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Quality evaluation of Algerian honeys: Rosemary, Tamarisk, Thistle and Multifloral.

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الملخص

يعتبر العسل مادة طبيعية حلوة ينتجها نحل العسل، من رحيق أزهار النباتات ونباتات العسل. لطالما اعتُبر العسل غذاء مفيد لصحة الإنسان مع وصف العديد من الصفات العلاجية. لا تزال جودة العسل مصدر قلق كبير للخبراء حيث لم يتم تحديد طريقة جيدة حتى الآن للكشف المتزامن عن أنواع مختلفة من العسل. وبالتالي، من المهم تطوير أدوات تحليلية سهلة وسريعة ودقيقة قد تعطي بيانات لتقييم أصالة العسل. لهذا السبب ، من الضروري إبلاغ المستهلكين عن سوء تسمية العسل بجودة أقل.الهدف من هذه الدراسة هو تقييم الخصائص الفيزيائية والكيميائية لتقييم جودة العسل الجزائري من أصول نباتية وجغرافية مختلفة. من أجل ذلك، سيتم تحليل عشر عينات من عسل من أصول نباتية مختلفة ، والتي ستشمل ثلاث عينات من عسل إكليل الجبل ، وثلاث من عسل الأثل ، وثلاث من عسل شوك الحليب (السدرة) ، وعسل واحد متعدد الأزهار. سيتم تحديد جودة العسل من خلال معايير مختلفة. سيتم إجراء تحليل ميليسوبالينولوجي وكيميائي فيزيائي (اللون ، الرطوبة ، درجة الحموضة ، الحموضة ، التوصيل الكهربائي ، مؤشر دياستاز ، البرولين ، محتوى 5-هيدروكسي ميثيل فورفورال والمعدن) ، وكذلك تقييم لمحات السكر والفينول. ، نشاط مضادات الأكسدة) تقليل الطاقة ، نشاط إزالة الجذور الحرة DPPH والنشاط المضاد للأورام .أخيرًا، سيتم فحص وجود المضادات الحيوية والمخلفات المتكررة في العسل، مثل التتراسيكلين والسلفوناميدات من خلال نظام فحص مستقبلات التحليلات المتعددة Charm II.

الكلمات المفتاحية: عسل، معلمات فيزيائية كيميائية ، إكليل الجبل ، أثل، شوك الحليب ، مضادات حيوية ، نشاط بيولوجي ، HMF 5-، سلفوناميدات ، تتراسيكلين ، ميليسوبالينولوجي ،DPPH

RÉSUMÉ

Le miel est considéré comme une substance douce naturelle produite par les abeilles, à partir des nectars des fleurs des plantes et du miellat. Le miel a toujours été considéré comme un aliment bénéfique pour la santé humaine avec plusieurs qualités thérapeutiques décrites. La qualité du miel reste une préoccupation majeure pour les experts car aucune bonne méthode n'a été définie jusqu'à présent pour la détection simultanée de différents types de miel. Par conséquent, le développement des outils analytiques simples, rapides et précis qui peuvent fournir des données pour évaluer l'authenticité du miel est important. Pour cette raison, il est essentiel d'informer les consommateurs dans l'étiquetage des miels sur sa qualité s'elle était inférieure. Le but de cette étude sera d'évaluer les caractéristiques physico-chimiques pour apprécier la qualité des miels algériens issus a des origines botaniques et géographiques différentes. Pour cela, dix échantillons de miels seront analysés, qui comprendront trois échantillons de miel du romarin, trois de miel du tamarix, trois du chardon-Marie et un miel multifloral. La qualité des miels sera déterminée à travers différents paramètres. Des analyses mélisso-palinologiques et physico-chimiques (couleur, humidité, pH, acidité, conductivité électrique, indice de diastase, teneur en proline, 5-hydroxyméthylfurfural et minéraux) seront réalisées, ainsi que l'évaluation des profils sucres et phénoliques, l'activité antioxydante (pouvoir réducteur, L'activité de piégeage des radicaux libres DPPH et l'activité anti-tumorale) seront également évaluées. Enfin, la présence d'antibiotiques, de résidus récurrents dans le miel, tels que les tétracyclines et les sulfamides sera criblée grâce au système de dosage des récepteurs multi-analytes Charm II.

Mots clés : Miel, paramètres physico-chimiques, romarin, tamarix, chardon-Marie, antibiotiques, bioactivité, 5-HMF, sulfamides, tétracyclines, mélissopalinologie, DPPH.

Abstract

Honey is considered as a natural sweet substance produced by honeybees, from the nectars of plant flowers and honeydews. Honey has always been regarded as a food which is beneficial for human's health with several therapeutic qualities described. The quality of honey is still a top concern for experts as no good method has been defined so far for the simultaneously detection of different types of honey. Consequently, the development of easy, quick, precise analytical tools that may give data for assessing the honey authenticity, are important. Because of that, it is essential to inform consumers from the mislabeling of honeys with lower quality. The aim of this study will be to evaluate the physicochemical characteristics to assess the quality of Algerian honeys from different botanical and geographical origins. For that, ten samples of honeys with different botanical origins will be analyzed, which will include three samples from rosemary honey, three from tamarix honey, three from milk thisle honey and one multifloral honey. The quality of the honeys will be determined through different parameters. Melissopalinological 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., 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 screened through the multi-analyte receptor assay system Charm II.

Keywords: Honey**,** physio-chemical parameters, rosemary, tamarix, milk thistle, antibiotics, bioactivity, 5-HMF, sulphonamides, tetracyclines, Melissopalinology, DPPH.

Resumo

O mel é considerado uma substância doce natural produzida pelas abelhas, a partir dos néctares das flores das plantas e de meladas. O mel sempre foi considerado um alimento benéfico para a saúde, com várias qualidades terapêuticas descritas. A sua qualidade ainda é uma das principais preocupações para os especialistas, pois não há um método ideal para a detecção simultânea de diferentes tipos de mel. Consequentemente, é importante o desenvolvimento de ferramentas analíticas fáceis, rápidas e precisas que possam fornecer dados que permitam avaliar a autenticidade do mel. Por esse motivo, é essencial informar os consumidores da incorrecta rotulagem de méis com baixa qualidade. O objetivo deste estudo será avaliar as características físico-químicas e desse modo aferir a qualidade de méis argelinos com diferentes origens botânicas e geográficas. Para isso, serão analisadas dez amostras de méis de diferentes origens botânicas, nomeadamente três de mel de alecrim , três de mel de tamarix, três de mel de cardo e um mel multifloral. A qualidade dos méis será aferida através de diferentes parâmetros. 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 e a atividade antioxidante (poder redutor, poder bloqueador de radicais livres e atividade anti-tumoral). Finalmente, a presença de antibióticos, resíduos recorrentes no mel, como tetraciclinas e sulfonamidas, será investigada através do sistema de ensaio Charm II.

Palavras-chave: mel, características físico-químicas,alecrim , tamarix, cardo, antibióticos, bioatividade, 5-HMF, sulfonamidas, tetraciclinas, Melissopalinologia, DPPH.

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List of abbreviations

CETOF-MS	Capillary Electrophoresis-Time-Of-Flight Mass Spectrometry
DHSTR	Dihydrostreptomycin
DPPH	2, 2-diphenyl-1-picrylhydrazyl-hydrate
EC	European Commission
EU	European Union
HPLC-RI	High-pressure Chromatography Coupled to a Refractive Index Detector.
IHC	International Honey Commission
GC-MS	Gas-Chromatography Coupled to Mass Spectrometry
LC-MS	Liquid Chromatography Coupled with Mass Spectrometry
MALDI-TOF MS	Matrix-Assisted Laser Desorption- Ionization Time of Fly Mass Spectrometry
MRL	Maximum Residue Level
NMR	Nuclear Magnetic Resonance Spectroscopy
W/W	Weight/Weight
5-HMF	5-Hydroxymethylfurfural

Introduction

Honey was the first and most consistent sweetener used by human beings. As a source of energy, the beneficial features of honey are its great nutritional value and the fast absorption of its carbohydrates during consumption. Furthermore, in many areas of daily life the importance of honey has been recognized for centuries and across civilizations for its good qualities and good benefits. The fact that Hippocrates, the father of medicine emphasizes that the nutritional and pharmaceutical value of honey is not accidental. Many researchers have found honey to be a useful alternative for healing wounds and burns, and for oral health; others have discovered its important role in cancer care and its antimicrobial characteristics ; as a natural, unprocessed and easily digested food, honey can be seen as an important part of our diet. For these reasons, honey still saves this natural representation and an augmentation in consumption can be attributed to the global increase in livings standard and makes people want to know more about its natural and beneficial health substances. The main concern of honey quality control groups is to make certain that honey is authentic in respect to the legislative requirements. Codex Alimentarius (2001) and EU (2002a)legislation are set to act for the minimum marketing value of the product and the need for consumer safety through correct denominations(Feás et al., 2010). Algeria is the second largest country on the African continent. It has a area of about 2.4 million $km^2$with circa 33.3 million populations. As the country is separated in the north by the Tell Atlas Mountains, which is parallel to the Mediterranean coast, and by the Saharan Atlas in the South, different diverse environmental and geologic conditions exist. The Tell Atlas region enjoys a Mediterranean climate in the coastal areas and is very good for beekeeping. The main honey flow is in April, May and the first part of June. There are several trees cultivated crops and wild plants, like Rosemary (*Rosmarinusoffi**cinalis*), tamarix (*Tamarix gallica L.*) and milkthistle (*Silybummarianum*) offer nectar and pollen for the bees .Also natural forests, incorporating pine trees, are suitable sources for the bees and it is possible to have honey all year round.Second,the centre part of the country contains of high plateaux with plains and some agriculture. The condition,in the north part of the country beekeeping plays an potentiel role. There is migratory beekeeping for the production of honey, but bees are generally not transferred for pollination. It is predicted that the yearly honey production hits 800 tonnes. Suitable quality honey is highly appreciated by the consumer but because of a limited knowledge by beekeepers a great quality product is not always obtained (Makhloufi et al., 2010).

In Algeria honey is used both for nutritive and healing purposes, and its price reaches quite great levels, while the data of the product is still deprived, and the quality control of local and imported honey is totally insufficient. This situation does not allow a sufficient safety of the consumer and leads to possible frauds. Indeed, at the scientific plane, only a few informations are available, To contribute more to the knowledge of Algerian honey, in the present study we further examined the subject, Trying to develop a previous University thesis , with the aim of evaluating The quality of ten samples belonging to different honey type (three samples from rosemary honey, three from tamarix honey, three from milk this honey and one multifloral honey) supplied from local producers from Algeria in order to verify its compliance with the standards of Codex Alimentarius, 2001b and the Council of the European Union (EU), 2002. The quality of the honeys will be determined through different parameters. Melissopalinological 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., 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 screened through the multi-analyte receptor assay system Charm II.

1.Literature Review

1.1 Definition of honey

Honey is a natural product obtained by *Apis mellifera* bees by sucking nectar from plant excretions. They collect and combine these liquids with specific products of their own metabolism and then stock it up in the comb to ripen and mature (Feás et al., 2010). Within nectar honeys, is possible to separate in two categories, monofloral or unifloral honeys (which predominantly come from a single floral species) and multifloral honeys (which is the result from bee's collecting nectar from different floral species) (Chergui, 1994).

1.2. Honey composition

1.2.1. Sugars

Honey incorporates simple sugars or monosaccharide, including fructose and glucose which are the major substances (65%) and around 18% of water. Additionally, there are small percentages of disaccharides present in honey composition (Bhandari et al.,1999). The percentage of sugars present in honey influence its viscosity, due to strong impact of the sugar's molecular chains (Bhandari et al.,1999). Fructose and glucose are present in a range between 85% to 95%, while sucrose (1.5%), maltose (7.5%) and other trace sugars are present in smaller amounts (Emmanuelle et al., 1996). In nectar honey fructose percentage are frequently higher than glucose (Zafar et al., 2008). The sum of fructose and glucose, fructose/glucose ratio and glucose/water ratio are also essential factors associated to the quality of honey. Fructose/glucose ratio shows the ability of honey crystallization. Honeys that have high amount of fructose, have less tendency for crystallization, while honeys rich in glucose frequently crystallize directly after harvesting or sometimes inside the comb cells (Dyce, 1931 and Maurizio, 1962). Previous studies in honey samples produced in different regions of Algeria (Makhloufi et al., 2007) revealed a sugar content in agreement with the international standards, with only two samples showing a level of fructose + glucose lower than 60 %, probably due to the presence of some honeydew (Makhloufi et al., 2007).

1.2.2. Protein content

Honey's protein content is mainly derived from the enzymes or amino acids and are depend upon the floral origins of pollen where bees collected the nectar, and storage time (Saxena et al., 2010). The amount of protein in honey range from 0.1 to 0.5%, however some honeys such as ling heather (*Calluna vulgaris*) show a higher protein amount (1–2%) (Chua et al., 2013 and Sáinz & Gómez, 2000). Previous studies on market Algerian honeys showed protein values up to 4g/kg (0.4%) which are in the range normally found for honeys around the globe (Khalil et al., 2012).

1.2.3. Vitamins

The main vitamins present in honey are the vitamins of B group and vitamin C (León-Ruiz et al., 2013), but there are others liposoluble vitamins like D, A, K and E also reported in smaller amounts (Sáinz & Gómez, 2000). The use of commercial filtration procedures and the presence of hydrogen peroxide, which naturally occurs in honeys (Ciulu et al.,2011) may contribute to the decrease of vitamin C levels in honeys. In Algerian honeys the levels of ascorbic acid reported (vitamin C) were around 160 mg/kg (Khalil et al., 2012).

1.2.4. Mineral content

The mineral content of honey has a significant linear relationship with its electrical conductivity and ash content and is influenced by the botanical origin and the type of soil in which the plant was situated. Besides, it can also provide information about the ecological pollution. (Anklam, 1998). Honeys with higher mineral content are generally darker (González-Miret et al., 2005) because of the formation of colorful compounds between transition elements and some organic complexes in honey (Harris, 2014). A high value of acidity in honey is also linked with honey samples with high mineral content. Honey contains varying quantities of mineral substances ranging from 0.02 to 1.03 g/100 g (Bogdanov, 2016). Recently, a study involving 22 multi floral Algerian honey samples described a mineral content that ranged from 0.019 to 0.518% (Amri & Ladjama, 2013). The most essential minerals exists in honeys are magnesium, calcium, sodium, and potassium, the less abundant minerals are chlorine, manganese, copper, iron and in minor quantities trace elements like, nickel, phosphorus, sulfur, silicon, bare and boron, and others (Doner, 2003).

1.2.5. Phenolic content

Phenolic acids and its derivatives are the major bioactive substances found in honey, with concentration varying from 5 to 1300 mg/kg (Alvarez-Suarez et al., 2012). The phenolic compounds are related to the geographical and botanical source of the flowers in which the bees collect the nectar. The healthy honey characteristics are linked to the presence of phenolic acids and flavonoids but are also related to other minor polyphenols (Da Silva et al., 2016). Some beneficial actions of flavonoids such as prevention of cardiovascular diseases (Abadio Finco et al., 2010) , makes honey a tool in alternative health treatment, known as apitherapy (Vit et al., 2004). The content of phenolic compounds is associated directly to the color, having the darker honeys higher content in phenolic compounds, sensory features, and antioxidant activity (Da Silva et al., 2016) . According to previous studies concerning Algerian honeys, where the phenolics where estimated by a modified spectrophotometric method, the honey samples present a phenolic content around 460 ± 2 mg gallic acid equivalents/kg and flavonoids in concentrations around 54.2 ± 0.6 mg catechin/kg (Ouchemoukh et al., 2017).

1.2.6. Organic acids

Organic acids, connected to honey flavor (Suárez-Luque et al., 2002), are present in small percentages in honey (0.5%) and impact in the honey acidity, which can be used as a quality parameter for the evaluation of deterioration due to storage, aging or for authenticity measure(Suárez-Luque et al., 2002). The acidity of honey helps the preservation against spoilage by microorganisms (White & Doner,1980). Diverse organic acids were described to be present in honey, including citric, lactic, acetic, malic, butyric, pyroglutamic, succinic, and oxalic acid (Crane et al., 1990), which can be used to characterize different honey types. The concentration of citric acid is useful as factor to distinguish between two types of honey : floral and honeydew honey (Talpay, 1988).

1.3. Honey botanical origin

Several advanced approaches have been proposed aiming at accurately assessing the botanical and geographical origins of honey, by targeting certain minor compounds in honey, such as phenolic acids, sugars, amino acids and other constituents, through the use of gas-chromatography coupled to mass spectrometry (GC-MS), liquid chromatography coupled with mass spectrometry (LC-MS), capillary electrophoresis-time-of-flight mass spectrometry

(CETOF-MS), matrix-assisted laser desorption-time of fly ionization mass spectrometry (MALDI-TOF MS), and nuclear magnetic resonance spectroscopy (NMR) (Schievano et al., 2013). Nevertheless, the assessment of these chemical markers can be affected by beekeeping techniques, environmental conditions, and climate changes, leading frequently to unreliable determination of its floral or geographical origin (Madesis et al., 2014). Honey contains a lot of pollen grains and honeydew substances giving a good fingerprint of the ecological area of the honey. For that reason, analysis of pollen in honey (melissopalynology) is very important for the determination and the control of the botanical and geographical origin of honeys. So far, melisso-palynological analysis based on the basic techniques of the botanical determination of honey, however the physico-chemical and sensory diagnosis are also important for an appropriate analysis of the botanical origin **(**Von Der Ohe et al., 2004). In Algeria, there are several types of monofloral honeys such as rosemary honey, tamarix honey, milk thistle honey, multifloral honeys and honeydew has been characterized. Here are some monofloral honeys that are present in Algeria and are within my research work.

1.3.1. *Rosmarinus officinalis*

Commonly known as rosemary, is a woody, perennial herb with fragrant, evergreen, needle-like leaves and white, pink, purple, or blue flowers. It is native to the Mediterranean and Asia but is reasonably hardy in cool climates, surviving even in the lack of water for lengthy periods. In temperate climates, the plant flowering period is between spring and summer; however, the plant can be in constant bloom in warm climates. Rosemary also has a propensity to flower outside its normal flowering season, it has been recognized to flower as late as early December, and as early as mid-February (Amin & Hamza, 2005).

Rosemary honey which are collected from Spain is a light color honey with sweet taste, with fruity notes and fatty sensation in palate. In generally, is a honey with low conductivity and acid content and values of fructose higher that glucose (Perez-Arquillué et al., 1994).The Analysis of some physiochemical parameters in 27 from spain are represented in **Table 1.**

Table 1. Analysis of some physiochemical parameters in 27 from Spain (Perez-Arquillué et al., 1994).

Parameter	Mean	Parameter	Mean
Moisture (%)	16.8	Lactone acidity (meq/kg)	1.06
Electrical conductance (104 S x cm ~)	1.55	Total acidity (meq/kg)	17.2
Ash content (%)	0.05	Fructose	36.6
HMF (mg/kg)	3.0	Glucose	31.2
Diastase activity (G°)	18.0	Sucrose	1.97
pH	3.71	Maltose	7.20
Free acidity (meq/kg)	16.2	Erlose	0.61

Figure 1. *Rosmarinus officinalis (international union for conservation of nature and natural resources. centre for mediterranean cooperation, 2005).*

1.3.2. *Tamarix gallica L.*

A high perennial shrub/little tree, densely ramified, 2-10 m. high. The purple-brown bark is initially smooth with huge elongated lenticels, after developing shallow splits and becoming rough when full-grown. The small, scale-like, 1-3 mm. -long leaves are grey-green

or green. The tiny flowers have 5 lavender pink or white petals 1.5-2 mm. Long, huge on long, very thin, spike-like racemes in terminal panicles. The fruits are tiny dry capsules have tiny cottony seeds. The capsules are conical, trigonous, tapering and pale pink. Flowering begins around March and ends until May. In the central Sahara it has been shown in full bloom in June (International Union for Conservation of Nature and Natural Resources. Centre for Mediterranean Cooperation,2005).

Tamarix honey which are collected from Iran is an intermedium colored honey with reddish tones and a taste of malt with overtones of citrus, with a slightly bitter after taste. The chemical composition is also characterized by low conductivity and medium acidity, with fructose and glucose values around 36 and 27 g/100g, respectively (Khalafi et al., 2016). The physicochemical properties of Iranian tamarix honey are represented in **Table 2.**

Table 2. Physicochemical properties of Iranian tamarix honey (Khalafi *et al*., 2016).

Parameter	**Mean (g)**	**Parameter**	**Mean**
Moisture (g/100 g honey)	15.1	Diastase activity (Gothe)	13.8
pH	4.1	HMF (mg/ kg)	2.2
Ash (g/100 g honey)	0.052	Total phenolic content (mg/100 g honey)	24.6
Electrical conductivity (mS/cm)	0.16	Total flavonoid content (mg/100 g honey)	2.1
Fructose (g/100 g honey)	35.9	Antioxidant activity (%)	46.7
Glucose (g/100 g honey)	26.7	Color intensity	0.342g
Ratio of fructose/ glucose	1.3		

Figure 2. *Tamarix gallica L (International Union for Conservation of Nature and Natural Resources, 2005).*

1.3.3. *Silybum marianum*

Silybum marianum is an annual to biennial plant, more than 2m high. The is stem 20-150 cm high, rarely smaller, slightly downy or glabrous, branched and erect in the superior part. The leaves are alternate, wide, white veined, glabrous with strongly spiky margins. The inflorescences are wide and circular capitula, solitary at the top of the stem or its branches, bordered by thorny bracts. The florets are hermaphrodite, tube-shaped with a red-purple corolla. The fruits are hard skinned achenes 6 to 8 mm long, usually brownish with a white silk like pappus at the top. The fruits are harvested in May - June, after blooming (International Union for Conservation of Nature and Natural Resources. Centre for Mediterranean Cooperation, 2005).

Milk thistle honey which are collected from Croatia has color ranges from pale yellow to deep amber, with a mild taste and sweet flavor reveal a slight bitterness and astringent aftertaste. It has a fresh floral aroma and slightly woody or mossy. The physiochemical parameters of milk thistle honey show slightly higher values of conductivity and acid content when compared with the former honeys, with high values of fructose and glucose (Mandić et al., 2006) . The physiochemical parameters in analysed of Croatian milk thistle honey are represented in **Table 3.**

Table 3. Physiochemical parameters in analysed of Croatian milk thistle honey (Mandić et al., 2006).

Parameter	**Mean**	**Parameter**	**Mean**
El. cond. [mS/cm]	0.22	Glucose/Water [%]	2.38
pH	3.72	Ksilose [%]	0.53
Free Acidity [meq/kg]	19.9	Maltose [%]	1.02
Water [%]	16.7	Melezitose [%]	0
Diastase [DN]	16.8	Raffinose [%]	0.05
HMF [mg/kg]	7.2	Total sugars [%]	78.34
Fructose [%]	36.4	Visual assessment	**Colour intensity:** medium **Colour tone:** bright yellow
Glucose [%]	39.8	Olfactory assessment	**Intensity of odour:** medium **Description:** floral-fresh fruit
Sucrose [%]	0.5	Tasting assessment	**Sweetness:** medium to strong **Acidity:** Absent **Bitterness:** absent **Intensity of aroma** : weak **Description of aroma:** – **Persistence/aftertaste:** absent **Other mouth perceptions:** –
Fructose+Glucose [%]	76.2	Physical characteristics	**Crystallisation rate :** slow

Figure 3. *Silybum marianum (International Union for Conservation of Nature and Natural Resources, 2005).*

1.4. Quality and physicochemical parameters of honey

To ensure the quality of honey, different international institutions, such as the International Honey Commission (IHC), the Codex Alimentarius and the European Commission suggest parameter levels and methodologies of analyses to assure the authenticity of honey (Draiaia et al., 2015) . Within those regulations we can find the following parameters:

1.4.1. Color

The color of honey is a parameter closely linked with the consumer acceptance of a particular sort of honey (González-Miret et al., 2005). The color can vary from colorless to dark-brown (Codex Alimentarius Commission, 2001a) and according to (Belay et al., 2015), it correlates with the flavor: honeys with light colors have a mild flavor, while dark honeys have an extra pronounced flavor (Bertoncelj et al.,2011). Also, throughout storage or under heating for an extended period, honey can undergo changes due to non-enzymatic browning reactions, like the Maillard reaction (Oroian & Ropciuc,2017). These reactions produce substances like furfural and 5-hydroxymethylfurfural (5-HMF), associated with the browning of honey (Da Silva et al., 2016) . Previous works on Algerian honeys reported honeys from dark amber to light amber color (Khalil et al., 2012).

1.4.2. Moisture content

The Moisture content in honey is correlated with the climatic and harvesting conditions and affects the physical parameters of honey, such as viscosity and crystallization, with

consequences in the quality (Gallina et al.,2010). Generally, the water amount in honey is less than 20%, except for heather honey, where the maximum can be up to 23% (Council Directive 2001/110/EC and FAO 2001). According to previous works on Algerian honey samples it was reported, for most of the samples, relatively low levels (average value 16.5%), with only one sample above the limit of 20% defined by the international standards. High values of water can lead to fermentation and, consequently, reducing the shelf life. These high levels can be related with a premature honey harvesting or by inadequate storage conditions (Makhloufi et al., 2007)

1.4.3. Ash and electrical conductivity

The honey's electrical conductivity is correlated with the mineral's concentration, proteins and organic acids, and so, is directly linked with the ash content. Usually, it is a characteristic estimated within the nutritional evaluation. Besides, it is a useful parameter for the differentiation on different botanical origin honeys (Krauze & Zalewski,1991). Concerning the Algerian honey, the literature reported a high electrical conductivity within the samples analyzed, with an average value of 0.65 ± 0.21 mS/cm. The international standards recommend a limit of 0.8 mS/cm for all nectar honeys (Makhloufi et al., 2007)

1.4.4. pH and acidity

The acidity of honey is due to the existence of organic acids, mostly gluconic acid (Terrab et al., 2004), and can be accessed by the evaluation of the free acids present in the sample together with the lactonic acidity, defined as the existing acidity when the honey is turned to alkaline (Terrab et al., 2003). Honey with low pH inhibits the existence and development of microorganisms. This factor is highly important during the storage and extraction of honey and is related to its stability, texture and shelf life (Terrab et al.,2004). Published reports show that honey pH ought to be between 3.2 and 4.5 and can be used to distinguish between nectar and honeydew honeys (Bogdanov et al.,1997). Free acidity limits are specified in European legislations as below 50 meq/kg, representing the non-existence of unwanted fermentation (Feás et al., 2010). Multifloral Algerian honeys from different regions were reported to have the pH in a range of 3.33 to 4.6 (Amri & Ladjama, 2013).

1.4.5. 5-Hydroxymethylfurfural (5-HMF)

Hydroxymethylfurfural is commonly identified as a freshness parameter for honey samples. Several parameters influence the formation of 5-HMF, like storage conditions (e.g. temperature) and floral origins. It is known that honey heating originates 5-HMF, which is synthesized throughout acid-catalyzed dehydration of hexoses, like glucose and fructose. According to the Codex Alimentarius and EU standards the 5-HMF maximum level is 40 mg/kg. Beekeeping organizations of some countries, e.g. Germany, Italy, Finland, Switzerland have set a highest limit of 15 mg/kg for particularly labeled "quality" or "virgin" honeys (Bogdanov, 2016). Regarding Algerian honeys, the literature revealed values for HMF around 18.5 mg/kg, in average, with 4 samples over the limit of 40 mg/kg (Makhloufi et al., 2007).The Building of HMF from a hexose sugar are represented in **Figure 4.**

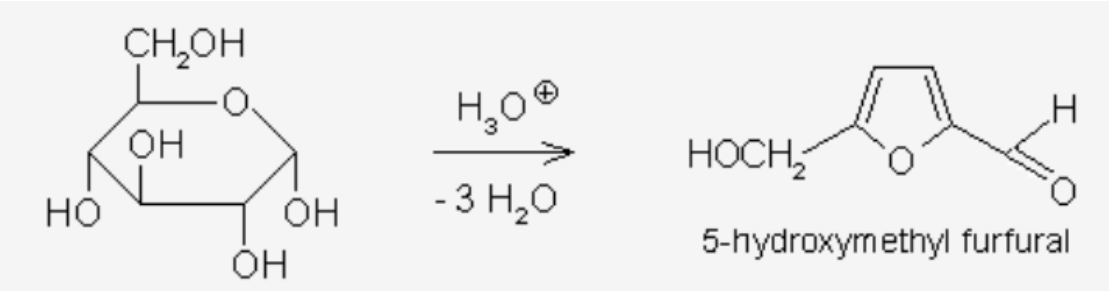

***Figure 4**. Building of HMF from a hexose sugar (Bogdanov, 2016).*

1.4.6. Diastase activity

Diastases are a group of enzymes that comprise α-and β-amylase, which are natural present in honeys. It is a parameter usually assign for honey freshness and can be quantified in Schade, Göthe or diastase units (Fechner et al., 2016). A minimum level of 8 diastase units is set by the Codex Alimentarius and the European honey directive (Bogdanov, 2016). For that reason, honeys with diastase activity under the permitted limits are linked to long storage periods and/or heating throughout its storage or processing (Fechner et al., 2016). Algerian honey samples reported to have the mean value of 17.4 ± 9.0 ranging from 4 to 40 Schade units (Makhloufi et al., 2007)

1.4.7. Proline content

Proline is the mostly abundant free amino acid in honey, varying from 50 to 85% of the total (Schieberle et al., 2009). It generally comes from salivary secretions of honeybee through the conversion of honeydew or nectar into honey (Bergner & Hansjörg, 1972) , and so is not a good indicator of the honey's botanical origin. However, (Biino,1971) published that high amount of proline were mainly found in honeydew honeys. Proline could be related to the content of enzymes, because of its important role of regulation of nectar enzymatic transfer, generally the secretions of invertase during the transformation of nectar in honey and the main content of proline in honeys should be more than 200 mg/kg (Bogdanov & Pascale, 2001). Some researchers analyze proline as quality indicator for ripeness of honey, and as a criteria of sugar adulteration, particularly when the levels of this amino acid are less than 180 mg/kg, the minimum level that has been established for genuine honey (Bogdanov et al., 1999). Proline was detected in higher amount (1692–2712 mg/kg) in all of Algerian honey samples evaluated (Khalil et al., 2012).

1.4.8. Bioactivity of honey

Honey has a natural antioxidant activity and it has proved to prevent food spoilage due to oxidative reactions (Gheldof and Engeseth, 2002). In vitro studies have shown that honey intakes block the oxidation of lipoproteins of human serum (Al-waili, 2003). This antioxidant potential of honey is due to the number of compounds that exist on it, both enzymatic (e.g., peroxidase, glucose oxidase and catalase) and non-enzymatic compounds (e.g., phenolic acids, carotenoids, α-tocopherol, proteins, amino acids, flavonoids, Maillard reaction products and ascorbic acid) (Gheldof and Engeseth, 2002). The amount and sort of these antioxidants is related with the honey floral source, and the antioxidant activity is related to phenolic content (Gheldof et and Engeseth, 2002). The radical scavenging activities of honey samples were evaluated by using the DPPH radical scavenging assay, The DPPH radical scavenging activities of Algerian honey five samples were measured at the following concentrations: 10, 20, 40, 60 and 120 mg/mL. (Khalil et al., 2012).

1.5. Antibiotics in honey

Antibiotics drugs are used by beekeepers to fight foulbrood diseases in honeybee colonies and so, they may contaminate honey if those colonies are used in production. Also,

the contamination of honey might occur during the regular apply of antibiotics like the streptomycin and its derivative dihydrostreptomycin which is frequently joint with tetracycline(Draiaia et al., 2015). According to the Codex Alimentarius and Council Directive of the European Union (EU), these bactericides are completely banned from honey(Van Bruijnsvoort et al., 2004), no maximum residue level (MRL) for tetracycline in honey has been established. This means that the existence of tetracycline residues in honey is not permitted. Although this determination, some countries have set action tolerated or limits amounts for tetracycline in honey. Currently, in Belgium, the limit for the group of tetracycline has been fixed at 20 µg kg‑1, France sets a non-conformity limit for tetracycline in honey of 15 µg kg‑1, the published limit in Great Britain is 50 µg kg‑1, however the tolerance amount in Switzerland is 20 µg/kg^{-1}. (Cara et al., 2012). Besides the fact that antimicrobials drug residues in honey can cause a potential danger to human health (Zai et al., 2013), it as a negative impact in the consumers perception on honey as a natural product. In one study concerning the quality evaluation of 36 samples of different honey types supplied by local producers from Algeria, it was shows that only two samples present 0.03 ppb of Oxytetracycline, with no residues of streptomycin or tetracycline (Draiaia et al., 2015).

2.THE OBJECTIFS

Taking into consideration the nutritional characteristics of honey and its lack in the Algerian market, it is unprotected to fraud. To make sure of its quality, many international institutions, like the International Honey Commission (IHC), the Codex Alimentarius and the European Commission suggest methods of analyses make sure that honey is authentic in respect to the legislative requirements. the production of Algerian honey is less than the needs of local consumption while it is said to be at the origin of a huge export. This low production affects the price and makes it remain high. For that reason, consumption remains as low as production. This absence of production is the result of many causes like the lack of national regulation, absence of a professional organization and insufficient quality control laboratory. Even so, Algerians researchers and scientists try to make an appropriate denomination that make sure of a minimum marketing value of the product.

The aim of the present study was to evaluate the quality of ten samples belonging to different honey type (three samples from rosemary honey, three from tamarix honey, three from milk this honey and one multifloral honey) supplied from local producers from Algeria in order to verify its compliance with the standards of Codex Alimentarius, 2001b and the

Council of the European Union (EU), 2002. The quality of the honeys will be determined through different parameters. Melissopalinological 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., 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 screened through the multi-analyte receptor assay system Charm II.

3. WORK PLAN

The aim of this study will be to evaluate the physicochemical characteristics to assess the quality of Algerian honeys from different botanical and geographical origins. The quality of the honeys will be determined through different parameters. Melissopalinological and physicochemical analysis previously cited. Finally, the presence of antibiotics, recurrent residues in honey, such as tetracyclines and sulphonamides will be screened through the multi-analyte receptor assay system Charm II. (**Figure 5.** describes the time plan to achieve the desired goals described above).

Figure 5. *Timeline of the current study*

4. Methodology

To perform the different tasks, we will follow some established methodologies as described below:

4.1 Sampling

The Algerian honeys under study were obtained directly from beekeepers in 2019 and correspond to four types of honey with different botanical origins, **Table 4**. which will be checked by melissopalynological analysis.

Table 4. Honey sample origin.

Sample Code	Botanical origin	Geographic origin	Month/year of collection
R1	*Rosmarinus officinalis* L.	Sidi Belabbes	June 2019
R2	*Rosmarinus officinalis* L.	Sidi Belabbes	June 2019
R3	*Rosmarinus officinalis* L.	Sidi Belabbes	June 2019
CH1	*Silybum marianum*	El Bayedh	June 2019
CH2	*Silybum marianum*	El Bayedh	June 2019
CH3	*Silybum marianum*	El Bayedh	June 2019
T1	*Tamarix gallica* L	El Bayedh	June 2019
T2	*Tamarix gallica* L	El Bayedh	June 2019
T3	*Tamarix gallica* L	El Bayedh	June 2019
MF	Multifloral	Sidi Belabbes	June 2019

4.2. Physicochemical characterization of the honey samples

The physicochemical parameters (color, moisture content, electrical conductivity, pH, free acidity (equivalence point at pH 8.3), lactonic and total acidity, 5-hydroxymethylfurfural, diastase activity and proline will be determined according to the Harmonized Methods of the International Honey Commission (International Honey Commission, 2009). Sugars will be analyzed by high-pressure chromatography coupled to a refractive index detector (HPLC-RI) and the phenolic compounds will be determined by liquid-chromatography coupled to mass spectrometry (LC-MS).

4.3. The bioactivity evaluation

The bioactivity of honey will be studied though the evaluation of the antioxidant activity, by the DPPH and reducing power assay, but also the cytotoxic potential using the sulforhodamine B (SRB) assay, and the anti-inflammatory potential by the murine macrophage (RAW 264.7) cell line , following the procedure described previously (Falcão et al., 2019).

4.4. Screening of antibiotics

The antibiotics sulphanamides and tetracyclines will be detected in the honey samples by Charm II kit for honey, which is a test based on radio labeling and microbial receptor created by Charm Sciences Co.(Lawrence, MA,USA), allowing a quick relative analysis (Salter, 2003).

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