabolites. These compounds have been used as chemotaxonomic markers in plant systematics. They have been recommended as potential markers for the determination of botanical origin of honey and for the differentiation between monofloral and multifloral honeys. In honey they are mainly derived from plants, as well as from pollen or propolis (Ferreres, Ortiz and Silva, 1992), being present in a range of 5–1300 mg/kg (Gheldof and Engeseth, 2002). According to the phenolic structural features, polyphenols are divided into two main groups, phenolic acids and flavonoids (Tomás-Barberan et al., 2001). Flavonoids aglycones are the polyphenols mainly found in honey. The loss of the sugar moiety of the glycosides present in nectar is due to the hydrolysis by bee saliva enzymes

(Tomás-Barberán et al., 2001). Dark honeys usually contain a higher quantity of polyphenolic compounds than the light ones. Dark honeys have been reported to contain more phenolic acid derivatives but less flavonoids than light ones (Tomás-Barberan et al., 2001).

3.10 5-Hydroxylmethylfurfuraldehyde (5-HMF)

5-HMF is a product of the decomposition of monosaccharides such as fructose, **Fig 2.** That is formed slowly and naturally during the storage of honey, and much more quickly when honey is heated. The 5-HMF amount present in honey is the reference used as a guide to the amount of heating that has taken place; the higher the 5-HMF value, the lower the quality of the honey (Bear, 2009). However, 5-HMF alone cannot be used to determine the severity of the heat treatment, because other factors can influence the levels of 5-HMF, such as the sugar profile, presence of organic acids, pH, moisture content, water activity and floral source. Therefore, the 5-HMF content gives only an indication of overheating or inadequate storage conditions (Bogdanov, 2014). As indicated by the Codex Alimentarius and EU standards, the 5-HMF maximum is 40 mg/kg for the mixture or processed honey, and a maximum of 80 mg/kg for honeys with a tropical origin. (Bogdanov, 2014).

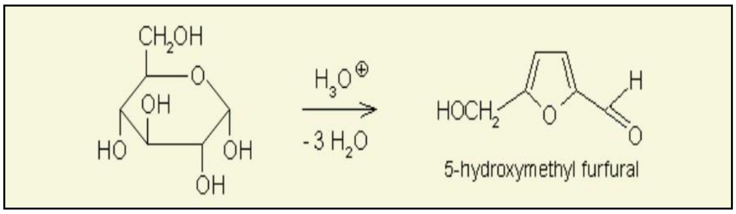


Figure 2.*5-HMF formation resulting from a sugar decomposition reaction (Bogdanov, 2014).*

3.11-Other physicochemical parameters

3.11.1-Color

Honey color can vary from practically colorless to brown dark, sometimes with green or reddish reflexes. These variations in the color of honey can related to its flavor: honey with lighter color have a gentle flavor while the darker honeys have a stronger flavor (Marchini, Sodré and Moreti, 2004). The color of honey depends on its floral origin, climate factors during nectar flow, soil conditions and the temperature at which the honey matures in the hive. Also, pollen, sugars, carotenoids, xanthophylls, anthocyanins, minerals, amino acids and phenolic compounds, mainly flavonoids (Bogdanov et al., 2004). Furthermore, honeydew honey is darker than bloom honey primarily because of mineral and phenolic substance and other components (Can et al., 2015).

3.11.2-Electrical conductivity

Electrical conductivity is a property related to the ability of a material to lead an electric flow. Honey contains minerals and acids, serving as electrolytes, which can conduct the electrical current, thus, the higher their content, the higher the resulting conductivity. It is an indicator often used in the quality control of honey that can be used to distinguish floral honeys from honeydew honeys at present it is the most useful quality parameter for the discrimination between floral honeys and honey dew honeys. As this parameter is directly related to the ash content, it was recently included in the Codex Alimentarius Standards, replacing the determination of the ash in honey. The standards recommend a maximum value of 800.00 mS cm^{-1} (Codex Alimentarius, 2001; Bogdanov, 2014).

3.11.3-Honey pH and acidity

The pH of honey ranges between 3.5 and 5.5 depending on its floral and geographical source, the pH of nectar, soil or plant association, and the amount of different acids and minerals (Crane, 1985). While pH analysis is useful as an auxiliary variable to estimate the quality of the product and as a parameter for evaluating total acidity, it is not directly related to free acidity due to the actions of the buffer acids and minerals present in honey (Pereira et al., 2009)**.** The acidity of honey can be assessed as free, lactonic, and total (free + lactonic) acidity (Navarrete et al., 2005). Free acidity is a parameter related to the deterioration of honey, being its limit established as 50 meq kg^{-1} (Codex Alimentarius, 2001; Eu Commission, 2002). Higher values may be indicative of fermentation of sugars into organic acids (Almeida et al., 2013).

4-Antibiotics residues in honey

According to Regulation (EC) No 470/2009; no veterinary medicinal product containing antibiotics is permitted in beekeeping. In fact, no antibiotic has ever had an MRL (Maximum Residue Limits) in honey (Cara et al., 2012).However some countries, like Switzerland, UK, and Belgium, have established action limits for antibiotics in honey, which generally lies between 0.01 to 0.05 mg/kg for each antibiotic group (Al-Waili et al., 2012). Some antibiotics have the potential to produce toxic reactions in consumers directly while some other can produce allergic or hypersensitivity reactions (Velicer et al., 2004). Antibiotic residues consumed along with food and honey can produce resistance in bacterial populations. Antibiotic resistance is a global public health problem and continues to be a challenging issue (Al-Waili et al., 2012). Two main approaches are used to determine the content of antibiotic residues in honey: screening tests and multi-stage analytical methodologies. The simple tests provide qualitative or quantitative information, enabling determination of a single target analyte. With multi-stage methods, a fairly broad spectrum of analytes can be determined in one analytical run. (Barganska, Slebioda and Namiesnik, 2011).

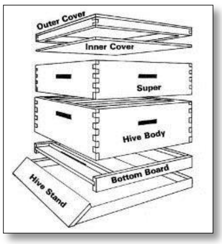
5-Biological properties of honey

Honey has been found to contain significant antioxidant compounds including glucose oxidase, catalase, ascorbic acid, flavonoids, phenolic acids, carotenoid derivatives, organic acids, amino acids and proteins (Beretta et al., 2005). Research showed a correlation between color and anti-oxidant capacity, with the darker honeys providing the highest levels of antioxidants (Jaganathan and Mandal, 2009).

Phenolic content in honey is responsible for anti-inflammatory effect (Al-Waili, Boni, 2003). These phenolic and flavonoids compounds cause the suppression of the pro-inflammatory activities of cyclooxygenase-2 (COX-2) and/or inducible nitric oxide synthase (iNOS). (Viuda, Ruiz, Fernandez, 2008). Furthermore, ingestion of diluted natural honey has produced reductions on concentrations of prostaglandins such as PGE2 (prostaglandin E2), PGF2α (prostaglandin F2a) and thromboxane B2 in plasma of normal individuals (Reyes, Segovia and Shibayama, 2007).

6-Beekeeping in Algeria

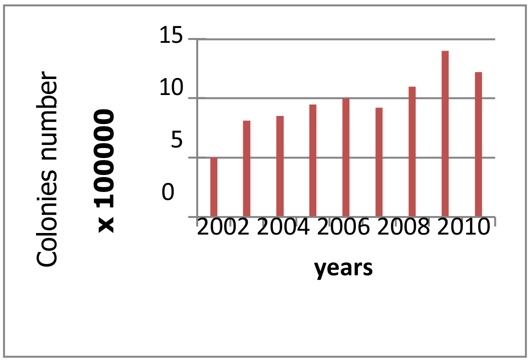
Beekeeping in Algeria is practiced mainly in the north of the country, where the floral diversity is ensured almost all the year. The honey bees need to be adapted to the desert climate and to be resistant to unfavorable environmental conditions such as high temperatures and strong prevailing winds. Hives which are best suited or adapted to the desert conditions must be used. Traditional hives made from rocks and muds are known from ancient times in Algerian deserts. Nowadays, Langstroth hive type is used in Algeria**, Fig.3**, with modifications due to the hot weather (Moustafa, 2001).



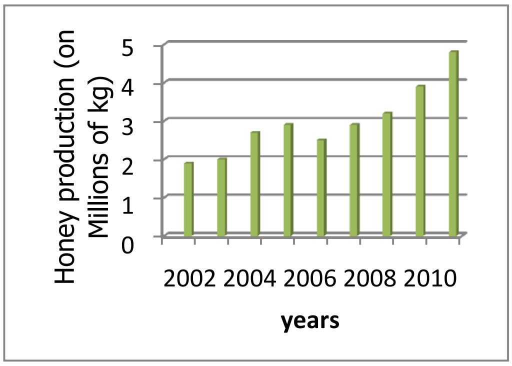
(A) **(B)**

Figure 3.*(A) The Langstroth hive and (B) the Langstroth hive different parts (John, 2014).*

The Algerian Beekeeping organization, counted around 1.2 million colonies, **Fig.4.A,** and 20,000 beekeepers, in 2010. The development of honey production shows a clear increase from 2002 to 2010**, Fig.4.B** (Adjlane et al., 2012)



(A)



(B)

Figure 4. *Number of honey bee colonies in Algeria from 2002 to 2010. (B) Honey production in Algeria from 2002 to 2010. Source: Ministry of Agriculture and Rural Development: MADR (2009-2010) (Adjlane et al., 2012).*

In Algeria, there are two main bee subspecies, the Tellian bee (*Apis mellifera intermissa*)**, Fig.5-(A).** This bee is native of the region located between the atlas and the Mediterranean which is known by the name of Tell. It is characterized by its black abdomen and its agressivity. The main advantages of this bee is its longevity, its remarkable ability to harvest pollen and a high production of honey which can reach up to 100 kg per colony provided that modern beekeeping methods are applied (Fresnay, 1981).

The Saharan (desert) bee (*Apis mellifera sahariensis*), **Fig.-5(B)**, better known as the Sahara bee, or locally the yellow bee, It is recognized for its many advantageous features such as ; the high breeding, the precocity, the extraordinary aptitude for nectar and pollen harvesting and good adaptability under difficult climatic conditions (Kessi, 2013).

A

B

Figure 5. *Images showing (A): Apis mellifera intermissa bee and a (B): Apis mellifera sahariensis bee (Tlemcani, 2013).*

6.1-Algerian Honey

In this research, Euphorbia (*Euphorbia bupleuroides*), jujube (*Ziziphus lotus*), Eucalyptus (*Eucalyptus globulus*) and multifloral honeys will be focused.

6.1.1-Eucalyptus Honey

The eucalyptus tree is a large, fast-growing evergreen that is native from Australia and Tasmania. The tree can grow to 125-160 meters. Eucalyptus belongs to the Myrtaceae family and more than 300 species of eucalyptus are described being the *Eucalyptus globulus*, **Fig 6.A**, is the most common and well-known (Catherin, 2020). Many of which produce enough nectar for honey bees to produce appreciable amounts of honey (Catherin, 2020; Persano, Baldi and Piazza, 2004). The main physicochemical parameters are shown in, **Table2**. It is a honey with a clear amber color, a wet wood, very intense and persistent aroma, a sweet with a slight acid note and a medium tendency for crystallization with fine crystals**.** (Orantes et al., 2018).

6.1.2-Euphorbia honey

Euphorbia is one of the largest flowering plant in the spurge family (*Euphorbiaceae*). With over 2,000 species, euphorbias can range from tiny annual plants to large and long-lived trees and look completely different. In the deserts of Africa and Madagascar, euphorbia adapted its physical characteristics becoming similar to cacti of America, although they are not cacti (Cherif et al., 2011). Recent inventory of native plants in Algeria identify over 51 species of *Euphorbiaceae*. *E. bupleuroides***, Fig.6.B,** is the main species used by bees to produce honey. (Le Houèrou, 1995; Quezel and Médail, 2003). The main physicochemical parameters are shown in, **Table2.** It is a honey with golden yellow to dark amber color, with a sweet, pinch in the throat with a typical light bit back flavor and with a spicy almost peppered aroma and pungent flavor (Cherif et al., 2011).

6.1.3-Jujube honey

Ziziphus lotus L. belongs to the family *Rhamnaceae*, which consist of about 135 species. The trees are medium-sized; growing 7-10 meters high, with shiny green leaves about 5 cm long. The edible fruit is a globose dark yellow drupe with 1–1.5 cm diameter, **Fig.6.C.** The wild jujube *Ziziphus lotus* is a species found in many habitats of arid and semiarid regions of the Mediterranean area, throughout Libya to Morocco and Algeria **(**Benammar et al., 2010). Jujube honey is a highly demanded product in Algeria and worldwide and is considered one of the most expensive honeys. Despite this commercial interest, this honey type has been scarcely described (Cherif et al., 2016).The main physico-chemical parameters of jujube honey are shown in **Table 2**. Its color is varied from light-amber to amber color.

Figure 6. *(A) Eucalyptus plant (Orantes, Gonell, Torres et al., 2018). (B) Euphorbia plant. (C) Jujube plant (Photograph by Andrii Salomatin, https://www.shutterstock.com/fr/g/Andrii%2BSalomatin retrieved on 24-05-2020.*

Table 2. Physicochemical properties of Jujube, Euphorbia, Eucalyptus honeys of arid and semi-arid zones in north Africa (Cherif et al., 2011; Cherif et al., 2016); (Makhloufi et al., 2010)

Botanical origin	**pH**	**Electrical conductivity s/cm**	**Water content %**	**Diastase Schade unit**	**Sucrose %**	**5- HMF mg/kg**	**References**
Ziziphus	4.45	673	16.65	15.63	0.61	8.71	(Cherif et al., 2016)
Euphorbia	4.23	411	17.06	12.67	0.97	12.08	(Cherif et al., 2011)
Eucalyptus	4.2	769	16.5	9.64		25.63	(Makhloufi et al., 2010)

7-Timeline

Tasks	March	April	May	Juin	July	September	October	November	December	January	February
Honey sampling	**X**										
Seminar writing	**X**	**X**		**X**							
Melissopalynological analysis						**X**					
Physicochemical characterization						**X**					
Protein content						**X**					
Sugars							**X**				
Mineral content							**X**				
Phenolic compounds determination by LC-MS								**X**			
Antioxidant activity									**X**		
Cytotoxic activity and anti-inflammatory activity									**X**		
Antibiotics residues detection in honey samples									**X**		
Statistical analysis									**X**		
Thesis writing									X		X

8-Methodology

8.1-Sampling

This study involved 10 honey samples from Algerian semi-arid regions (Ainsafra, El Bayadh, Sidi bel Abess) including three monofloral Ziziphus honeys (*Z. lotus* L.), three Euphorbia honeys (*E. bupleuroides* L.), two Eucalyptus honey (*Eucalyptus globulus*) and also two multifloral honeys. The information regarding the harvesting date, the region and the floral origin is shown in **Table 3.**

Table 3. Honey samples botanical origin, geographical origin and harvesting time.

Sample code	Botanical origin	Region	Harvest time
Z1	Ziziphus L	Ain Safra	June 2019
Z2	Ziziphus L	Ain Safra	June 2019
Z3	Ziziphus L	Ain Safra	June 2019
Ef	Euphorbia B	El Bayadh	June 2019
Ef	Euphorbia B	El Bayadh	June 2019
Ef	Euphorbia B	El Bayadh	June 2019
Ec1	Eucalyptus G	Sidi Belabes	June 2019
Ec2	Eucalyptus G	Sidi Belabes	June 2019
Mf1	Multifloral	Sidi Belabes	June 2019
Mf2	Multifloral	Sidi Belabes	June 2019

8.2- Determination of botanical origin

Mellissopalynology is the methodology that will be used in pollen analysis and is of great importance in the process of determining botanical and geographical origin. The determination of pollen analysis is carried out by microscopic counting of pollen grains contained in honey and the results are expressed as a percentage of representation of each type of pollen and according to their frequency: I, important pollen (3% - 15%); A, accompanying pollen (15% - 45%); and D, dominant pollen (equal to or greater than 45%). If the pollen prevalent in the constitution of honey is present in a proportion equal to or greater than 45%, honey is classified as monofloral (Schweitzer, 2004).

8.3-Physicochemical characterization of the honey samples

The physicochemical parameters (color, moisture content, electrical conductivity, pH, free acidity (equivalence point at pH 7), lactonic and total acidity,5-hydroxymethylfurfural after White, diastase activity after Phadebas and proline is determined according to the Harmonized Methods of the International Honey Commission (International Honey Commission, 2009). All Physicochemical analyses are performed in triplicate to provide greater reliability for the results.

8.4-Proteins content

For the determination of proteins, the Kjeldahl method will be applied, which consists of indirect determination, based on the quantification of total organic nitrogen (Lopez et al., 2018).

8.5-Sugars

Sugars will be analysed and quantified by high-pressure liquid chromatography coupled to a refractive index detector (HPLC-RI) (Lopez et al., 2018).

8.6-Mineral content

For the analysis of the minerals, the following elements will be were evaluated: potassium (K), sodium (Na), calcium (Ca) and magnesium (Mg) using a flame atomic absorption spectrophotometer. The determination of manganese (Mn), copper (Cu) and cadmium (Cd) will be made by atomic absorption spectrophotometry in a graphite chamber.

8.7-Phenolic compounds characterization

The phenolic compounds will be analyzed through ultra-pressure liquid chromatography coupled with diode-array detection and electrospray ionization tandem mass spectrometry (UPLC/DAD/ESI-MS) after extraction.

8.8- Antioxidant activity

Antioxidant activity will be determined by the total phenolic content determined using a UV Vis spectrophotometer, the scavenging capacity of the free radical DPPH (2, 2-diphenyl-1-picrylhydrazyl) and reducing power assay (Lopes, Falcão, Dimou et al, 2018).

8.9- Cytotoxic activity and Anti-inflammatory activity

The cytotoxic effects will be evaluated in five human tumor cell lines: MCF-7 (breast adenocarcinoma), NCI-H460 (non-small cell lung cancer), HeLa (cervical carcinoma) and HepG2 (hepatocellular carcinoma) and MM127 (human malignant melanoma). The cytotoxic potential of the honey samples will be evaluated using the sulforhodamine B (SRB) assay, while the anti-inflammatory potential will be evaluated by the murine macrophage (RAW 264.7) cell line, according to the procedure described previously (Falcão et al., 2019).

8.10- Antibiotics residues

Antibiotics of the class of sulphonamides and tetracyclines will be screened by the system Charm II, which is a scintillation based detection system for a wide range of residues including antibiotics utilizing class specific receptors or an antibody in immuno-binding assay formats (Salter, 2003).

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