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Topic

Characterization of selected Algerian honeys and identification of some potential biomarkers

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Abstract

The objective of the present work was an evaluation of the quality of Algerian monofloral honeys by the analysis of physicochemical parameters, color, total phenols and flavonoids content, antioxidant capacities and apha-amylase inhibition as a preliminary study, as well as the search for a particular fingerprint that characterizes them via a non-targeted metabolomic approach of their volatile profile by the HS-SPME method coupled with GC-MS. Fifty-nine samples of monofloral honey were collected from beekeepers from different regions of the mediterranean, semi-arid and arid parts in Algeria. According to the preliminary results, the samples as a whole, are of good commercial quality. In combination with the microscopic results of the pollen and the chemometric analysis, a reclassification of the samples was made. Eleven types were revealed citing *Acacia* (Mimouza), *Arbutus unedo* (Lenj), *Atractylis serratuloides* (Sor), *Bupleurum*, *Capparis spinosa* (Merkh), *Eruca sativa* (Harra), *Eucalyptus*, *Genista*, *Hedysarum*, *Retama sphaerocarpa* (Retem) and polyfloral, of which several samples did not come from the declared origin. For the biological properties (antioxidant capacity and α-amylase inhibition), darker samples showed higher electrical conductivity, phenol, flavonoid content, and antioxidant activity than the lighter samples of honey. *Arbutus* honey sample showed the highest inhibition of α-amylase. In addition, *Eruca sativa* and *Retama* samples also stood out for this property. The volatile fraction of the three selected types (*Atractylis*, *Retama* and *Eruca*), revealed exclusive components to each type: 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl-, (E); 1,6-octadien-3-ol, 3,7- dimethyl ; phenol, 2-methoxy and 2-naphthalene methanol, decahydro-α,α,4a- trimethyl-8-methylene-, [2R-(2α,4aα,8a,8aβ)] for *Atractylis* honey, lilac aldehyde and lilac aldehyde D for *Retama* honey, and dimethyl trisulphide for *Eruca* honey. Sensory analysis allowed to distinguish three different profiles among which the honeys of *Atractylis* of crystalline nature, sweet smell and aroma, with clear color. Those of *Retama* of viscous aspect, a color between dark amber and dark, caramelized vegetable odor, a salty sweet savor. Finally, those of *Eruca sativa*, in the form of crystallized honeys, straw color, animal, vegetable and floral odor with an important persistence. These perceptions allowed to distinguish the three main types of honeys, which in case of smell and aroma explains the variation of the volatile composition already mentioned.

Key words: Monofloral honey; Physicochemical analysis; Antioxidant activity; Inhibition of a-amylase; Volatile compounds; HS-SPME/GC-MS.

Résumé

L'objectif du présent travail était l'évaluation de la qualité des miels monofloraux algériens par l'analyse des paramètres physico-chimiques, de couleur, de teneur totale en phénols et en flavonoïdes, de capacités antioxydantes et de l'apha-amylase inhibition comme étude préliminaire. Ainsi qu'à la recherche d'une empreinte particulière qui les caractérise via une approche métabolomique non ciblée de leur profil volatil par la méthode HS-SPME couplée à la GC-MS. Cinquante-neuf échantillons de miel monofloral ont été collectés auprès des apiculteurs de différentes régions méditerranéennes, semi-arides et arides d'Algérie. D'après les résultats préliminaires, les échantillons dans leur ensemble sont de bonne qualité commerciale. En combinaison avec les résultats microscopiques du pollen ainsi que l'analyse chimiométrique, une reclassification des échantillons a été faite. Onze types ont été révélés citant *Acacia* (Mimouza), *Arbutus unedo* (Lenj), *Atractylis serratuloides* (Sor), *Bupleurum*, *Capparis spinosa* (Merkh), *Eruca sativa* (Harra), *Eucalyptus*, *Genista*, *Hedysarum*, *Retama sphaerocarpa* (Retem) et polyfloral, dont plusieurs échantillons ne provenaient pas de l'origine déclarée. Pour l'analyse des propriétés biologiques (capacité antioxydante et inhibition de l'a-amylase), les échantillons plus foncés ont montré une conductivité électrique, une teneur en phénols et en flavonoïdes ainsi qu'une activité antioxydante plus élevée que les types de miel plus clairs. L'échantillon d'*Arbutus* a montré la plus forte inhibition de l'a-amylase. En outre, les échantillons d'*Eruca sativa* et de *Retama* se sont également distingués pour cette propriété. La fraction volatile des trois types choisis (*Atractylis*, *Retama* et *Eruca*), a révélé des composants exclusifs à chaque type : 1,6,10-dodécatrien-3-ol, 3,7,11-triméthyl-, (E) ; 1,6-octadien-3-ol, 3,7-diméthyl ; phénol, 2-méthoxy et 2-naphtalène méthanol, décahydro-α,α,4a- triméthyl-8-méthylène-, [2R-(2α,4aα,8a,8aβ)] pour le miel d'*Atractylis*, aldéhyde lilas et aldéhyde lilas D pour le miel de *Retama*, et trisulfure de diméthyle pour le miel d'*Eruca*. L'analyse sensorielle a donné trois profils différents distingués parmi lesquels les miels d'*Atractylis* de nature cristalline, odeur et arome douces, couleur claire. Ceux de *Retama* d'aspect visqueux, de couleur entre ambre foncé et foncé, odeur caramélisée végétale, une saveur douce salinée. Enfin, ceux d'*Erica sativa*, comme des miels cristallisés, de couleur paille, odeur animale, végétale et florale avec une persistance importante. Ces perceptions ont pu distinguer les trois principaux types de miels, dont par rapport à l'odorat et l'arome, ils expliquent la variation en composition volatile déjà mentionnée.

Mots clés : Miel monofloral ; Analyses physicochimiques ; Activité antioxydante ; Inhibition de l'a-amylase ; Composés volatils ; HS-SPME/ GC–MS.

الملخص

الهدف من العمل الحالي هو تقييم جودة العسل الجزائري أحادي الزهرة من خلال تحليل المعلّمات الفيزيائية والكيميائية واللون ومحتوى الفينولاتوالفلافونويد الكلي والقدرات المضادة للأكسدة وتثبيط انزيم الألفا أميلاز كدراسة أولية ، وكذلك البحث عن بصمة معينة للتمييز عن طريق نهج التمثيل الأيضي غير المستهدف للمكونات المتطايرة بواسطة طريقة HS-SPME مقترنة ب GC-MS .

جُمعت 59 عينة من العسل أحادي الزهرة من النحالين من مناطق مختلفة من شمال الجزائر والمناطق شبه القاحلة والقاحلة.

وفقًا لنتائج الدراسة الأولية، فإن العينات ككل ذات جودة تجارية جيدة. بالاقتران مع النتائج المجهرية لحبوب الطلع والتحليل الكيميائي ، تم إجراء إعادة تصنيف للعينات حيث تم الإعلان عن تم أحد عشر نوعًا (*Eruca sativa*،)كبار(*Capparis spinosa*، *Bupleurum* ،) صر(*Atractylis serratuloides*،)لنج(*Arbutus unedo*،) ميموزا(*Acacia* ، *Hedysarum*،)مرخ(*Genista saharea* ، *Eucalyptu)s*،) (حارة / جرجير .رتم(, التي لم تأت عدة عينات منها من المصدر المعلن(Retama sphaerocarpa

، أظهرت العينات الأكثر قتامة موصلية) α-amylase لتحليل الخصائص البيولوجية)القدرة المضادة للأكسدة وتثبيط ال كهربائية أعلى ، ومحتوى الفينول والفلافونويد ، ونشاط مضاد للأكسدة أكبر مقارنة بأنواع العسل فاتحة اللون. أظهر عسل *Retama* و *Eruca sativa* بالإضافة إلى ذلك ، برزت عينات . α -amylase أعلى تثبيط ل *Arbutus* .أيضًا لهذه الخاصية

- عن مكونات حصرية لكل نوع: 1،6،10) *Eruca* و *Retama* و *Atractylis* (كشف الجزء المتطاير من أهم ثلاثة أنواع - ثنائي ميثيل ؛ الفينول ، - 2 ميثوكسي و 7 ، 3، (E 1،6 - octadien-3-ol ؛)، 11-trimethyl- ، 7 ، 3، dodecatrien-3-ol لعسل أتراكتيليس]) 8aβ، 8a، 4aα، 2α) -Rنفثالين الميثانول ، ديكاهيدرو ألفا ، ألفا ، - - 4 أ تريميثيل - - 8 ميثيلين ،] - - 2 2 ل عسل ريتاما ، وثنائي ميثيل ثلاثي كبريتيد لعسل الجرجير. سمح التحليل الحسي بتمييز D ، ألدهيد الليلك ، ألدهيد الليلك ذو الطبيعة البلورية، ذات الرائحة السكرية و اللون الفاتح. عسل الرتم Atractylis ثلاثة أشكال مختلفة من بينها عسل ذو القوام اللزج ، واللون الداكن ورائحة الخضار مع الكراميل كذلك النكهة الحلوة ذات ملوحة محسوسة. أخيرًا ، Retama ذو القوام المتبلور ، لون القش ، ورائحة حيوانية ، ونباتية ذات الثبات المهم. سمحت هذه التصورات Eruca sativa عسل .بالتمييز بين الأنواع الرئيسية الثلاثة للعسل ، وهو ما يفسر اختلاف التركيبة المتطايرة الكيميائية التي سبق ذكرها

الكلمات الأساسية: عسل أحادي الزهرة ؛ تحليل فيزيائي كيميائي النشاط المضاد للأكسدة؛ تثبيط الأميليز. مركبات متطايرة HS-SPME GC-MS

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List of Abbreviations

2-D PAGE : Two-Dimensional Polyacrylamide Gel Electrophoresis

AAS : Atomic Absorption Spectrophotometric

ABTS. +: Acide 2,20-azino-bis-3-éthylbenzthiazoline-6-sulfonique

ANOVA: Analysis of Variance

CACTI: Centro de Apoio Científico-Tecnolóxico á Investigación

COI : The mitochondrial Cytochrome Oxidase subunit 1

CRBt: Centre de Recherche et de Biotechnologie

DNA : Deoxyribonucleic Acid

DPPH: 2,2-Diphenyl-1-picrylhydrazyl

DSE: Dynamic Solid Phase Extraction

EC: Electrical conductivity

ET-AAS : Electrothermal Atomic Absorption Spectrometry

GC/FID: Gas Chromatography with Flame Ionization Detection

GC/MS: Gas Chromatographic Mass Spectrophotometry

HCA: Hierarchical Cluster Analysis

HD: Hydrodistillation

HgTe: Mercury Telluride

HMF: Hydroxylmethyl furfural

HPAEC-PAD: High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection

HPLC: High Performance Liquid Chromatography

LC/MS: Liquid Chromatography–Mass Spectrometry

LLE: Liquid-Liquid Extraction

LNSDE: Likens-Nickerson Simultaneous Distillation Extraction

LRIc: Linear Retention Index calculated

MALDI-MS: Matrix-Assisted Laser Desorption/Ionization Mass Spectrophotometry

MRJP2: Major Royal Jelly Protein 2

MS: Mass Spectrophotometry

MSDE: Micro-simultaneous steam and solvent distillation extraction

NGS: Next-Generation Sequencing

NMR: Nuclear Magnetic Resonance

OPLS-DA: Orthogonal Partial Least Squares Discriminant Analysis

PCA: Principal Component Analysis

PCR: Polymerase Chain Reaction

pH: Potential of Hydrogen

PLS: Partial Least Squares Regression

rbcL : Ribulose-Bisphosphate Carboxylase Gene

RSA: Radical Scavenging Activity

SALDI-MS : Surface-Assisted Laser desorption/ionization mass spectrometry

SDE: Simultaneous Steam Distillation Extraction

SDS-PAGE : Sodium Dodecyl Sulfate–Polyacrylamide Gel Electrophoresis

IC_{50}: The half maximal inhibitory concentration

ID: Index of Diastase

IHC: International Honey Commission

ISO: International Organization for Standardization

ITS2: Internal Transcriber Spacer

USE: Ultrasonic Extraction

UV : ultraviolet

UV-VIS-NIR : Ultraviolet, Visible, Near-Infrared.

VIS/NIR : Visible, Near-Infrared.

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Introduction

Honey is one of the most sacred natural products. For primitive man, it was not only an important source of food, but was also attributed with many magical powers. According to historical references, Romans were responsible for the expansion of beekeeping in the Mediterranean, but it is from the Arab domination that we find references, which continue almost uninterruptedly until our days. Arabs, like most oriental people, made extensive use of honey, its use being very frequent in the elaboration of magistral formulas for medicinal purposes and in many culinary recipes. Their penchant for honey-based desserts and sweets is well known, some of which gave birth to our nougat. Therefore, we can think that the importance of beekeeping was great and that the number of hives and people dedicated to this activity at that time was very high (Mateu-Andrés *et al.*, 1993). The Moors practiced beekeeping and were considered good consumers (Skender, 1972).

Scientifically and with regard to its composition, although sugars are the most abundant compounds in honey, many other nutrients such as enzymes, amino acids, minerals, organic acids, phenols and various volatile compounds, among others, also characterize the chemical composition of the product. Since many of these properties are plant-based, the botanical origin of honeys is an important factor in determining the minor components of honey (Da Silva *et al.*, 2016).

In addition, and according to Johnston *et al*. (2018), some medicinal properties of plants can be incorporated into their nectar, so many of them could be transmitted to the human body through honey. This is why it is so interesting to study its chemical composition.

Often, alternative treatments using natural products to solve health problems are preferred due to the side effects of pharmaceutical drugs. Honey is among the most widely used unprocessed natural products in this field (Samarghandian *et al.*, 2017; Meo *et al.*, 2017; Khan *et al.*, 2018).

In modern science, and despite its long history as an alternative medicine, honey has not always been recognized as a therapeutic and/or alternative agent. Its biological properties and the mechanisms underlying its health-promoting attributes are still not clearly understood, which is why more and more research is being conducted on the medicinal use of honey (Khan *et al.*, 2018).

Since botanical and geographical origins have become a more important task than ever, considering the development of the world honey market, and in order to link the healthy properties of honey to the corresponding botanical and geographical origin, a good characterization of honey is necessary. It is a difficult task that has been approached with different strategies. Some of them are based on the identification of components or compounds acting as a single marker (Gerhardt *et al*., 2018) or as a fingerprint in combination with other chemical or biotic markers (Manyi-Loh *et al*., 2011).

Numerous studies have been conducted to evaluate honey samples from various botanical origins using metabolomic sub-approaches, trying to find useful chemical markers for monofloral honeys, based on the analysis of compositional data of honey volatile compounds, phenolic acids, flavonoids, carbohydrates, amino acids and some other constituents (Karabagias *et al*., 2014; Seisonen *et al*., 2015; Tette *et al*., 2017; Rodríguez-Flores *et al*., 2021).

Research on volatile compounds in foods has shown promising results for characterization, as some of them are related to a particular sensory profile, such as flavor, odor and aroma, as well as to the presence of undesirable compounds or substances giving an unpleasant odor (Nieminen *et al*., 2008). It is therefore very important to identify this fraction in food products in order to characterize them and to study variations according to geographical origin, production technology, seasonality, drying or interaction with the packaging material.

Volatile compounds in honey began to be researched and considered since the 1960s, and it was found that they could come from a variety of sources, including the botanical origin, the processing of plant compounds by the bees, the activity of the bees, compounds generated during processing, storage of the honey, and even some microbial interactions or environmental contaminations (Baroni *et al*., 2006; Machado *et al*., 2020).

These compounds are present in honey at very low concentrations as complex mixtures of different chemical classes (Manyi Loh *et al*., 2011; Da Costa *et al*., 2018). They can be extracted using a variety of different sampling techniques, both conventional and innovative, but due to the advantages of solvent-free sample processing, and high sensitivity and reliability, headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC/MS) has been widely used for the analysis of this chemical fraction (Dou *et al*., 2020).

However, the volatile fraction of honeys from this geographical origin has not been well advanced so far, only a recent study on seven samples from the Algerian territory has been published (Neggad *et al*., 2019). Some monofloral honeys produced in semi-arid, arid areas near to the Sahara or even Mediterranean are still little studied, this is the case of those of Retem, Sor, Harra, kebbar, Merkh and others, as they are named locally. These monofloral honeys are currently available, well appreciated by tasters, but they remain rare and poorly known.

For this concept, the present thesis work falls within the framework of the thematic of food sciences of the University Mouloud Mammeri, Tizi Ouzou, Algeria, of which one of the structuring projects is the research of biomarkers for the prediction of food quality. Thus, in order to deepen the research on the traceability of local natural products, a contribution to the valorization of some types of Algerian honeys, more precisely less known and less marketed, by their basic characterization and the comparison between their polinic spectrum and their volatile profiles. Then, the sensory analysis could be adjusted for further distinctions.

I.Bibliographic part

I. 1. Honey bee

The most important bee for beekeeping and the basis of the beekeeping industry is the one belonging to the races and strains of *Apis mellifera*, which originated in Europe, and were introduced to America, Australia, New Zealand and the Pacific Islands, as well as to North Africa. Outside these temperate zones, there are tropical African sub-strains of *Apis mellifera* that are not as suitable for domestication in hives, unlike the oriental bee *A. cerana* which can be domesticated in hives and is almost similar to *A. mellifera* in many ways, except that it is often smaller and less productive. In tropical Asia, abundant honey is also collected from large wild nests of *A. dorsata*, and from smaller but more accessible nests of *A. florea* (Qamer *et al*., 2013). In addition to the species mentioned, there are many species of stingless bees (Meliponinae) (Crane, 1990), which produce honey with a long tradition of consumption and various medicinal uses attributed to them (Souza *et al*., 2006; Tomás-Barberán *et al*., 2013).

Honey bees of the sub-species *Apis mellifera* were first classified according to their morphological and behavioral characteristics and their geographical distribution. Thus, morphometric analyses conducted on large datasets have established four different evolutionary lineages of honey bees: (M) in Northern and Western Europe, (A) in Africa, (C) in South-Eastern Europe, and (O) in Western Asia (Ruttner, 1988; Achou *et al*., 2015). In Algeria, two different bee subspecies have been recorded: *A. mellifera intermissa* as it is dominant in North Africa (Tunisia, Algeria and Morocco) and extends in the northern Sahara from Libya to the Moroccan coast of the Atlantic Ocean, and *A. mellifera sahariensis* extends along the Jebel Amour and Ain Sefra in Algeria to various oases from Figuig to Ouarzazate in Morocco (Achou *et al*., 2015). However, in the oases of the Eastern Sahara, only *Apis mellifera intermissa* is present (De la Rua *et al*., 2009).

I. 2. Beekeeping situation in Algeria

In Algeria, beekeeping is considered an integral part of the agricultural and rural routine. It is practiced in several regions, but due to the favorable climatic conditions and the great floral biodiversity that provides melliferous resources during most of the year, the practice has gained in importance in the north of the country (Hussein, 2000). In recent years, beekeeping in Algeria has experienced remarkable growth and development throughout the

country. Although the import and export of bees is highly regulated by Algerian law, some foreign queens bees are still introduced into the country as it is believed that they could provide better honey production and have superior disease resistance than local bees (Achou *et al*., 2015).

There are more than 20000 beekeepers with 700000 hives across Algeria (Hussein, 2000), mainly modern hives of the so-called Langstroth and Dadant types, and rarely traditional ones. About 90% are independent and approximately 10% are professionals (Ghorab *et al*., 2021). However, honey production in Algeria is relatively low, ranging from 5 kg to up to 20 kg of honey per hive, despite the increased use of modern hives (Laallam *et al*., 2015).

According to the head of the beekeeping sector at the Institut Technique de l'Élevage ITELV, (Technical Institute of Livestock) in January 2020, the national production of honey has almost doubled in the last ten years while consumption remains limited.

However, this estimate as well as the one stated by Laallam *et al*. (2015) are not exhaustive, as there are also quantities produced and marketed by informal networks, and other honey-producing regions that have yet to be identified. Thus, some challenges must be met to continue promoting this activity in rural areas. One of the most critical is the absence of a legal framework for the quality of honey intended for trade and competition on the conventional market with imported honey (Ghorab *et al*., 2021).

According to Skender (1972), the inhabitants of Maghreb, including Algerians, were considered to be major consumers of honey, but more recently, consumption levels in Algeria and the Maghreb countries are still generally considered to be very low compared to the figures for European and American countries. According to the same study, honey in Algeria is consumed as a medicine, and consumption per individual is about 0.200 kg/year/h. These consumption levels remain very low, giving honey an insignificant share in the diet, also the Algerian consumer’s culture has been so restricted with regard to the notion of honey quality (Haderbache, 2015). They prefer dark, liquid honeys, and especially those from the random hives of trusted beekeepers. Jujube, eucalyptus and polyfloral honeys are the most demanded and beekeepers have had no choice but to condense the production of these types of honey and neglect the other types. Nevertheless, the diversified production of honey in Algeria has increased in recent times and even the variation is well noted at the level of occasional exhibitions of the products of the hive, the consumption of which is still restricted.

I.3. Honey production and technologies

Honey formation starts with the collection by bees of nectar (plant product) and/or honeydew (plant or insect product on plants) in the fields. In Algeria, it is assumed that there are about 4000 plant species distributed over the whole territory of which flowering plants constitute the most diverse flora (Véla and Benhouhou, 2007), and most of them are considered as melliferous plants, those that are important for honey bees to produce honey (Koçyigit, 2014; Ghorab *et al*., 2021). Traditionally, and practically in Algeria, the distribution or local marketing of honey is done by the beekeeper himself directly to his surroundings. With the advancement of food technology, a honey technology sector has been established, from extraction to labelling.

I.3.1. Honey production (bee work)

To obtain honey, and although bees may forage on fruit juices, extrafloral nectaries and other sweet secretions, the main raw materials for honey are nectar and honeydew, both of which come from the phloem of higher plants. The phloem transports sugars as well as other nutrients such as amino acids, electrolytes, plant hormones, phenolic acids, vitamins, organic acids, etc… (Crane, 1975). Multiple transformations take place on the predigested material (nectar and/or honeydew) either in the bee's digestive tract or during shared work between the carrier bees.

I.3.1.1 In the digestive tract of the bee

Honey production begins when the bees collect nectar and/or honeydew by suction with their tubes and store it in their crop, the addition of saliva which enriches it with enzymes by transforming polysaccharides into simple sugars, so that the sugary secretions collected by the bees mixed with the saliva have been diluted, and thus the nectar contained in the bee's honey sac generally has a lower sugar concentration than originally. The glandular secretions provided by the bees contain small amounts of lipids, vitamins and enzymatic proteins that will play a fundamental role in the transformations that the nectar and/or honeydew will undergo to become honey (Mateu *et al.*, 1993). These compounds will appear in the final product produced by the bees, entering its composition.

I.3.1.2. Honey dehydration

Once near the hive, an act of sharing by successive regurgitation with other workers, called "trophallaxis", takes place. The transmission rate depends on several factors such as temperature, age of the bees, breed, hive size and supply of raw material.

The honey bees store the regurgitated nectar in their crop where it undergoes a complex transformation. The partially ripened honey is deposited in the cells where its maturation takes place. Aeration should be carried out until a liquid with a water concentration of more or less 19% is obtained. Once the honey has become ripe, it will be sealed by the bees with a layer of wax in contact with the hot and dry air, which allows it to lose water (Huchet et al., 1996). This process takes place in two stages: the bees actively participate in the first stage and only the honeybees participate in the second. The parallel role of the beekeeper during the foraging and honey production period is to provide favorable conditions for the bees, harvest the honey, preserve it, and keep it in good condition (Lequet, 2010). Thus, this product is stable over a long period of time and not very sensitive to fermentation.

I.3.2. Harvesting and storing honey (Beekeeper's work)

After the bees have produced honey, the role of the beekeeper continues, including the harvest period after the honey has matured. Some of the early techniques are still in use, new methods have been devised by each generation of beekeepers.

I.3.2.1. Honey harvesting

Honey should be collected by the beekeeper when it is ripe. A frame can be removed from the hive when three quarters of it is covered with wax, after which it is imperative to leave a quantity of honey for the bees. The bees should be smoked beforehand to limit their movements. Other techniques are also used such as the use of a bee catcher, a blower to temporarily propel the bees into the air and the use of a small broom to manually remove the bees from the comb. The next step is dehumidification, which some beekeepers apply because humidity can increase for reasons such as uncapped cells, transport and handling. Using a frame lifter, the beekeeper removes the frames filled with honey, which are then transported in a sealed vehicle to the honey house where the cells of the frames are uncapped. This can be done manually or mechanically with the help of a machine. The uncapped frames are then placed in a centrifuge, either manual or automatic, for the extraction phase, so that the centrifugal force pushes the honey out of the machine, and then in a clean and dry place.

Maturation stage is set up, of which a filtration and a decantation are put successively in a maturator (Figure 1). As honey is a foodstuff that can spoil over time, good storage, preservation and packaging conditions must be in place (Biri, 1986).

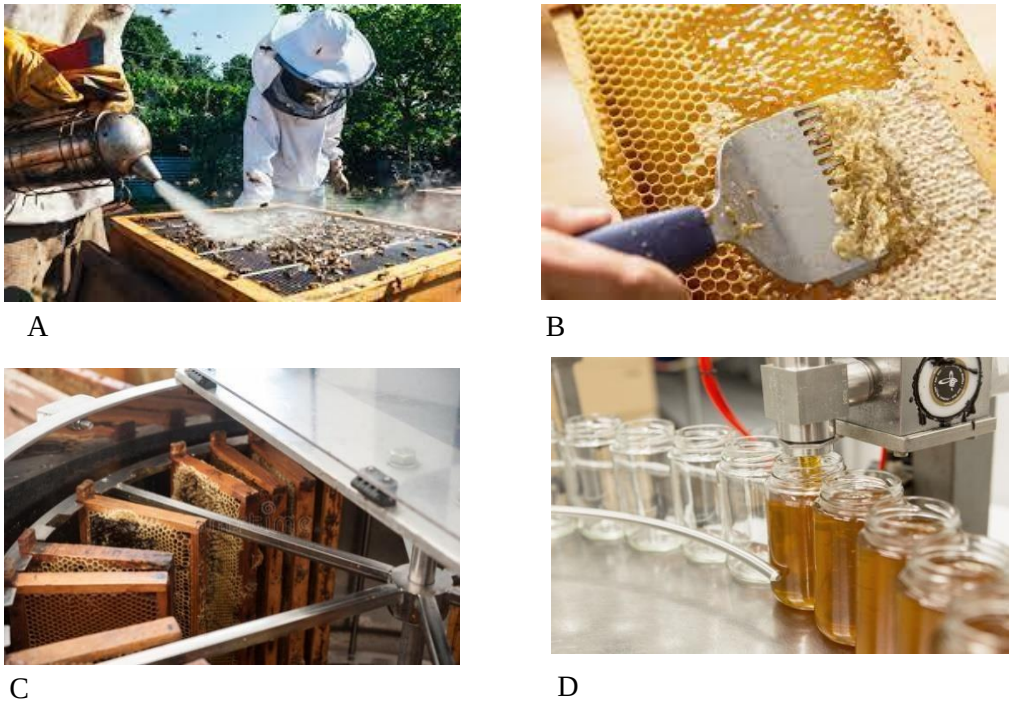


Figure 1: Honey recovery A: Smothering of bees, B: Uncapping of cells, C: Extraction of honey by centrifugation, D: Honey packaging.

Finally, honey can be packaged in jars with capsules that ensure their watertightness and are labelled with all relevant legal information. But its labelling based solely on the beekeeper's experience, thus a good transhumance practices is no longer sufficient for a declaration of botanical origin in the case of monofloral honey. Immediate analytical intervention before packaging is also essential before any form of labelling. It remains to be clarified which type of analysis is most effective in these cases.

Crystallization phenomenon

Honey crystallization is a very common natural phenomenon, which also refers to its authenticity. However, most consumers consider crystallization to be an alteration or adulteration of the product. The crystallization process does not lead to any change in nutritional value if the honey is correctly crystallized, but incorrect crystallization can lead to an increase in water activity and thus to fermentation. This is a desired phenomenon in the

production of creamy honeys, spreadable honeys that are recently becoming popular with some consumers (Krishnan *et al*., 2021).

The tendency of honey to crystallize is directly related to some sensitive parameters (crystallization indicators), such as water content, glucose content, ratios: glucose/water (D/W), glucose-water/fructose (D-W/L) and fructose/glucose (L/D) in favor of glucose, as well as melecitose content (Maniskis and Thrasyvoulou, 2001; Laos *et al*., 2011). Some types of honey crystallize naturally and correctly just after the extraction.Many beekeepers and in particular in Algeria report this phenomenon and express their concern about the bad marketing of these types of honey.

I.4. Honey and its sources

Honey is the most common edible product of the bee. According to the Codex Alimentarius Commission (2001), it is defined as a natural sweet substance produced mainly by bees of the species *"Apis mellifera"*, from the nectar of plants, and/or secretions from living parts of plants, or excretions left on them by sucking insects, which the bees collect, process, combine with specific materials of their own, deposit, dehydrate, store and ripen in the combs of the hive. Honey is therefore considered a sweet, viscous natural product with intermediate humidity, their properties, as well as the high osmotic environment, do not favor microbial growth in the standards, which makes honey more stable (Machado De-Melo *et al*., 2018). Two main types of honey are distinguished according to international standards: flower honey (or nectar honey) and honeydew honey (EC Directive 2001/110).

I.4.1. Nectar honey

Obtained mainly from a substance called "nectar" from the flowers of so-called "nectariferous" plants. Nectar is the most common resource for honey production. It is formed from plant sap in specific organs of flowering plants called nectaries or nectar glands, of which there are two types: Some are located at the base of the petals, in the heart of the flower, called "floral nectaries", others are located on other parts of the plant (leaves, stems or others...), and are called "extra-floral nectaries". By its biochemical composition, nectar is intended to attract pollinating insects such as bees. It is a more or less viscous aqueous solution depending on its water content, which can vary greatly, and whose dry matter represents between 5 and 80% of the nectar.This dry matter is composed of 90% sugars, the most common of which are sucrose, glucose and fructose., also organic acids and proteins including enzymes and amino acids, aromatic substances and inorganic compounds

(phosphate, etc.), which give honey its aroma and color (Hoyet, 2005).When collecting nectar, the bees will cover themselves with pollen, a substance produced by the male organs, and each flower leaves its identity card in its nectar (pollen, pigments, aromas, etc.). The bee then continues on its way to its hive to produce its honey, called "nectar honey".

Monofloral honeys

The hives of the bees, well established within the natural flora, spontaneous and diversified according to the geographical areas, are sometimes directed by professional beekeepers towards specific fields, natural or programmed, in order to direct the bees for their foraging, this is what we call "transhumance" or "pastoral beekeeping".

This movement of hives is carried out over short or long distances (500 km), but always at least three kilometers from the initial location in order to benefit from more generous honey flows. According to Bérard and Marchenay, (2007), transhumance is carried out late in the evening or before sunrise, so that the whole colony is back at the hive. Certain precautions must be taken during this journey because of the sensitivity of the bees and the ventilation conditions to avoid asphyxiation of the colonies.The desired product of this transhumance is a honey mainly from a single flora, called "monofloral honey".

According to the preliminary study of Louveaux *et al*. (1978), and until now, a honey sample is generally considered to be monofloral, which can be governed by the physicochemical and sensory properties of a main nectar, when the pollen of the main melliferous plant from which it originates, exceeds 45%. Elamine *et al.* (2019) cite two exceptions to this rule, including lavender honey, for which the presence of 15% lavender pollen is sufficient to be labelled as monofloral honey (Estevinho *et al.*, 2016), as well as chestnut honey, which is only labelled when it contains more than 90% chestnut pollen (Louveaux *et al.*, 1978).

The authors thus confirm that these exceptions underline the importance of establishing a specific threshold for a given type of honey, especially when it is newly introduced in the scientific community. Several other exceptions are also cited in other works when studying new types of honeys.

I.4.2. Honeydew honey

For the elaboration of this type of honey, the bee takes directly the secretions of plants such as the genera (*Pinus, Abies, Castanea* and *Quercus*, etc.) or the exudates of certain

sucking insects, deposited on the living parts of the plant, mainly of the family Aphididaes. In honeydew, pollen of anemophilous plants remains of hyphae of fungi, spores, green algae, etc, are abundantly present (Persano Oddo and Piro, 2004). Therefore, the composition of honey is closely related to its botanical origin. It also depends on the geographical area, as the characteristics of the soil and the climate determine the honey flora.

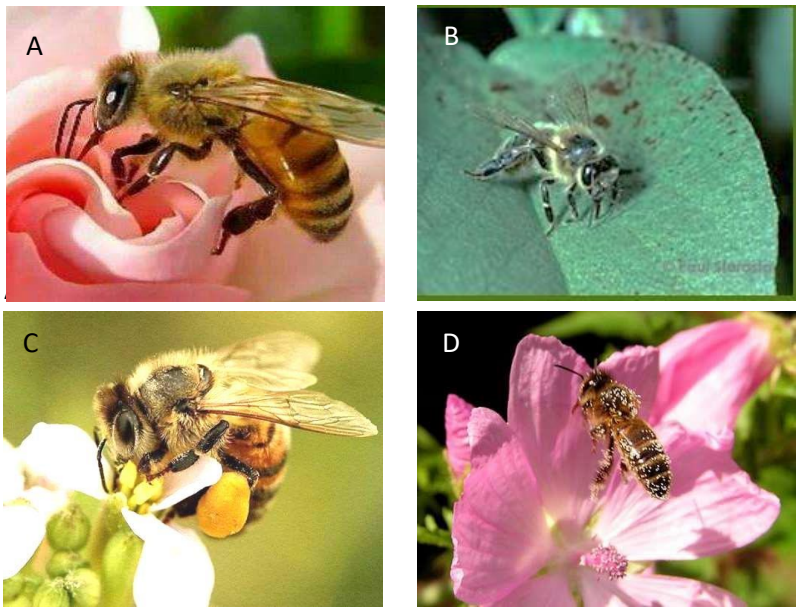


Figure 2: Foraging bee (A): A forager bee of *Apis mellifera* foraging only for nectar from a flower (B): Honeydew collecting bee, (C): A forager bee foraging for pollen as well as nectar from the flower, (D): A forager bee foraging only for pollen on the flower (Sihag and Kaur, 2018).

I.5. Honey composition

For its composition, honey contains about 200 different substances, mainly sugars, water and other substances like proteins (enzymes), amino acids (proline, etc.), organic acids, vitamins (including vitamin B6, thiamine, niacin, riboflavin and pantothenic acid), minerals (calcium, copper, iron, magnesium, manganese, phosphorus, potassium, sodium and zinc), pigments, phenolic compounds, a wide variety of volatile compounds and solid particles from the harvest as well as incorporated

pollen (Alqarni *et al.,* 2012; Da Silva *et al.,* 2016).

This complex chemical composition will depend mainly on their botanical origin and other external factors, such as geographical origin and climatic conditions. The listed components highlight both the physical properties and the nutraceutical characteristics of the product itself. Honey is already known for its nutritional and medicinal values. As a natural

sweet, it is intended for direct consumption, widely used in the food industry, especiallyas a preservative (Wen *et al.*, 2017).

I.6. Honey typification methods: Conventional, modern and complementary

This involves the application of certain tests to ensure the quality of honey for consumption.

A. Quality notion

Etymologically, quality comes from the Latin root *qualitas*, which means: way of being, nature of a thing. The Latin word *qualitas* itself derives from *qualis*, which expresses the relationship to a being or thing that it determines: what, of what kind, of what nature. It is what makes one thing more recommendable to human use and taste than another of the same nature (Reid, 2018). A honey is of good quality if it is able to meet the needs of the body, without harming the health of the individual who ingests it, providing maximum satisfactionBee honey intended for human consumption must be of sufficiently high quality. The requirements of each country include certain parameters.

Nowadays, in order to characterize a honey, several types of methods could be used: Basic (conventional) methods, modern and/or complementary methods, the first type of which is constituted by the methods previously implemented to verify the authenticity and basic quality of honey, based on the recommendations of the Codex Alimentarius and the International Honey Commission (IHC: 2002-2009), the second type consists of methods that check the same parameters but with more sophisticated tools, while the third and according to Kaškoniene and Venskutonis (2010), are complementary methods that are used to identify molecules of the chemical composition of the product. These molecules can often reveal specific biological markers (biomarkers).

The authenticity of honey through identification or differentiation by botanical and/or geographical origin has been studied by many authors, using different methods such as: pollen analysis and analysis of intrinsic components (volatile compounds, phenolic compounds, amino acids, carbohydrates, etc.), as well as other parameters such as: electrical conductivity, color, enzyme activity, among others.

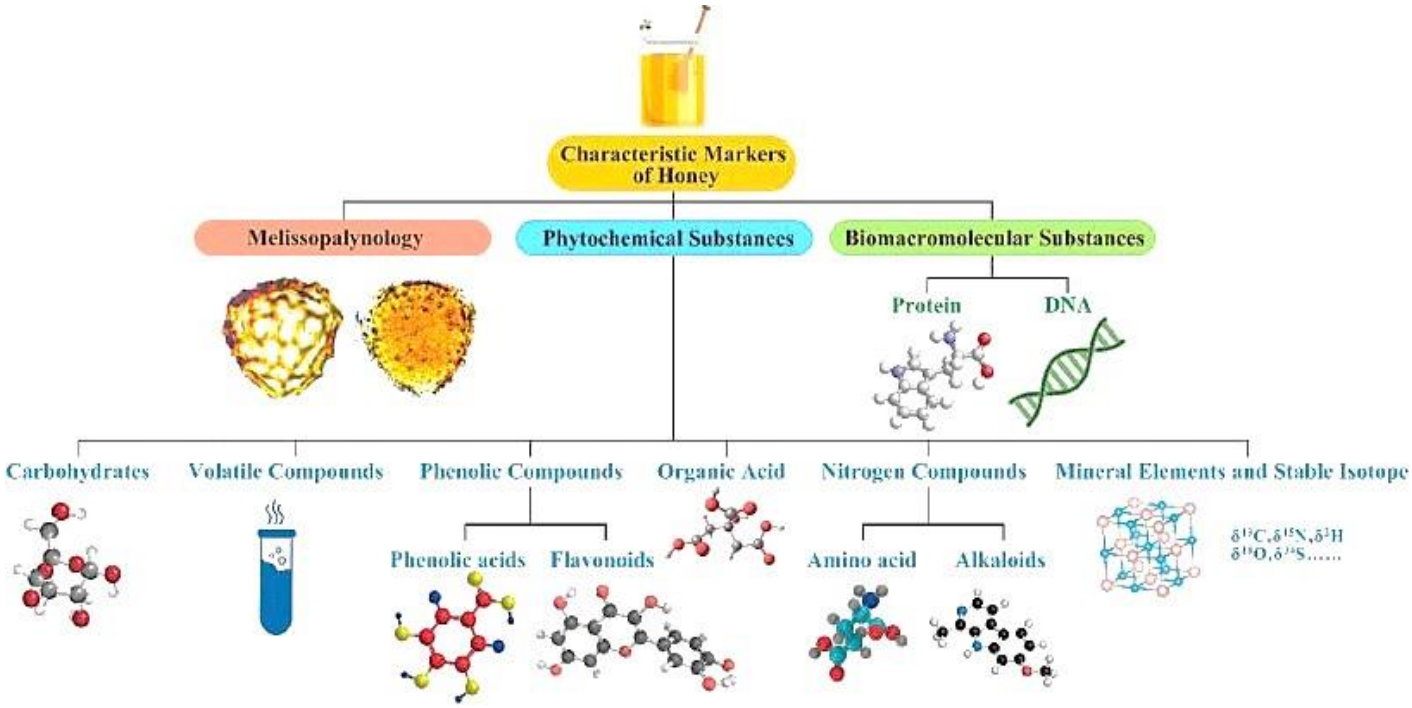


Figure 3: Potential characteristic markers for honey from different origins (Wang *et al.*, 2022).

B. Biomarker notion

A biomarker, or "biological marker", is a generic term that has been used for several years in many scientific fields, especially in the clinical area (Hulka *et al.*, 1990). It can be broadly defined as a biological molecule associated with a particular phenotype that can be readily used to characterize that phenotype (Picard *et al.*, 2015).

They generally refer to a measurement that can be used as a trace of a biological state or condition. Many research papers discuss the use of some of the so-called "omics" technologies, separately or in combination, not only for the analysis of food constituents, but also for food authentication and safety assessment (Balkir *et al.*, 2021).

I.6.1. Microscopic analysis of pollen

Traditionally, the search for plant pollen in honey is the only method used for the detection of its floral origin. This technique, called "melissopalynology", was developed in 1895 by Pfister and improved since then. It is currently the most widely used technique, which determines the proportion of predominant pollen grains in a particular honey, on the basis of which the honey variety is named, (Maurizio *et al.*, 1951; Puścion-Jakubik *et al.*, 2020). The analysis of pollen in honey is done in two steps: Quantitative analysis and qualitative analysis.

I.6.1.1. Quantitative pollen analyses

The quantitative analysis of the pollen is based on the counting of the pollen grains detected by the optical microscope, then the determination of the pollen grain richness of each sample studied and then the classification of the honey samples in different classes according to their pollen richness based on the classification of Maurizio (1939), which groups the samples in different classes, according to the absolute content of pollen (N) in 1 g of honey:

Class I: $N<2000$, includes pollen-poor nectar honeys and honeydew honeys;

Class II: $2000<N<10000$, has an ordinary pollen margin, including most honeys;

Class III: $10000<N<50000$, includes pollen-rich honeys;

Class IV: $50000<N<100000$, corresponds to honeys very rich in pollen;

Class V: $N>100000$, corresponds to honeys that are extremely rich in pollen, or press honeys.

I.6.1.2. Qualitative analysis of pollen

This technique, also based on the optical microscope, allows the recognition of the different pollens present in honey and consequently, their most important types in honeys, as well as the existence of characteristic elements or combinations of elements that can be used as geographical indicators (Feller-Demalsy *et al.*, 1989). This identification is based on a botanical database. However, according to some authors; the melissopalynology technique has some disadvantages, such as being expensive, time-consuming and highly dependent on the skills and judgement of the analyst. In addition, a pollen library is required; industrial infiltration can also affect the accuracy and precision of the technique, and there is great variability in the contribution of a particular flower's nectar to the amount of its pollen found in honey. Although a specific pollen may be present in a honey, its presence may be low in some types of honey (citrus, lavender and rosemary).

It has already been pointed out by Guyot *et al.* (1999) that in some cases pollen analysis may not be useful, especially when the honeys are from sterile plants. It is also more difficult to determine the floral origin of a honeydew honey because palynological analysis cannot be carried out given its source of production.Therefore, there is a tendency to replace pollen analysis by analytical and/or physicochemical profiling of markers for honey discrimination (Stanimirova *et al.*, 2010).

I.6.2. Sensorial analysis

Sensorial characteristics are the first attributes distinguished by consumers and together with melissopalynology; they allow the study of the botanical and geographical origin of honey. They can therefore be used as a tool for researching the variety and/or type of honey, as well as for distinguishing the organoleptic properties of different samples and the hedonic preferences of tasters.

They are based on the evaluation and scoring of organoleptic properties through visual, olfactory, gustatory and tactile perceptions (Marcazzan *et al.*, 2018). Originally, and at the professional level, sensory analysis was generally carried out by specialists who had extensive experience of the products and gave their opinion based on their own knowledge (the so-called traditional method), it was a useful, fast, simple and inexpensive method, but it cannot be considered a true method of analysis according to the same authors because it did not

really meet scientific requirements (reproducibility, repeatability, reliability). According to Pangborn (1964), the first method was developed by himself on the basis of a panel of evaluators as well as defined and controlled experimental protocols and statistical techniques to process the results. This procedure was then standardized to allow for an objective evaluation. The obtained results are reproducible but the methods are more complex, more time-consuming and require many more people.

As far as honey is concerned, the first scientists to apply traditional sensory analysis gave scientific relevance to the methodology (Gonnet and Vache, 1979; 1985; 1992). Their ideas and methods were quickly adopted in Italy where they spread throughout the country with great success, among scientists and beekeepers. An Italian register of experts in honey sensory analysis, officially registered with the Italian Ministry of Agriculture (Sabatini *et al*., 2007). This methodology has been successfully extended to many other countries, where new proposals for its modern application have been published.

During the last decade, several working groups have been created and have produced work on the analysis of the sensory profile of honey. Most of this work deals with the characterization and description of honey, but very little with methods for the sensory evaluation of honey itself. In general, researchers refer to ISO standards that give general indications applicable to all products.

In 1998, a working group (sensory group) was created by the International Honey Commission (IHC) to study sensory analysis applied to honey. As a result of the earlier studies of the sensory group, a harmonized glossary was developed, including a retronasal olfactory wheel for honey, a selection of descriptors and their intensity scale.In addition, a method for assessing botanical correspondence and the presence of defects has been proposed. These methods are based on the ability of trained assessors to evaluate the conformity of a declared single-flower honey to a typical profile that they have memorized during their training (Piana *et al*., 2004). However, the sensory group stated that these methods were used because of the need for control techniques and the lack of scientifically determined sensory profile models.

I.6.2.1. General conditions for the sensory analysis of honeys

As any case of food sensory analysis, the honey must be taken with care and attention. A first step consists in inviting a tasting panel, according to the objective of the analysis. This

panel is a real "measuring device", whose analyses can be made by three different types of jury.

- Naive subjects: the person who does not meet any particular criteria (ISO 8586-2, 1994), of which there are many.
- Qualified subjects: the subject chosen for his or her ability to carry out a sensory test (ISO 8586-2, 1994).
- Experts: the person who, by virtue of his or her knowledge and experience, is competent to give an opinion in the fields on which he or she is consulted (ISO 8586-2, 1994). They can be composed of five to nine persones.

The called persons must meet certain requirements such as: being in good health, it is recommended to exclude anyone who has taken medication or eaten too spicy food or any very tasty, smelly or exciting food or drink on the day of the test. Do not do the test after smoking, chewing gum. The use of scented cosmetics is not recommended.A well-equipped testing room is essential, with sufficient separation between tasters (Marcazzan et al., 2018).

I.6.2.2. Organoleptic criteria for honey

The term organoleptic generally represents all descriptions that can be perceived by the human sensory organs, so in the case of honey, it refers to its texture, color, taste and smell.

I.6.2.2.1. Color

For its color, honey is available in different degrees: white, amber, red, brown and almost black (Eleazu *et al*., 2013; Ndife *et al*., 2014). This characteristic is the first detected by the eye of the consumer whose preferences are based on.The multicoloring of honey varies due to certain internal factors related to its ingredients such as the type of polyphenols, the presence, quality and quantity of mineral salts and certain components like HMF, produced when the honey is exposed to high heat or sunlight or during the ageing of the honey, and other external factors such as contaminants of which dust is a good example as well as the storage time (González-Miret *et al.*, 2007), the types of plants from which the nectar is extracted also, black seed honey is dark, while citrus honey is light yellow and sometimes transparent. This difference in color is mainly due to the difference in the plant source, i.e. the source of the nectar. Differences between ecological regions and altitudes also influence the color of honey of the same floral type; in places above sea level, honey colors tend to be

lighter and more transparent. The quality and type of beeswax used is also an influencing factor (Da silva *et al*., 2016; Szabó *et al*., 2016).

I.6.2.2.2. Taste, flavor, aroma and smell

Once in the mouth, the senses are awakened and detectable on the palate. Among the five senses, **taste** is the only one that appeals to the other four senses through the encounter with food, and it allows us to distinguish the major families of **savors**: sweet, salty, sour and bitter. Other sensations can be added to it such as: astringent, spicy, pungent, metallic and umami. Detectable in the mouth, or by its intervention in the nostrils (aroma), as well as by the nose with regard to the smell. The aroma of honey is one of its most typical characteristics, and consumers often determine their choice according to this characteristic.The so-called volatile composition in honey is responsible for the aroma and the odor originating mainly from plants, of whichdistinct flavors and aromas have been found among different types of honey due to their floral origin (Ruisinger and Schieberle, 2012).

I.6.2.3. Analysis by electronic tongue

The electronic tongue is a matrix equipped with sensors and pattern recognition software that attempts to mimic the human sense of taste to classify products. This is a recent technique cited by Ulloa *et al.* (2013) to distinguish honey samples in conjunction with optical spectroscopy, ultraviolet visible and near infrared spectroscopy (UV-VIS-NIR), and statistical analysis methods, which are supposed to replace sensory analysis. According to the same authors, the combination of these techniques with the multidirectional principal component analysis allows a correct classification of the samples.

I.6.3. Research of the basic quality of honey (legislation on physico-chemical parameters)

The quality criteria of honey are defined by the Codex Alimentarius, (2001) which is an international reference that has been used as a basis for the elaboration of more specific standards at national level. It defines, among other things, the sensory and physicochemical properties of honey, as well as the minimum or maximum quantity related to the parameters of maturity, purity and alteration of honeys. The following table presents the compositional characteristics and quality parameters of honey, according to the Codex Alimentarius.

Table 1: Compositional characteristics and quality parameters of honey, according to Codex Alimentarius (2001)

Sugar content	
Fructose and glucose content (sum of the two)	
Flower honey	≥60 g/100 g
Honeydew honey, mixtures of honeydew honey and flower honey	≥ 45 g/100 g
Saccharose content	
In general	≤5 g/100 g
False acacia (*Robinia pseudoacacia*), Luzerne (*Medicago sativa*), Banksia of Menzies (*Banksia menziesii*), Sulla (*Hedysarum*), Red *Eucalyptus* (*Eucalyptus camaldulensis*), *Eucryphia lucida, Eucryphia milliganii, Citrus pp.*	≤10 g/100 g
Lavender "*Lavandula spp.*", borage "*Borago officinalis*".	≤15 g/100 g
Water content	
In general	≤20%
Calluna heather honey and honey for general industrial use	≤23%
Calluna vulgaris heather honey for industrial use	≤25%
Water insoluble solids content	
In general	≤0.1 g/100 g
Pressed honey	≤0.5 g/100 g
Electrical conductivity	
Honey not listed in the two paragraphs below, and mixtures of such honeys	<0.800 mS/cm
Honeydew honey and chestnut honey, and mixtures thereof, except with the honeys listed Below	≥0.800 mS/cm
Exceptions: *Arbutus unedo,* argan tree*"Erica", Eucalyptus,* lime tree *"Tilia* spp.*",* heather , *Calluna vulgaris,* manuka or agar "*Leptospermum*", tea tree *Melaleuca* spp.	
Free acids	
In general	≤50meq/kg
Honey for industrial use	≤80meq/ kg
Diastatic index (Schade scale)	
In general, with the exception of honey for industrial use	Not less than 8
Honeys with low natural enzyme content (e.g. citrus honeys) and an HMF content not exceeding 15 mg/kg.	Not less than 3
HMF	
In general, with the exception of honey for industrial use	≤40 mg/kg
Honey of declared origin from regions with a tropical climate and blends of such honeys	≤80 mg/kg.

The physico-chemical analysis of honey aims to determine the presence or quantity of certain chemical compounds or physical properties of honey that are useful mainly for the recognition of its quality and degree of freshness. These compounds also complete the process of recognition of the botanical origin of honey, because some physical or chemical characteristics are specific to certain types of honey.

I.6.3.1. Water content and activity

The water content of honey is defined, according to Bogdanov and Martin (2002), as a quality criterion that determines the capacity of honey to remain stable and to resist alterations due to fermentation by yeasts. Since honey is hygroscopic, it absorbs moisture from the environment, and therefore the storage conditions and treatment of the product must be adequate to avoid this problem. Indeed, excess water accumulates in the upper layers of honey, causing the formation of foam, an acidic smell and a characteristic taste. On the other hand, yeasts of the genus *Torulopsis* cause fermentation which is manifested, for example, by the escape of honey from its packaging (Wilde, 2013). Prabucki, (1998) states that the susceptibility of honey to the development of microorganisms increases in samples with water content higher than 17%.

This parameter is measured in honey by refractometry, sometimes a percentage is read directly on the instrument or via the refractive index value. The requirements of the European countries are based on the regulation that the water content of honey should not exceed 20 g/100 g, except in some cases where the content can reach 23% and sometimes 25% (Puścion-Jakubik *et al.*, 2020). This exception is well applied for honeys of tropical origin as well.

I.6.3.2. Ash and mineral salts

The ash and mineral content is a useful parameter. These two elements are more abundant in darker honeys. The minerals in honey come almost exclusively from the nectar, they react with the organic matter present to form brown compounds, so the higher the amount of minerals in the honey, the darker it will be.

Minerals, amino acids and organic acids, which are minor compounds of honeys, present in bee honeys, form ionic forms in aqueous honey solutions, which consequently affect the conduction of electric current and the measurable parameter called electric conductivity (Prabucki, 1998; Puscion-Jakubik *et al*., 2020). Minerals, after the combustion of the honey are transformed into ashes under a temperature of oven between 350-400 C°, during at least 1 h, repeated until obtaining a constant weight. Its quantity is expressed in g/100 g, and its determination is useful to evaluate the type of honey (IHC, 2009).

I.6.3.3. Acidity

The acidity of honey is a criterion that contributes greatly to its characteristic taste and may be responsible for its antiseptic properties and stability against microbial growth. There

are three types of acidity in honey: free acidity, lactonic and total acidity, the latter being the sum of the other two. Honey contains free organic acids and lactones, which give rise to the corresponding acids when the honey is alkalinised, thus constituting a potential acidity reserve. Free acidity is an important parameter related to honey spoilage. It is characterized by the presence of organic acids in equilibrium with lactones, internal esters and some inorganic ions such as phosphates, sulphates and chlorides (Da Silva *et al.*, 2016). Codex Alimentarius (2001) allows a maximum value of 50 meq/kg for free acidity. Higher values may indicate fermentation of sugars into organic acids. However, the presence of other organic acids, geographical origin and time of harvest can also affect the acidity of honeys (Codex Alimentarius, 2001; Da Silva *et al.*, 2016).

The pH is the measure of the coefficient characterizing the acidity or basicity of a medium, it represents the concentration of H+ ions in a solution. Honey is considered a buffer, i.e. its pH does not change with the addition of small amounts of acids and bases. The buffering capacity is due to the content of phosphates, carbonates and other mineral salts. Most honeys have a relatively acidic pH, ranging from 3.5 to 4.5 for nectar ones and from 4.5 to 5.5 for honeydew ones, with a few exceptions (Bogdanov, 2016).

I.6.3.4. Freshness (HMF content)

Hydroxymethylfurfural is a cyclic aldehyde formed in honey by the spontaneous dehydration of sugars, especially fructose and glucose, in an acidic medium. Freshly extracted honey with good handling practices contains low percentages of this aldehyde (0-7 mg/kg), thus, its concentration increases with prolonged storage or under inadequate conditions, by excessive heat treatment, and this is even more pronounced the more acidic the honey is. The legislation establishes that the values of this parameter should not exceed 40 mg/kg, and a maximum of 80 mg/kg for honey from tropical climates and their mixtures (Codex Alimentarius, 2001).

Spetrophotometric methods can be used for this measurement (White method and Winkler method). Others are based on the measurement of 5-(hydroxymethyl-) furan-2-carbaldehyde in honey by reversed phase HPLC and UV detection (IHC, 2009).

I.6.3.5. Diastase enzyme content

Diastase is one of the main enzymes present in honey, mainly produced by the bee, although pollen and nectar also provide small amounts of this enzyme. Its activity is a well-used criterion for assessing product quality, as it is used as an indicator of loss of freshness,

due to both aging and excessive heating during processing (Thrasyvoulou, 1986). Noting that fresh unprocessed honeys from different floral sources can show large variations in their diastasic activity, these variations are related to differences in pH between honeys, the amount and collection period of nectar processed by foraging bees, as well as the physiological state of the colony (Juan-Borrás, 2015).

Legislation provides for a minimum of 8 ID (diastase index according to the Schade scale) for diastase activity, except for honeys with low enzyme content, which may have a minimum value of 3 ID, provided that the value of hydroxymethylfurfural does not exceed 15 mg/kg (Codex Alimentarius, 2001).

I.6.3.6. Estimation of sugars

Sugars are the main constituents of honey produced by enzymatic hydrolysis of sucrose and transglycosylation and are responsible for several of its qualities, such as viscosity, thermal properties, taste, tendency to granulate, hygroscopicity, rotatory power, etc. (Da Silva *et al*., 2016). May be one of the key factors to establish the botanical origin and, indirectly, to allow an adequate classification (Ruiz-Matute *et al.*, 2007).

The content of fructose, glucose, sucrose (Sacharose), as well as maltose, turanose, erlose, raffinose, melezitose and isomaltose is determined by high performance liquid chromatography (HPLC) with infrared (IR) detection, whose peaks are identified according to the standards used. Their retention times and heights and the results are presented in g/100 g (IHC, 2009), of which the total glucose and fructose content must not be less than 60 g/100 g for nectar honeys, while for honeydew and honeydew nectar it must not be less than 45 g/100 g.

The sucrose content should not be higher than 5 g/100g, except for some known exceptions where it can reach 15g/100g. In addition, the amount of sucrose is a very important parameter for assessing the ripeness and/or adulteration of honeys. A high level of this sugar can indicate: adulteration/fraud (by adding sweeteners or syrups), early harvest (because sucrose has not been completely transformed into glucose and fructose), artificial feeding (with sucrose syrups) (Escuredo *et al*., 2013). The fructose/glucose ratio of honeydew honeys is normally higher than that of nectarhoneys. In addition, melezitose is a sugar that is exclusively found in honeydew honey (Wilde, 2013).

A less frequently used but still reliable method is the titration technique in which methylene blue is used as an internal indicator. The difference in sugar concentration before and after inversion, multiplied by a factor of 0.95, gives the sucrose content (IHC, 2009).

Other methods considered more sophisticated for the estimation of sugars in honeys will be presented later in this chapter.

I.6.4. Other methods

I.6.4.1. Supplementary methods

In addition to the mentioned conventional methods, confirming the basic quality of the honey, and in order to go further in the research of the different quality vectors as well as the authenticity and the traceability of the honey, the researchers use for some parameters additional or sometimes more sophisticated methods in order to confirm their results or to give them more reliability, so the identification of the biomolecules can play the roles sometimes of markers of certain criteria by introducing the "omics" approaches.

I.6.4.1.1. Honeys differentiation

In order to differentiate flower honeys from honeydew honeys, characteristics derived from conventional basic quality parameters can guide researchers, but some authors confirm the differentiation by determining the specific optical rotation of honey of which nectar honeys are generally with negative values, while honeydew honeys are with positive values (Wilde, 2013). The specific optical rotation, [α]D20 is the angle of rotation of polarized light at the sodium D-line wavelength at 20°C of a 1 dm deep aqueous solution containing 1g/ml of the substance(IHC, 2009).

Some attempts have been used to distinguish honey varieties such as the CIE (Commission Internationale d'Eclairage) light scale. The advantage of the proposed method is the small sample size (about 2 g) and the lack of destructive effect (samples can be reused in further analyses).

Principal component analyses (PCA) and hierarchical cluster analysis (HCA) were used to distinguish between the different varieties and classifications of honey. Other methods can be used such as the spectrophotometric method of Pfund value indicating the degree of color which can differentiate the varieties of honeys as well as their geographical origin. The last two colorimetric methods are a good complement to optical sensory analysis.

I.6.4.1.2. Evolution of the composition of honey

For the assessment of ripeness, a multi-physicochemical parameter method combined with chemometric analysis was adopted on honey samples. During the ripening process, honey changes its chemical composition, ripens at a moisture content below 18% and at a sugar concentration above the saturation point, and is enclosed in honeycomb cells.

The main differentiating variables revealed by analysis of variance were total sugar content (fructose, glucose and sucrose), total protein content and total phenolic content. PCA, CA and orthogonal partial least squares discriminant analysis (OPLS-DA) were used for classification (Huang *et al*., 2019).

I.6.4.1.3. Freshness

For its freshness the parameter of HMF measurement can be carried out by an alternative method proposed in 2013 by Hoštalková *et al.*, by the use of high performance thin layer chromatography (HPTLC) and reflectoquant spectrophotometric assay, both of which techniques allow for a short analysis time (2.5 min) and the deviation between the methods was 15%. The HPTLC method was characterized by a higher accuracy. The advantage of both techniques is the absence of harmful reagents, which is in line with the principles of green chemistry. The HPTLC method is characterized by greater precision. The advantage of both techniques is the absence of harmful reagents, which is in line with the principles of green chemistry.

An alternative method to determine the diastasic activity of honey bee was developed by Sak-Bosnar and Sakac (2012), using a platinum redox sensor and based on the potentiometric measurement of free triiodide, which is released from the triiodide-starch complex during degradation. Comparing the new rapid measurement technique with the two methods of Schade and the method based on incubation with Phadebas tablets, the analysis time of a sample in the proposed technique is only 5 min, and thus much shorter. A paper on the determination of the number of diastases by near infrared and visible spectroscopy (VIS/NIR) was already published by Huang *et al*. (2019).

I.6.4.1.4. Carbohydrate composition

For its carbohydrate composition, more methods are recommended to determine the sugar content of bee honey, such as: HPLC, then HPAEC with pulsed amperometric detection which is based on the principle that at high pH levels, sugars behave like very weak acids,

they are totally or partially ionized, and can therefore be separated using the ion exchange mechanism (IHC, 2009). Gas chromatography (GC), where the sugars are silylated, and then the derived fraction is quantified. Mannitol is used as an internal standard. Brazilian scientists have proposed a new method described as a rapid capillary electrophoresis method for the determination of carbohydrates in honey samples to determine the fructose, glucose and sucrose content of bee honey samples.

The advantages of this method are the high resolution, the short sample preparation time, the small sample size and the short duration of the analysis itself. Within two minutes, the three tested compounds were completely separated, which guarantees repeatability and linearity (Rizelio *et al.,* 2012).

Another method based on Raman spectroscopy followed by advanced statistical techniques of PLS, PCA and ANN for the analysis of glucose, fructose, sucrose and, additionally, maltose content was proposed by Turkish researchers. A high correlation coefficient was obtained between the determined values and those predicted by the models (Ozbalci *et al*., 2013).

For the quantification of saccharides (monosaccharides and disaccharides) in honey samples, laser-assisted desorption mass spectrometry using HgTe nanostructures as matrix (SALDI-MS) was used. Sucralose is used as a standard. The authors point out that this method does not require tedious sample preparation and that the analysis time is only 30 minutes. Furthermore, it is characterized by its high repeatability (Wang *et al*., 2014). The HPAEC-PAD (high performance anion exchange chromatography/pulsed amperometric detection) method is the most recently used.

I.6.4.1.5. Detection of contaminants

The impurities present in the bee honey can penetrate in the final product. Due to the prevalence of diseases causing mass extinction of bees, techniques for detecting undesirable residues of various compounds in natural bee honey are gaining popularity. Among the xenobiotics, sulfonamide residues must be mentioned. The presence of these substances in bee products may present a risk to consumers (Genersch *et al*., 2010).

Short C-18 column extraction by high performance liquid chromatography with fluorescence detection was used to detect sulphonamides, high resolution mass spectrometry was also used to detect xenobiotics and statistical tools useful for metabolomic techniques

(Sajid *et al*., 2013). Electrothermal atomic absorption spectrometry (ET-AAS) technology has also been used to determine cadmium and chromium in water. Thus, the determination of the concentration of these elements in honey may be useful as a bioindicator of environmental contamination.

I.6.4.1.6. Detection of adulteration

In order to detect adulteration of honey, as well as any type of addition and/or falsification of origin, and in addition to the basic characterisation methods, other techniques can also be used such as: High performance thin layer chromatography with image analysis can be used, a technique which has allowed the authors to analyze the basic sugars present in honey: fructose, glucose and sucrose.

More recent methods allow the detailed examination of honey composition, including the content of macroelements, microelements and toxic elements. The capillary electrophoresis method with UV detection used by the authors allowed the determination of certain metal cations present in bee honeys and affecting the quality of bee honey (Puscas *et al*., 2013).

I.6.4.2. Analysis by "Omics" technologies

Recent advances in omics technologies for the study of bee products have enabled easier, faster and more reliable determination of the botanical and geographical origin of honeys. The cascade of omics technologies in modern biology is "genomics, transcriptomics, proteomics and metabolomics in addition to lipidomics" as well as combined approaches, as each of the mentioned research areas represents a level of system study.

I.6.4.2.1. Genomic analysis in honey

Genomics is a multidisciplinary field of modern biology that studies all the genes in an organism, organ or tissue. In contrast to genetics, which studies specific genes and their inheritance, genomics is concerned with the characterization and quantification of all genes that are responsible for the production of proteins in an organism (Chial, 2008).Various authors have proposed the use of genomic tools such as DNA barcoding as an alternative to melissopalynology (Valentini *et al*., 2010).

According to Hawkins *et al.* (2015) and as honey contains traces of DNA from the bee and plant of origin, DNA-based markers are recognized as very effective and accurate identification methods. For example, sequences based on the Internal Transcriber Spacer

(ITS2), rbcL and COI genes have been used to determine the origin of honey (botanical and bee species) (Richardson *et al*., 2015).These barcoding approaches can be followed by Sanger sequencing according to several authors, to identify plant species from the pollen DNA contained in honey, then and in order to distinguish honey from different floral and geographical origins and, therefore, to determine its botanical signature, the cited approach was developed using NGS to accelerate the generation of sequence data that were analyzed using specially designed bioinformatics pipelines (Bruni *et al*, 2015; Prosser *et al*., 2016; Utzeri *et al*., 2018; Kafantaris *et al*., 2021).

The MRJP2 (Major royal jelly protein 2) gene was also used to distinguish two types of honey: honey produced by *Apis mellifera* and honey produced by *Apis cerana*. In order to correctly identify them, the authors designed two pairs of species-specific primers. The amplification products of *A. mellifera* and *A. carena* honeys were 560 and 212 base pairs (bp) respectively.The primers obtained were characterized by high species specificity. The MRJP2 gene was detected using the PCR method and the selected primers. The differences in this gene allowed the origin of the honey to be established. The PCR method was able to detect the addition of *A. mellifera* honey which was only 1% (Zhang *et al*., 2019). Thus several other methods have been adopted and more are in progress.

Transcriptomics, which is the second step of the omics approach and is defined as the study of all elements resulting from the transcription of DNA into ribonucleic acid (RNA) (transcriptome), has also been used to study the antimicrobial activity of honey (Kafantaris *et al*., 2021).

I.6.4.2.2. Proteomic approaches analyses

Proteomic is the study of all the elements resulting from the translation of messenger RNAs (the proteome), the aim of which is to describe and quantify the expression of proteins and their modifications under the influence of biological perturbations (Wasinger *et al*., 1995). Proteomic analyses have been considered as a complementary tool in the search for authenticity of honeys. According to Chua *et al*. (2015), the honey proteome is poorly studied, mainly due to the low amount of protein present in honey (0.1-0.5%), which makes protein extraction difficult (Kafantaris *et al*., 2021). Despite this, proteins in honey are widely used as markers of honey authenticity and adulteration, as well as quality indicators (Won *et al*., 2008; Chua *et al*., 2013; Bilikova *et al*., 2015).

The research topic of Rossano *et al.* (2012) was the study of the presence of proteolytic activities in commercial unifloral honeys from Italy, produced in two different geographical areas for each type, by creating a specific fingerprint by two-dimensional zymography, which is a technique that allows the detection of the whole proteolytic network present in a biological sample. Since honey proteins can originate either from nectar, pollen grains or bee gland secretions, and the most important ones in honey are nine major types of royal jelly proteins, two-dimensional zymography is considered an important aspect when proteins are used as chemical markers of the geographical and floral origins of honeys (Marshall and Williams, 1987; Bilikova and Simuth, 2010), which was confirmed also by Rossano *et al*. (2012).

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) as well as MALDI-Ms were used for the identification and quantification of honey proteins as well as for their floral and geographical traceability (Di Girolamo *et al*., 2012; Azevedo *et al*., 2017).

I.6.4.2.3. Metabolomic approaches analyses

Metabolomic is the science that studies the metabolome of an organism under given biological conditions, identifies and quantifies it. It refers to compounds involved in metabolism as substrates, products or effectors, which are considered to be any organic compound not resulting directly from gene expression, including amino acids, sugars, organic acids, fatty acids, nucleotides, conjugated metabolites, vitamins, steroids, etc. (Wishart, 2008). Xenometabolites and xenobiotics are also included, while polymerised structures such as proteins or nucleic acids are excluded.

The food metabolome therefore consists of a variety of different components from a large number of chemical classes. Moreover, among the different compounds included in the food metabolome, the quantitative differences are even more important. Some compounds are very common and abundant, and can be found in millimolar concentrations (e.g. sugars). Others exist in very small quantities, such as vitamins, and may be present at concentrations as low as femtomolar. However, this wide range of concentrations implies a significant analytical challenge, as the characterization of some of the components of food metabolites could interfere with the correct characterization of compounds present in much lower amounts. In view of these difficulties, two main different but complementary approaches are used to characterize the food metabolome. The first is based on **profiling** (non-targeted

methods); this approach depends on prior knowledge of the sample to be analyzed and focuses on the analysis of a group of related metabolites, whose relationship is often based on chemical classes.

The other approach, commonly called **fingerprinting** (targeted methods), focuses on comparing metabolite profiles between samples. The subsequent statistical processing of the results obtained can ideally allow different samples to be grouped according to their nature, treatment or any other desired characteristic.Therefore, the main objective of fingerprinting is not to identify all the compounds involved, but to establish patterns between them.

Profiling and fingerprinting can be used alone or in combination, as they can offer complementary information. With regard to the objective of this chapter, both approaches are used in different applications concerning food safety, quality and traceability (Herrero *et al.*, 2012). Regardless of the type of metabolite studied, two approaches can be used:

The first is a **targeted approach**, which aims to detect and, more importantly, to quantify in absolute terms a limited number of predetermined metabolites, i.e. known molecules, and then to perform customized extractions and analyses specifically targeting this class of molecules. This can be used to test a hypothesis or to explore a particular metabolic pathway, for example, the metabolism of lipids in a specific biological state (Hollywood *et al*., 2006).

The second approach is called **non-targeted** that aims to obtain the most complete metabolic profile possible. It is also called the **global approach**. It is an enumeration of known or unknown molecules present in a sample, with the aim of quickly identifying a maximum number of metabolites (Lv, 2012), for certain reasons depending on the case. This approach often involves the use of different analytical systems to detect as many metabolites as possible.

In the field of food science and plant-based products, according to Esteki *et al*. (2018), metabolomics can be used to assess/confirm the quality, safety and traceability of products, as well as the factors that influence their composition (such as genetic origin, environment and final product development processes). Honey is a good example of how this approach can be applied to investigate or confirm its authenticity, regardless of its botanical or geographical origin.

Among these different "-omics" sciences, the present research will focus on the metabolomic approach, in particular the non-targeted approach to the identification of volatile compoundsof various chemical classes in the honey samples.

Metabolomics began as an analysis using gas chromatography coupled with mass spectrometry. Soon after, the idea of using metabolic profiling in science began to spread and the use of nuclear magnetic resonance (NMR) took precedence over mass spectrometry for metabolic profiling. It was not until the late 1990s, with the advent of sequencing and functional genomics that the concept of metabolomics took off (Nicholson *et al.*, 1999).

Other methods were then considered, such as infrared spectroscopy or 2D thin layer chromatography. Thus, given the complexity of the samples studied, metabolite detection techniques were often coupled with a separative technique (UV, laser-induced fluorescence or mass spectrometry). Currently, published scientific studies in metabolomics mainly use two techniques for the detection of metabolites: nuclear magnetic resonance and mass spectrometry (Figure 5). The latter, often coupled with a chromatographic technique, has become the majority in recent years (Gowda and Raftery, 2017).

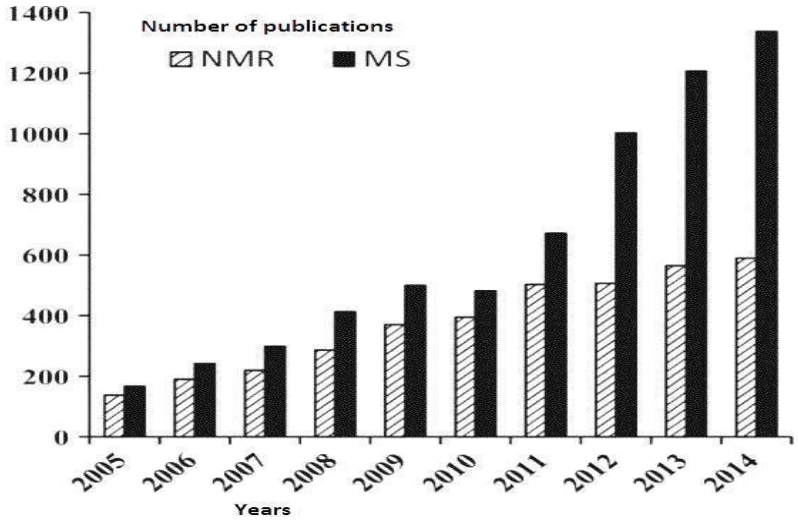


Figure 5: Number of publications on metabolomics involving either nuclear magnetic resonance spectroscopy or mass spectrometry (Gowda and Raftery, 2017).

A. Nuclear magnetic resonance (NMR)

As mentioned earlier, NMR was one of the first technologies used for metabolome analysis, with 1H NMR being the most widely used.

It is a technology based on the physical properties of certain atoms that have a non-zero magnetic moment. Thus, in a strong magnetic field, the spins involved will align themselves parallel to the magnetic field and by applying a radio frequency pulse, the spins will tilt perpendicular to the magnetic field. After excitation, the system returns to equilibrium by inducing a radio frequency field which is detected and recorded. The frequency will be converted and recorded into a spectrum which can then be interpreted.

This innovative high-throughput technique allows the simultaneous detection (screening) of a large number of parameters for a rapid and comprehensive control of honey authenticity and has been successfully used to discriminate and classify honey from different floral sources and geographical origins (Beretta *et al*., 2008; Consonni and Cagliani, 2008).

The coupling of this technique with chemometric analysis has successfully allowed the distinction of honeys of different botanical origins. Similarly, the application of chemometric methods to 1H-NMR profiling has allowed the distinction of honeys of diverse geographical origin, produced in Greece, Brazil, South Africa, Zambia and Slovakia (Karabagias *et al*., 2018). In addition and according to Luong *et al*. (2019), NMR metabolomic fingerprinting was implemented to characterise and classify Vietnamese honey samples according to their origin and quality.

B. Mass spectrometry (MS)

A technique used to measure the mass-to-charge ratio (m/z) of different chemical species. To do this, the molecular compounds in the biological sample must be ionized by an ionization source, which vaporizes the sample on arrival at the spectrometer. An analyzer that separates the different ions formed at the source is also installed in addition to a signal processing system that converts the recorded signals into m/z ratios.

The present work as already mentioned above will focus on the non-targeted metabolomic approach and more precisely the (GC/MS), notably because of its importance in the study of the botanical origin of honeys. As known, monofloral honey is produced from nectar originating entirely or mainly from a single species or plant and is therefore provided

with specific volatiles (Jerković and Marijanović, 2010). Therefore, volatile compounds could be used to distinguish monofloral honeys of different floral origins, as their high number gives a distinct profile that could represent the fingerprint of each honey type.

B.1. Gas chromatography-mass spectrometry (MS) for the detection of the volatile fraction of honey

More than 600 volatile compounds have been identified in honey, belonging to different biosynthetic pathways. In particular, monofloral honeys have been studied in search of a common volatile fingerprint that facilitates the distinction of one type of honey from another (Da Costa *et al.*, 2018). Due to the low concentration of these volatile compounds, it is necessary to remove sugars that are the main components of honey before isolating the volatiles for analysis.

B.1.1. Volatile compounds extraction

Several techniques are used for the isolation of volatile compounds from honey **prior** to their analysis by Gas Chromatography-Mass Spectrometry (GC/MS) or sometimes by Gas Chromatography with Flame Ionization Detection (GC/FID), all of which have various advantages and disadvantages. It has been found that the composition of honey volatiles shows a large variability depending on the extraction techniques, since the volatility and polarity of each compound significantly affect the percentage of recovery (Jerković *et al.*, 2015).

B.1.1.1. Humidity and heat based extraction procedures

Some of these methods, e.g. hydrodistillation (HD), liquid-liquid extraction (LLE), simultaneous steam distillation extraction (SDE) or Likens-Nickerson simultaneous distillation extraction (LNSDE) and micro-simultaneous steam and solvent distillation extraction (MSDE), use heat. However, the heat treatment used in the mentioned methods can lead to the formation of furan and pyran derivatives due to the effect of heat on sugars or amino acids (non-enzymatic browning reaction/Maillard reaction) (Cuevas-Glory *et al.*, 2007). This alters the sensitive compounds and facilitates their oxidation and decomposition, leading to the appearance of new compounds that do not belong to the honey flavor.

B.1.1.2. Solvent-based extraction procedures

The use of solvent extraction methods for volatile compounds in honeys, such as the ultrasonic extraction technique (USE), does not require heat and also allows the isolation of

low and high molecular weight compounds, which can provide interesting markers for honey origin determination. However, the use of solvents and the above techniques can also affect the extraction of volatiles and analysis by the gas chromatograph, as solvents can solubilize non-volatile compounds, thus impairing the operation of the gas chromatograph by contamination. In addition, some volatiles may be lost during solvent extraction and some analytes may be masked by the solvent, preventing their detection (Manyi-Loh *et al*., 2011; Soares *et al*., 2017).

B.1.1. 3. Other techniques for extracting volatile compound from honey

In addition to the techniques already mentioned, other methods have been proposed to minimize the consumption of organic solvents and reduce the amount and time of sample preparation. Dynamic Solid Phase Extraction (DSE) is one example that can completely eliminate solvents, although it requires some modification of gas chromatography.

Another method considered the most popular and appropriate technique for the analysis of volatile compounds in different food matrices, including honey, is headspace solid phase microextraction (HS-SPME). It has some advantages over the methods already mentioned, as it protects, according to Cuevas-Glory *et al*. (2007), the fiber from undesirable effects caused by non-volatile compounds present in the sample matrix (such as sugars in the case of honey) and allows the pH of the sample to be modified without any effect on the fiber. This method has been widely used as it offers good sensitivity and selectivity for the determination of non-polar to medium polar volatile compounds, including aromas. According to some authors, it is considered that the composition of volatile compounds may vary depending on the chosen extraction method, so that the application of separate techniques may be advisable in order to obtain more relevant and complete results (Ouradi *et al*., 2020). Regardless of the extraction method, injection is performed by the appropriate tool into the GC/MS device pending mass spectrometric identification.

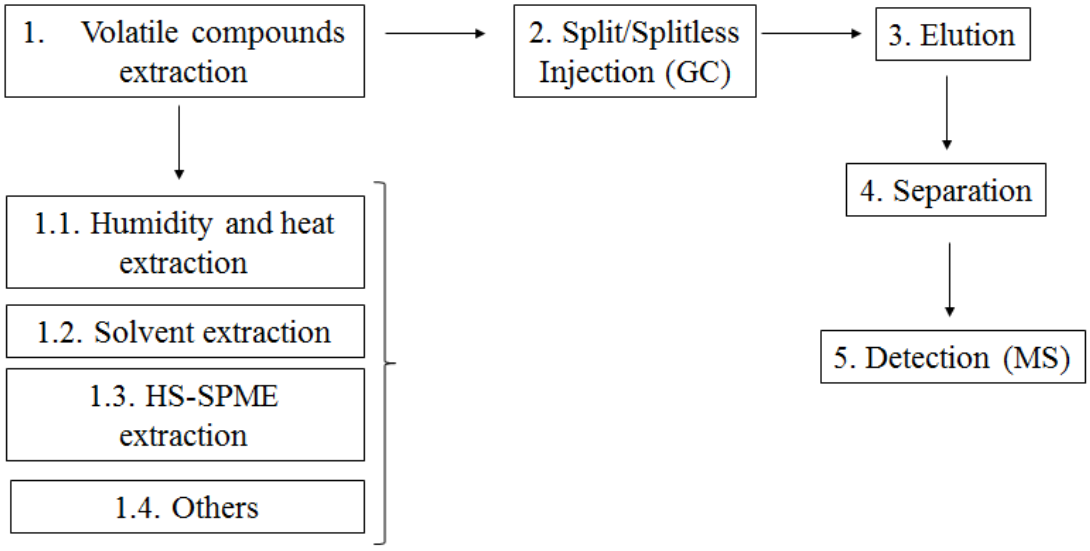


Figure 06: Different methods of extraction of volatile compounds and injection into the GC-MS

I.7. Research on the biological quality of honey

Due to the side effects of pharmaceutical drugs, people are still turning in some cases to natural and quality products with both nutritional and health benefits in recent decades to solve their health problems, largely due to the increased perception of the importance of wellness in human life, especially after the COVID-19 pandemic.

Among these unprocessed products, honey is one of the most widely used in this field, due to its history as a health food and the therapeutic effects attributed to it (Meo *et al*., 2017; Khan *et al*., 2018; Cucu *et al*., 2021).

The use of honey as a nutritional food and medicine has been reported since ancient times. However, despite its long history of use as an alternative medicine, modern science has not always recognized it as a therapeutic agent. Its biological properties and the mechanisms behind its beneficial health attributes are still not clearly understood, which is why currently more research is being conducted on the medicinal use of honey. Honey has been associated with improved antioxidant capacity, antimicrobial activities, regulation of glycaemic responses, modulation of the immune system, influence on lipid values (through antihypercholesterolemic effects) and others (Khan *et al*., 2018).

I.7.1. Antioxidant activity

Several studies have shown that honey consumption can improve defences against oxidative stress (Kuś *et al*., 2014; Al-Farsi *et al*., 2018; Nakib *et al*., 2021). This property is related to the inhibition of free radicals that are responsible for oxidative reactions within the human body and can damage cells and cause various disorders (Tong *et al.*, 2015; Cucu *et al*., 2021). This function has been attributed mainly to natural phenolic compounds (e.g. flavonoids) in honey, but also to other components such as organic acids, amino acids, carotenoids, proteins, products of the Maillard reaction or certain enzymes (glucose oxidase, catalase.Thus, according to current knowledge, honey, due to its high antioxidant content, can protect the human body against the harmful effects of free radicals and thus contribute to the prevention of certain chronic diseases (cancer, cardiovascular diseases, diabetes, obesity, etc.) (Cucu *et al*., 2021).

I.7.2. Antimicrobial activity

Considering that some microbial strains are pathogenic to the human organism and resistant to different drug treatments, the valorisation of honey as a therapeutic substance has been addressed in many scientific researches for its possible use in alternative medicine in this case (Haderbache *et al*., 2020). Thus, the low water activity and the presence of organic acids

and enzymatic substances in honey play an important role in inhibiting the growth of pathogenic microorganisms, making it a microbiologically safe product.

The factors influencing this effect are mainly the botanical source of the plants, the metabolism of the bees, the species of bees and the climatic, processing and storage conditions to which the honey is subjected (Cucu *et al*., 2021).

I.7.2.1. Antibacterial activity

Bacterial inhibition processes in honeys are associated with certain actions, namely H_2O_2 formed during honey ripening, whose main function is to prevent alteration of honey caused by the action of micro-organisms by producing minimal inhibitory concentrations in the bacteria and thus damaging its cell walls, this component thus constituting a good defensive barrier against bacteria in the exogenous environment. The literature reports that phenolic compounds have a well-structured antibacterial mechanism, as they are able to decrease the antibiotic resistance of infectious bacteria and prevent the formation of biofilms.

A recent study, revealed how vitamin C supplementation can amplify the antimicrobial potential of honey against a wide range of bacteria. Furthermore, the side effects of using honey as an adjuvant therapy have not yet been investigated in in vivo studies (Wang *et al*., 2018; Majtan *et al*., 2020; Al-Ghamdi *et al*., 2021). Nakib *et al*. (2022) have examined the influence of honey dilution levels on bacterial inhibition of certain Gram-positive and negative strains, of which some honeys at 1/3 and 1/2 concentrations can give strong inhibitions compared to more concentrated honey levels.

I.7.2.2. Antiviral activity

Although the antiviral activity of honey has not been extensively studied, its mechanism of action could be explained by the existence of various compounds (copper, ascorbic acid, flavonoids and H_2O_2) capable of inhibiting viral growth by interrupting viral transcription and replication. The antiviral potential of honey could be linked to specific pathways, including nitric oxide (NO), a molecule that has shown beneficial activities in viral infections by slowing the spread of viral lesions and stopping their replication (Mehta *et al*., 2012).

I.7.2.3. Antifungal activity

Fungi are known to be more pathogenic than bacteria, and their resistance to antifungal drugs is therefore more complex and requires new therapeutic approaches. Several studies

have reported that high sugar content can act as an inhibitor of fungal growth by osmotic pressure. Phenolic compounds play a key role in its antifungal effects.

These compounds have the ability to denature proteins and thus cell membranes by altering their stability. In a recent study, the free acid content was correlated with the highest *Candida* inhibition effect, thus underlining the importance of acids in influencing the antifungal effect of honey (De Groot *et al*., 2021; Nakib *et al*., 2022).

I.7.3. Effect on blood sugar regulation

The term "glycaemic regulation", also known as glycaemic homeostasis, refers to various phenomena leading to the correct regulation of blood glucose levels (i.e. blood sugar), which is one of the mechanisms used by organisms to keep the properties of their internal environment constant, its disruption is described by diabetes mellitus which is defined as a well-known chronic disease, can lead to numerous complications, which manifest in various organs and cause serious health problems (Little *et al.*, 2011).Acarbose is the most widely used treatment for type II diabetes, which has significant side effects manifesting mainly as abdominal distension, diarrhoea, nausea and flatulence (D and Vgm, 2014).

Honey has been so much considered useless for diabetic subjects as it contains a considerable proportion of sugars, while other studies have given interesting results positioning honey as a nutritional supplement substituting white sugars in subjects with glucose homeostasis disorders (Zamanian and Azizi-Soleiman, 2020), based on its significant low glycaemic index compared to other sweet products. Bobiş *et al*. (2018) reported that various studies demonstrate the hypoglycemic effect of honey, but the mechanism of this effect remains unclear. However, more studies are needed to better understand the use of honey to help diabetics. Krishnasree and Ukkuru, (2017) suggested that phytochemicals in some honeys may be responsible for reducing hyperglycaemia through competitive inhibition of the enzyme α-amylase, which is found in the brush border epithelium of the gut that is responsible for starch hydrolysis. It is also said to promote regeneration of the intestinal mucosa, stimulate new tissue growth and act as an anti-inflammatory agent. Honey also improves the skin and may be useful in the treatment of infectious processes such as acne due to its antibacterial properties.

The components of honey that may have a particular relationship with nerve and brain function are choline and acetylcholine, which the bee incorporates into honey during the production process.

I.7.4. Interests of monofloral honeys

The quality and price of honey is determined by its floral and geographical origins, and consumers are more interested in honey with a specific origin label. Monofloral honeys are well suited in this context because of their economic, nutritional, organoleptic and also therapeutic interests.

According to Mărgăoan *et al.* (2021), in recent years, monofloral honey has aroused consumer interest, especially in the medicinal field, due to the presence of phytochemicals from a specific floral origin, directly related to health benefits, wound healing (Sunflower honey), antioxidant (Eucalyptus honey), anticancer and anti-inflammatory activities. Some monofloral honeys are also declared by their moderate glycemic index, which makes them more suitable for diabetics (e.g. *Acacia* honey, *Arbutus* honey, etc.).

In terms of beekeeping, plant diversity is strongly correlated with the production of a wide variety of honey. Therefore, based on the existing plant diversity in each country, multiple varieties of honey are produced with different health characteristics. If the beekeeping potential and consumer preferences are reflected in the variety of products, this leads to an increase in the economy of the region and to important exports.

Finally, research on Algerian monofloral honeys and their characterization for the first time is a good task for the local production, consumer benefit and the economy of the country.

II. Material and methods

The practical analyses of the present work were shared between three sections:

1. Pharmacology and Toxicology Laboratory and Food Quality Control Laboratory, Biotechnology Research Center (CRBt), Constantine, Algeria.
2. Aerobiology and Apiculture Laboratory, Department of Plant Biology and Soil Science, Faculty of Science, University of Vigo, Ourense, Spain.
3. Center for Scientific and Technological Support to Research (CACTI) of the University of Vigo, Spain.

Honey samples were collected directly from beekeepers in Algeria. Their pollen spectra were analyzed under the microscope in order to confirm or orientate towards the botanical origin of each sample.

The number of the analyzed samples, and according to certain criteria, was reduced in order to rely on less known and new honey samples for scientific characterization.

The overall analyses performed are presented in the following figure (Figure 6).

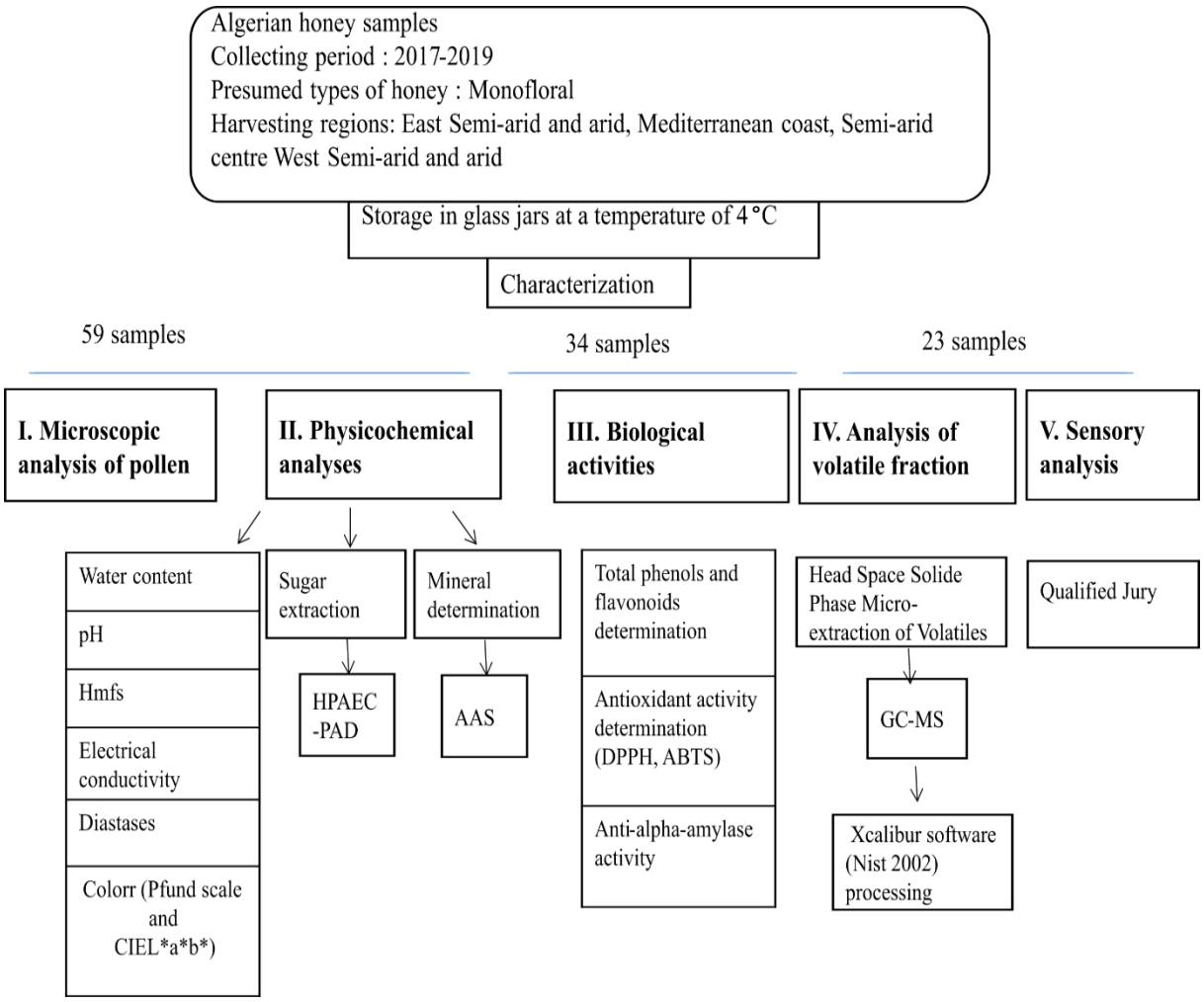


Figure 7: Experimental methodology adopted for the characterization of the studied honeys

II.1. Samples collection

Sampling was carried out during the period (2017-2019), and samples were provided directly by beekeepers in some regions of Algeria (East Semi-arid and arid; Mediterranean coast; Semi-arid centre; West Semi-arid and arid).

For each honey sample, a data sheet was elaborated, with relevant data such as: sample code, geographical origin, beekeeper's name, number of hives, transhumance (yes/no), type of hive (traditional, modern), production period, harvest date, type of extraction (manual/centrifugation), quantity of product, botanical origin (according to the beekeeper), visual color, crystallization (yes/no), odor and other comments.

A total of 59 honey samples were collected, including five monofloral types (Retem, Merkh, Sor, Harra and Eucalyptus) according to the nomenclatures declared by the beekeepers and sometimes labelled, as well as other samples obtained at random. The first four types (Retem, Merkh, Sor and Harra) are considered to be new locally, currently available from Algerian beekeepers and presented at beekeeping events etc., and are less known, especially as they have never been characterized, as is the case for several other types of honey in the world.

The collected honeys considered as those of Retem, were from semi-arid and arid regions (Setif, Laghouat and Biskra), very viscous in appearance, dark in color and with a particularly strong odor. Those of Merkh, considered by beekeepers to be typical of the arid region of Oued souf, characterized by its less viscous appearance and its light greenish and transparent color.

The honeys of Sor, obtained in the semi-arid regions (Naama, Tlemcen and El Bayadh) from western Algeria, had a crystallized appearance under normal conditions, a beige color. Those of Harra, another type that has never been so well characterized, less consumed in Algeria, but recently known for its positive effect on male fertility according to some consumers. It is distinguished by particular rheological characteristics such as its creamy appearance and its light color accentuated by floral and mineral odors.

Eucalyptus honey samples from the east-mediterranean region, considered as a type well known locally, characterized by its viscous aspect and dark color. The rest of the samples are considered as controls or references, they contain various samples such

as bitter honey, Acacia honey as declared by the beekeeper, and other types, whose floral origin of any sample still needs to be confirmed by microscopic study of the pollen as a preliminary step.

The following illustration shows the geographical origins of the samples.

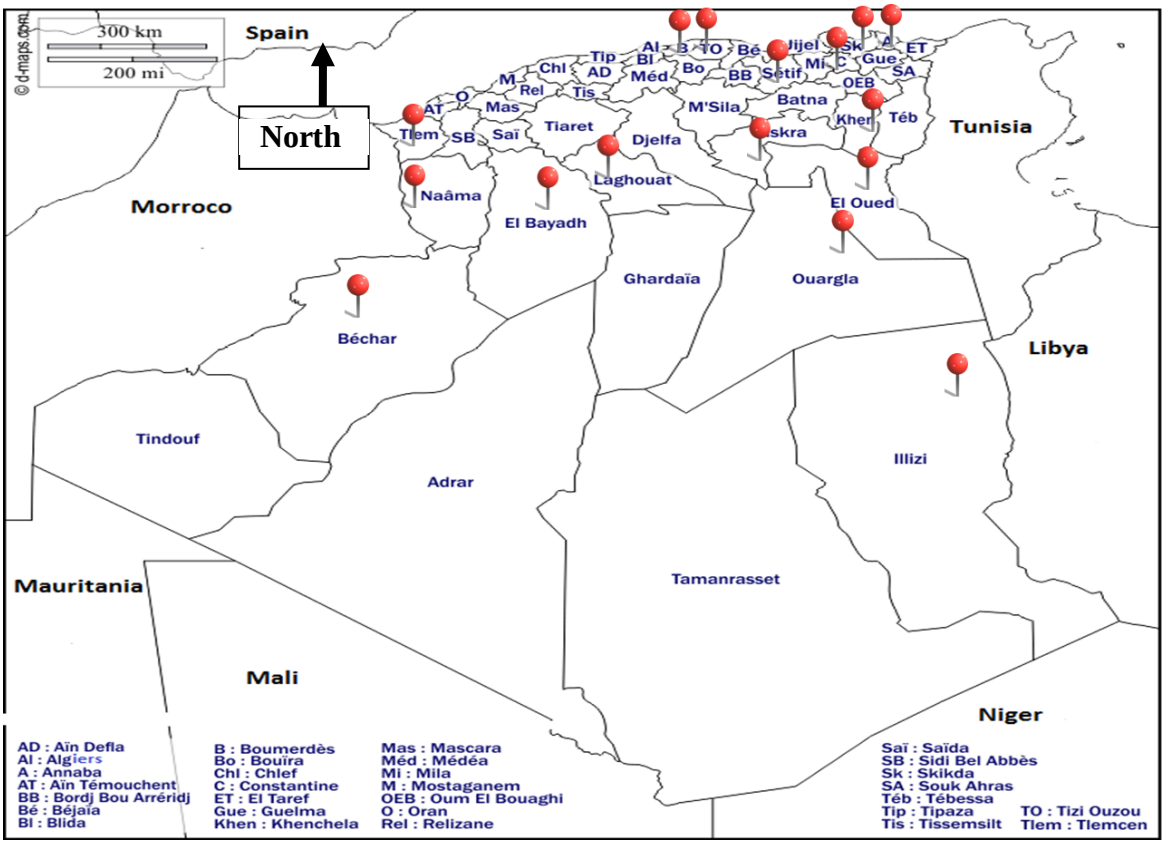


Figure 8: Different points (Wilayas) of honey samples collection

Table 2: Geographical origins of collected honey samples

N	Sample code	Presumed botanical origin	Geographical origin	Collection year
1	R1	Retem	Biskra	2018
2	R2	Retem	Biskra	2018
3	R3	Retem	Biskra	2018
4	R4	Retem	Biskra	2018
5	R5	Retem	Laghouat	2018
6	R6	Retem	Biskra	2018
7	R7	Retem	Setif	2019
8	R8	Retem	Setif	2019
9	R9	Retem	Oued souf	2019
10	R10	Retem	Laghouat	2018
11	R11	Retem	Laghouat	2019
12	M1	Merkh	Oued souf	2018
13	M2	Merkh	Oued souf	2018
14	M3	Merkh	Oued souf	2018
15	M4	Merkh	Ouargla	2018
16	M5	Merkh	Oued souf	2018
17	M6	Merkh	Oued souf	2018
18	M7	Merkh	Oued souf	2018
19	M8	Merkh	Oued souf	2018
20	M9	Merkh	Oued souf	2018
21	M10	Merkh	Oued souf	2018
22	M11	Merkh	Oued souf	2018
23	M12	Merkh	Oued souf	2018
24	M13	Merkh	Oued souf	2019
25	S1	Sorr	El Bayadh	2017
26	S2	Sorr	El Naama	2018
27	S3	Sorr	Tlemcen	2018
28	S4	Sorr	Tlemcen	2018
29	S5	Sorr	Tlemcen	2018
30	S6	Sorr	Tlemcen	2018
31	S7	Sorr	El Naama	2018
32	S8	Sorr	Tlemcen	2018
33	S9	Sorr	Tlemcen	2018
34	S10	Sorr	Naama	2019
35	S11	Sorr	Naama	2019
36	S12	Sorr	Naama	2019
37	S13	Sorr	Naama	2019
38	S14	Sorr	Naama	2019
39	S15	Sorr	Naama	2019
40	S16	Sorr	El bayadh	2019
41	H1	El Harra	Khenchela	2018
42	H2	El Harra	Khenchela	2018
43	H3	El Harra	Illizi	2019
44	H4	El Harra	Bechar	2018
45	H5	El Harra	Bechar	2018

46	E1	Eucalyptus	Blida	2017
47	E2	Eucalyptus	Blida	2017
48	E3	Eucalyptus	Constantine	2018
49	E4	Eucalyptus	Constantine	2018
50	E5	Eucalyptus	Constantine	2018
51	E6	Eucalyptus	Annaba	2018
52	E7	Eucalyptus	Boumerdes	2018
53	E8	Eucalyptus	Tizi ouzou	2018
54	E9	Eucalyptus	Boumerdes	2018
55	E10	Eucalyptus	Skikda	2018
56	X4	Thapsia	Laghouat	2017
57	Am	Lenj	Skikda	2018
58	A	Acacia	Mostaganem	2018
59	R'	Unknown	Biskra	2018

II.2. Characterization of collected samples

Some analyses are essential and fundamental for honey to verify and/or confirm its conformity to the standards. The study of the pollen could serve to confirm the floral and sometimes geographical origin, while several parameters fixed by the International Honey Commission (IHC) as physicochemical or biochemical analyses are applied to confirm the organoleptic and commercial quality of this honey.

II.2.1. Microscopic analyses of pollen

Microscopic analysis of pollen or melissopalynology was performed using the OLYMPUS BX50 tool: a light microscope (400× or 1000×, depending on the case) following the methodology proposed by Louveaux *et al*. (1978), modified by Rodriguez-Flores *et al*. (2019). The objective of this analysis is to reveal the set of pollen types present in the samples, the number of pollen grains per gram of honey (quantitative results) which allows us to calculate the pollen richness, and the pollen spectra of the honey samples (qualitative results), which indicates the types of pollen in each sample apart, in combination with the quantitative and qualitative results, a confirmation/proposal or suggestion of honey type will take place.

Ten grams of honey was mixed with 40 mL of warm distilled water (not above 40 °C) and completely dissolved. The solution was centrifuged for 10 minutes at 4500 rpm and the supernatant was removed. The sediment was dissolved again in distilled water and another centrifugation was performed under the same conditions. The supernatant was again discarded until a volume of 5 mL and then the sediment was vortexed. Using a micropipette, 10 µL of sediment was placed on a slide and spread over an area of approximately 24 mm × 24 mm. The samples were prepared in duplicate. Total number of pollen grains in each drop was counted and the results were expressed as number of pollen grains per g of honey considering the mean value of both drops. At least 500 pollen grains were counted in the two slides.

Once obtained the number of pollen grains present in 10 µL of honey solution that is came from 10 mL of honey solution, the number of pollen grains in 1 g of honey can be calculated as follows:

$$\frac{A.\,10\ mL}{0.01\ mL} = B\ pollen\ grains$$

Where:

A = the number of pollen grains present in 10 μL of honey solution that is came from 10 mL of honey solution.

B = the number of pollen grains present in 10 mL of honey solution containing a given amount of honey.

Therefore, in 1 g of honey, we will have:

$$\frac{B\ pollen\ grains}{X\ g\ of\ honey} = B\ pollen\ grains/\ g\ of\ honey$$

Where:

X = the grams of honey used to make the honey solution.

Once the data in pollen grains per gram of honey was obtained, the samples were grouped according to the classes of pollen grains per gram of honey. The samples were grouped according to Maurizio's classes, already mentioned in bibliographic part.

Regarding the qualitative analyses the obtained sediment for quantitative one was centrifuged again and the supernatant was discarded. After vortexing, two drops (100 μL) of sediment were placed separately on a slide and distributed over an area of about 24 × 24 mm. Examination of pollen slides was performed using the optical microscope. The percentage of representation for each type of pollen was calculated by counting at least 500 pollen grains per sample, we used as reference the pollen library of the bee flora used in the Laboratory of Aerobiology and Apiculture of the Faculty of Science, University of Vigo, as well as different guides and keys for the identification of pollen (Punt, 1976; Moore and Webb, 1978; Punt and Clarke, 1980; Punt and Clarke, 1984; Valdés *et al*., 1987; Punt *et al*., 1988; Punt and Blackmore, 1991). Once the pollen was recognized, it was classified taxonomically into species, genus or type. The latter indicates a related morphology for different genera, mainly within the same genus, different genera, mainly within the same family.

Pollens that definitely correspond to a plant species are named with the genus and species, and the generic name is adopted when the pollen described is common to several species of the same genus.

The nomenclature of Louveaux *et al*. (1978) was used to express the relative abundance of each pollen type in honey, grouping pollen types into dominance classes:

D: dominant pollen represents more than 45% of the total.

A: Secondary pollen represents from 15% to 45% of the total.

I: Important pollen represents from 3 to 15% of the total.

R: minority pollen represents 1% to 3% of the total.

P: Pollen present, less than 1% of the total.

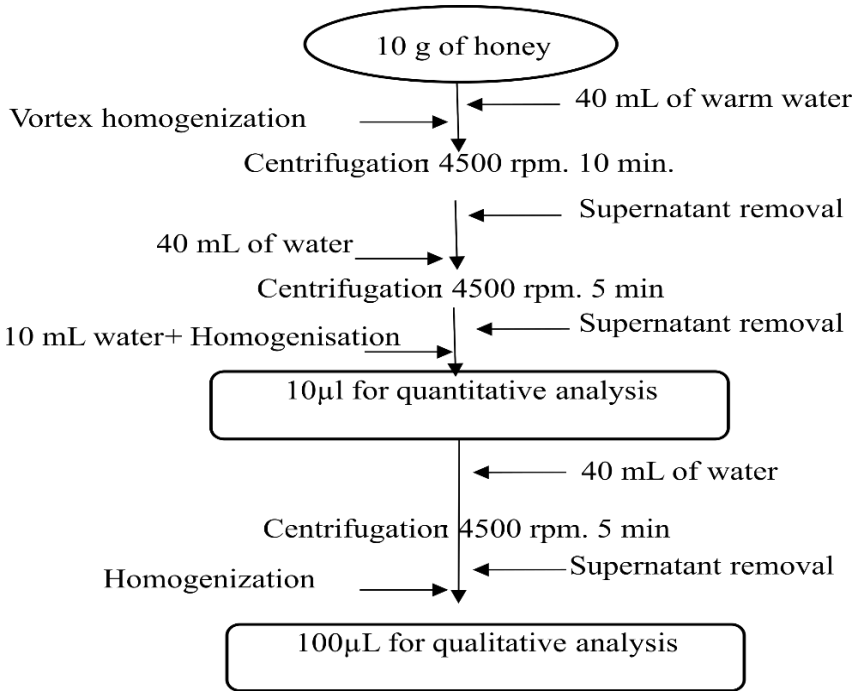


Figure 9: Quantitative and qualitative analysis of pollen

II.2.2. Physicochemical and biochemical analysis

II.2.2.1. Water content

Using a portable refractometer (ATAGO HHR-2N), a drop of honey is placed on the prismatic plate (previously calibrated with distilled water) and spread in a thin layer. The measurements were repeated three times. The reading is taken directly through the eyepiece of the refractometer at the horizontal separation line between a clear and a dark area (blue) and the results are expressed in percentage.

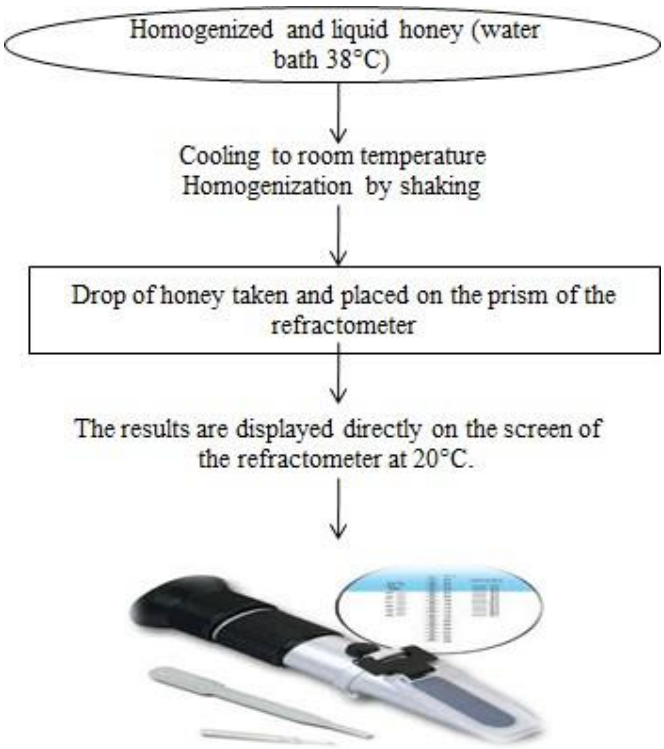


Figure 10: Moisture content measurement

II.2.2.2. Electrical conductivity measurement

The electrical conductivity was measured with a portable conductivity meter (Knick Portamess® 913 Conductivity, Berlin, Germany), on a sample of 20 g of honey dry matter in 100 mL of distilled water at 20°C. The weight of the honey used was calculated according to the following formula: g (dry matter)= [(20*100)/(100-A)], where A is the water content of the sample. The results were read on the instrument and expressed in microsiemens per centimeter (µs/cm).

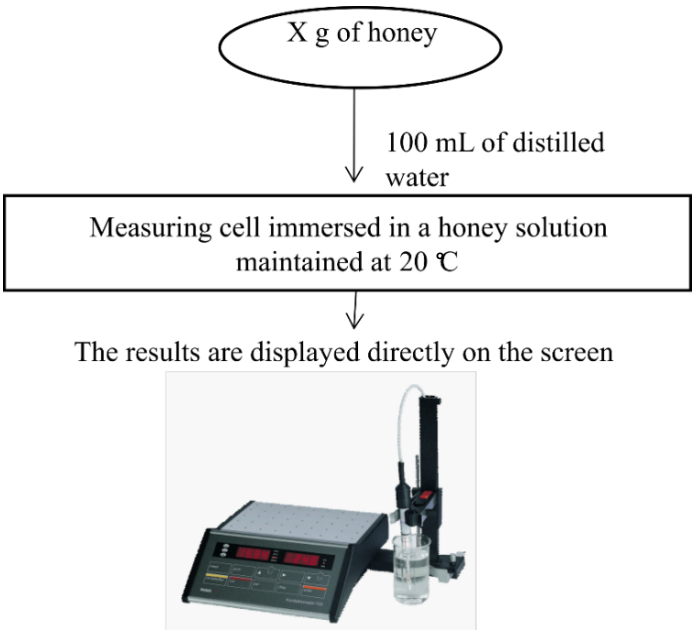


Figure 11: Electrical conductivity measurement (IHC, 2009)

II.2.2.3. pH measurement

Using the same previous instrument (Knick Portamess® 913 Conductivity, Berlin, Germany), the pH value was measured for a honey solution prepared by dissolving 10 g of honey in 75 mL of distilled water according to IHC (2009). The pH value is displayed directly on the instrument.

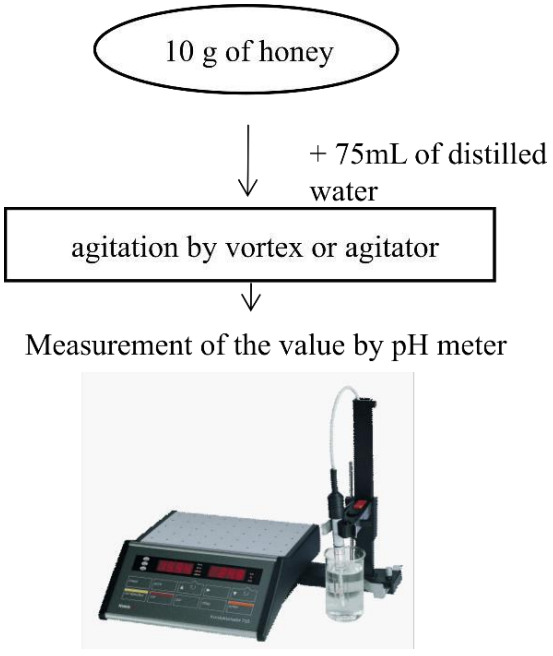


Figure 12: pH measurement (IHC, 2009)

II.2.2.4. Hydroxylmethyl furfural (HMF) measurement

The detection of HMF in honey according to IHC (2009) by the method of White is performed by measuring the absorbance of honey and reference solutions at two wavelengths (284 nm and 336 nm) using a spectrophotometer. A mass of 5 grams of honey is dissolved in 25 mL of distilled water. A volume of 0.5 mL of carrez I (15% potassium hexacyanoferrate) solution and a volume of 0.5 mL of carrez II (30% zinc acetate) solution are added. The mixture is made up to 50 mL with distilled water and drops of ethanol are added to remove foam. After filtration, the first tenths of the filtrate are removed. A volume of 5 mL of each initial solution is introduced in two test tubes: in the first tube, 5 mL of distilled water is added (sample solution), in the second tube, 5 mL of sodium metabisulfite solution (0.2%) is added (reference solution).

The absorbance is read at 284 nm and then at 336 nm using a UV spectrophotometer and the HMF content is given by the following equation:

HMF (mg/kg) = (A284 – A336) x 149.7 x 5 x D/M

D: Dilution factor. When the absorbance is greater than 0.6, the assay and reference aliquots are diluted with distilled water and sodium metabisulfite solution, respectively.

M: Mass of the honey sample

A284 and A336: Absorbances at 284 nm and 336 nm, respectively.

149.7: constant

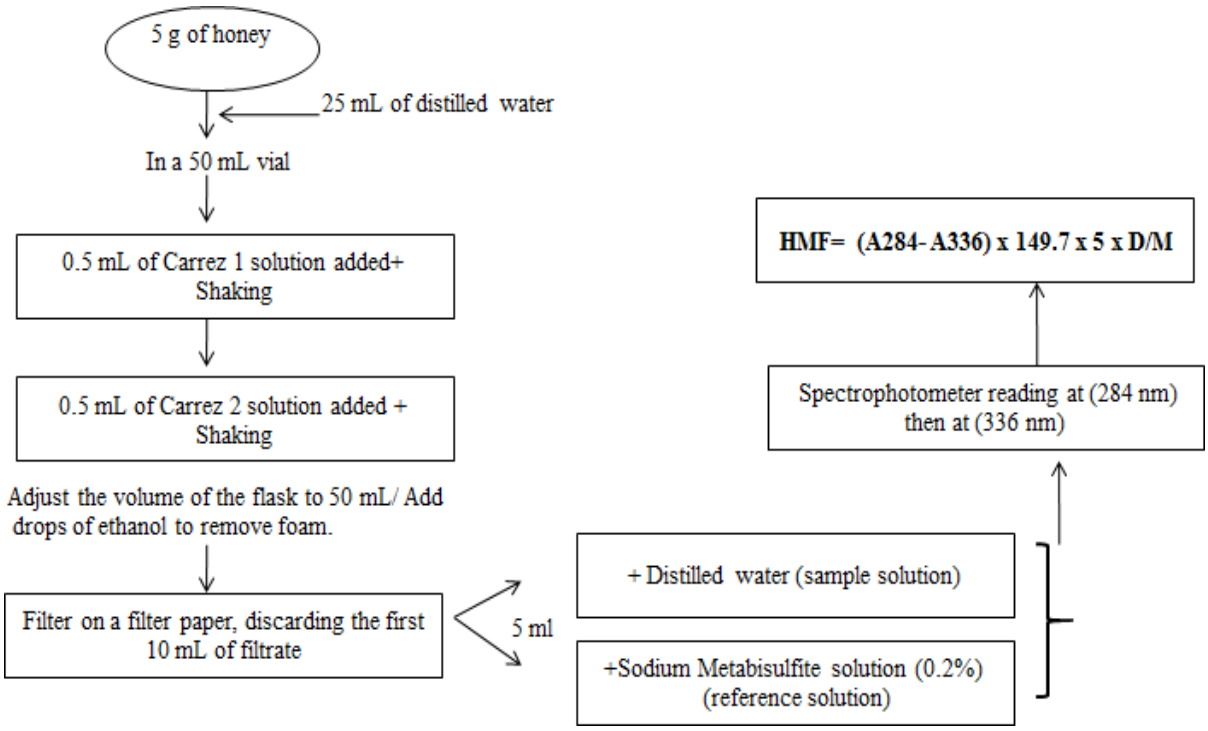


Figure 13: Hydroxylmethyl furfural (HMF) measurement (IHC, 2009)

II.2.2.5. Diastase number measurement (ID)

The determination of diastatic activity was based on the method of Shade *et al.* (1958), with some modifications. It is carried out according to a spectrophotometric technique, based on the hydrolysis rate of a starch solution by the amylase present in a buffered honey solution, measured at an absorbance of 660 nm. The results are expressed in Schade units per gram of honey (ID).The method requires a preparation of some solutions beforehand.

Iodine standard solution: 8.8 g of resublimed iodine are dissolved in 30-40 mL of distilled water containing 22 g of potassium iodide and diluted to 1 L in a volumetric flask.

Iodine solution 0.02 N: 20 g of potassium iodide was dissolved in 30-40 mL of distilled water. This solution was transferred to a 500 mL flask and 143 mL of the standard iodine solution was added. Finally, it was mixed with water. This solution remained stable for 24 hours.

Iodine solution 0.0007 N: In a volumetric flask, 10 g of potassium iodide are dissolved in 20 mL of distilled water; 2.5 mL of standard iodine solution are added and diluted to 250 mL. This solution is stable for 48 hours.

Acetate buffer pH 5.3 (1.59 M): 87 g of sodium acetate trihydrate was dissolved in 400 mL of distilled water, 10.5 mL of glacial acetic acid was added in a little water and brought to a volume of 500 mL. The pH was then adjusted to 5.3 with sodium acetate or acetic acid, as appropriate, using a pH meter.

0.5 M sodium chloride solution: 14.5 g of sodium chloride was dissolved in boiled distilled water and diluted to 500 mL.

Starch solution: A soluble starch was used with a blue index between 0.5 and 0.55 was used. An amount of soluble starch equivalent to 2 g of anhydrous starch was then weighed.

The equivalent of 2 g of anhydrous starch was weighed (humidity was previously controlled by drying at 130 °C), mixed with 90 mL of water and brought to a boil with continuous rapid stirring. Boiled gently for three minutes, covered and allowed to cool, transferred to a 100 mL volumetric flask and placed in a water bath at 40 ºC until the liquid reached this temperature.

Blue index determination: An amount equivalent to 1 g of anhydrous starch is dissolved as above, the solution is allowed to cool, 2.5 mL of acetate buffer is added and the volume is made up to 100 mL. To a 100 mL volumetric flask, 75 mL of water, 1 mL of 1 N hydrochloric acid and 1.5 mL of 0.02 N iodine solution are added. Then 0.5 mL of starch solution is added and made up to 100 mL with distilled water.

The solution is allowed to stand for one hour in the dark, and then the absorbance is read on a spectrophotometer at 660 nm against a control containing all of the above compounds except the starch solution. The reading on the absorbance scale is the blue index.

The honey solution to be determined is then prepared as follows: 10 g of honey is weighed, and then dissolved in 20 mL of distilled water, 5 mL of acetate buffer solution is added. Once the sample is dissolved and buffered, 3 mL of 0.5 M sodium chloride is added, then the whole is transferred to a 50 mL vial and completed to the level of the vial with distilled water. Using a micropipette, 10 mL of the honey solution is

transferred, into two tubes of about 60 mL each, and then placed in a water bath at 40°C with the vial containing the starch solution.

Separately, several tubes, of appropriate capacity, were prepared with 10 mL of 0.0007 N iodine solution each and the volume of water obtained during normalization of the starch solution. After 15 minutes, 5 mL of distilled water is poured, using a pipette, into one tube containing the honey solution (blank) and 5 mL of starch solution into the other tube containing the honey solution (test). Mix well and start a stopwatch.

0.5 mL of the blank solution poured into one of the tubes containing the iodine solution, this is the reading blank. At five-minute intervals, using a pipette, take 0.5 mL of the control (test) solution and pour it into the previously prepared tubes and mix well. Immediately, the absorbance is determined at 660 nm in the spectrophotometer against the blank. Then, 0.5 mL aliquots are taken at known time intervals until an absorbance of less than 0.235 is obtained.

The following table shows the approximate time required to reach the end point, based on the absorbance measured in the reading obtained five minutes after mixing.

Table 3: Estimated time for the sample to have an absorbance below 0.235 nm.

Absorbance (λ)	Approximate time (min)
0.70	8-9
0.65	9-10
0.60	11-12
0.55	13-15
0.50	16-20
0.45	25-30
0.40	30 or more

For calculations and expression of results, the equation of the regression line between absorbance and time was then calculated, determining the time (t) at which the mixture reaches the absorbance of 0.235, including:

$$\text{ID}= \left(60\frac{mins}{tx}\right) X \left(\frac{0{,}10}{0{,}01}\right) X \left(\frac{1}{2}\right) = 300/tx$$

With: tx= the time in minutes needed to reach an absorbance equal to 0.235.

ID = the diastase index on the Gothe/Shade scale.

The following diagram summarizes the dosing procedure:

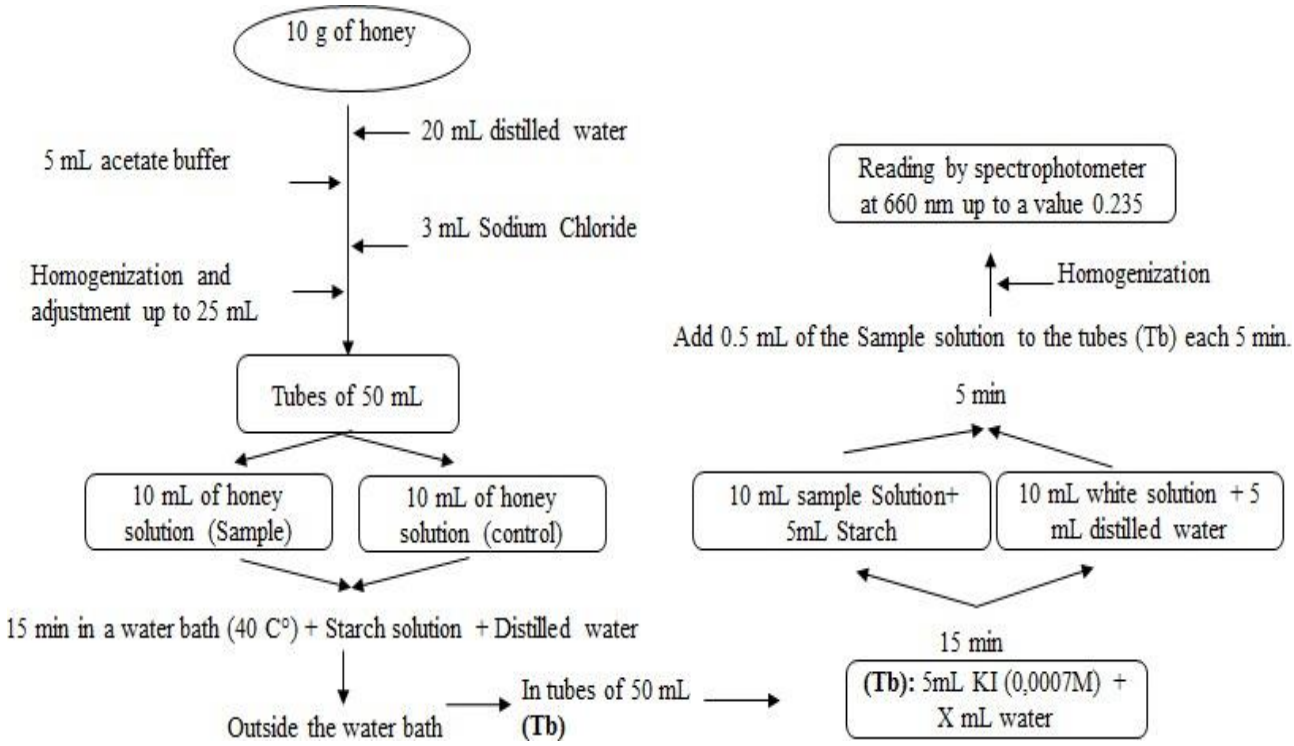


Figure 14: Diagram of diastasis measurement (IHC, 2009).

II.2.2.6. Color estimation

The color of honey depends on its botanical origin, ranging from almost transparent water white to dark brown almost blackish.

II.2.2.6.1. Pfund's scale

To measure the intensity of the color according to the Pfund scale, about 4 mL of honey are placed in smooth-walled plastic cuvettes with a span of 1 cm. The sample must be fluid for the measurement to be correct, in case of turbidity or crystallization; the samples are placed in an ultrasound until foam of water bubbles appears which will be eliminated afterwards.

Once the sample is ready, the cell is placed in a colorimeter, (HANNA Honey Color C221 Colorimeter) (Woonsocket, Rhode Island, USA) pre-calibrated with glycerine (Escuredo *et al.*, 2019). The instrument gives the value in millimeters using the Pfund scale presented in the following table:

Table 4: Pfund scale values

Color	Millimeters
Water white	0-8 mm
Extra white	8-16 mm
White	16-34 mm
Extra light amber	35-50 mm
Light Amber	51-84 mm
Amber	85-114 mm
Dark	115-140 mm

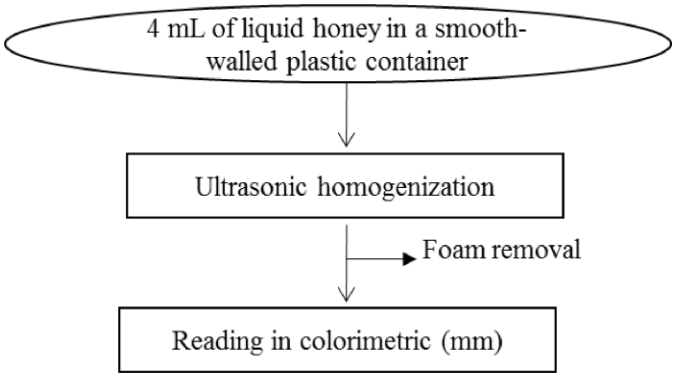


Figure 15: Color measurement according to the Pfund scale (Escuredo *et al*., 2019).

II.2.2.6.2. CIEL*a*b* coordinates

CIEL*a*b* tristimulus determination was performed using a Minolta CR-210 Chroma Meter (Konica Minolta, Tokyo, Japan).This device is a portable measuring instrument designed to evaluate the color of objects, especially with smoother surface conditions. It uses diffuse lighting and must be calibrated beforehand with a calibrated plate. The method uses cartesian coordinates to calculate the chromatic attributes in a color space.

The color space is based on a Cartesian sequential or continuous representation with three orthogonal axes: L*, a* and b*. L* represents the luminosity (L*=0, black, and L*=100, incolore), a* the green/red color component (a*>0, red, and a*<0, green), and b* the blue/yellow color component (b*>0, yellow, and b*<0, blue). Samples (5 ml) were measured in Petri dishes (3.5 cm diameter and 1 cm height) on a white background

(Escuredo *et al.,* 2019). All procedures were performed in triplicate and results were expressed as mean values.

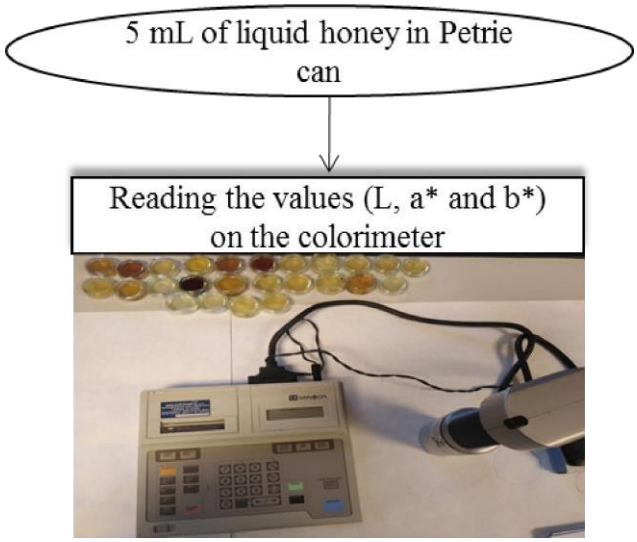


Figure 16: Representation of the measurement of the colorimetric coordinates of CieLa*b* (Escuredo *et al*., 2019).

II. 2.2.7. Mineral composition characterization

The extraction of minerals from honey samples was carried out by microwave digestion according to the methodology indicated in Caroli *et al.* (1999). The physical condition of the honey samples required prior homogenization before analysis. To overcome this difficulty, honey samples were quietly heated to approximately 50 °C and dissolved by ultrasonic agitation.

Aliquots of 0.5 g of honey were taken and transferred in a CEM MARSX model microwave press oven, where they were subjected to hydrolization by 9 mL of nitric acid and 2 mL of hydrogen peroxide. Finally, the residue was made up to 25 mL with distilled water and Mg, Cu, Ca, Fe, P and Zn were quantified by Atomic Absorption (Varian SpectrAA-220 Fast Squencial) and Na and K by Atomic Emission using Atomic Absorption Spectrophotometer VARIAN SPECTRA A-220 FAST SQUENCIAL. The results were expressed in mg/100g.

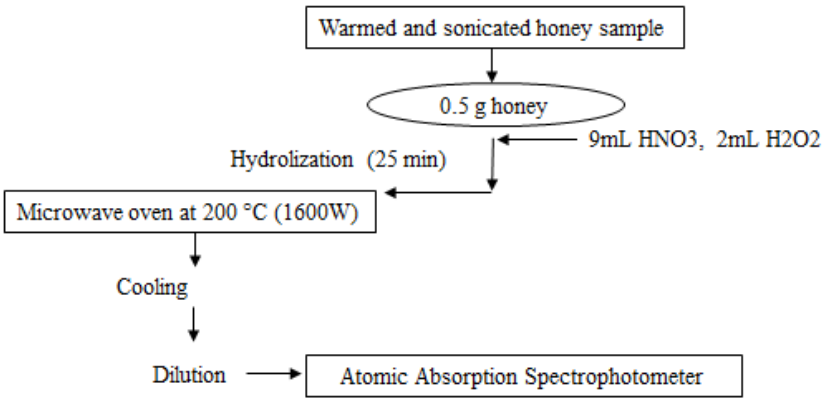


Figure 17: Determination of minerals by AAS method (Caroli *et al.*, 1999).

II.2.2.8. Sugar composition by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD)

One g of honey was dissolved in 100 mL of milli-Q water to give a honey solution concentration of 10 mg/L. This was followed by a 0.5 mL dilution of this solution a to a final volume of 100 mL and about 5 mL of this was filtered through a 0.45 µm diameter pore filter to remove any impurities or large pollen grains that might be present (Escuredo *et al.*, 2014).

Using a Dionex ICS-3000 ion chromatography system (Sunnyvale, Calif., USA) incorporating an analytical column, guard column, and pulse amperometric detector (PAD), all data for identification and quantification of sugars in honey were provided.

Separation of sugars from concentration was performed with a CarboPac PA1 column (3 X 250 mm) (polyvinylidene/polyvinylbenzene column suitable for mono-, di-, tri- and oligosaccharide analysis).A pulsed amperometric detector was used to detect sugars, with a gradient of two mobile phases (A and B). Phase A was ultrapure water, while phase B was 200 mM NaOH (HPLC grade, Merck). The sugar content of the honey samples was calculated using standard solution calibration curves for each pure sugar (Sigma-Aldrich).

The concentration of the standard solution for glucose and fructose was 25 mg/mL and for sucrose, melezitose, and maltose 0.2 mg/mL.

The acquisition of all chromatograms was performed with the software CHROMELEON chromatography management system (Escuredo *et al.*, 2014).

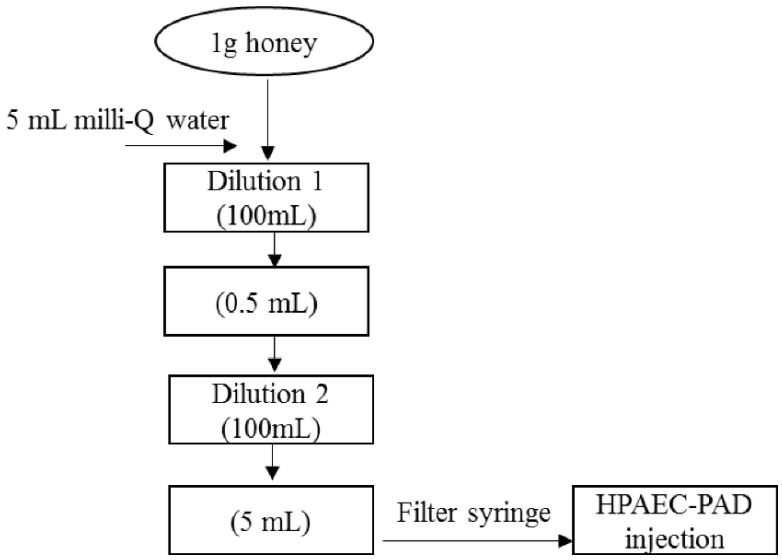


Figure 18: Honey sugar extraction (Escuredo *et al.*, 2014).

II.2.2.9. Statistical analysis

Using the software Statgraphics Centurion V18 (The Plains, USA) for windows, two clusters that are groups of observations with similar characteristics, in order to group similar samples for their tipyfication, were presented, by using the variables that are suitable, once according to the honey samples (1st cluster), the other time according to the geographic origin (2nd cluster).

A Principal Component Analysis was carried out to analyze the interrelations between the inserted variables.

After grouping the samples according to their botanical origin, each basic characteristic parameter is presented by a box-and-whisker diagram in order to compare the types of honeys, so the significant differences in case of their presence between the means and the standard deviations of the groups of honeys for each parameter are studied by the F-tes of the ANOVA and the Leven's test respectively.

Advanced characterization

A research in deep term of quality is currently indispensable on certain criteria in honey. The *in vitro* biological activities for each type of honey as well as the identified chemical composition are two more advanced components in the term of honey characterization.

II.3. Biological activities

The *in vitro* research on the biological capacities of honeys of different floral and geographical types using chemical standards (gallic acid and quercetin) and controls (Ascorbic acid and acarbose),is requested in order to know the value of the therapeutic and nutritional quality of the present samples. The applied protocols are common in the field of in vitro biology for similar tests.

II.3.1. *In vitro* determination of antioxidant activity

Antioxidants have been defined as any substance that delays, prevents, or suppresses oxidative damage to a target molecule. They include enzymatic and non-enzymatic substances in honey, including flavonoids and phenolic compounds (Khalil *et al*., 2012). In this context the determination of polyphenols as well as flavonoids especially is essential for the estimation of antioxidant power.

These analyses were carried out on 34 of the total samples of honey studied. The samples were then selected according to their botanical origins, they are listed here according to the nomenclatures declared by the beekeepers, including six samples of retem (R1, R2, R3, R4, R5 and R6) seven samples of Merkh (M3, M4, M5,M6, M1, M2 and M7), seven samples of Sor (S1, S2, S3, S4, S5, S6 and S7, two of Harra (H1 and H2), nine samples supposed also of *Eucalyptus* (E3, E4, E9 E1, E2, E5, E6, E7 and E8) as well as two samples of two other types (A: *Acacia*) and (Am: *Arbutus*).

II.3.1.1. Total phenol content

The total phenolic content (TPC) of the selected honey samples was determined using the Folin-Ciocalteu reagent according to Singleton and Rossi, (1985) by the microplate assay method described by Muller *et al*. (2010) with modifications.

The Folin-Ciocalteu Reagent (FCR), consisting of a mixture of phosphotungstic acid ($H_3PW_{12}O_{40}$) and phosphomolybdic acid ($H_3PMo_{12}O_{40}$), is reduced, during the

oxidation of phenols, to a mixture of tungsten (W_8O_{23}) and molybdenum (Mo_8O_{23}) oxides. The blue coloration produced is proportional to the content of total phenols and has a maximum absorption around 750 -765 nm. Twenty-five µL of honey solution (100 mg/mL) was mixed with 100 µL of Folin-Ciocalteu reagent and 75 µL of sodium carbonate (7.5%).

After incubation for 2 h in the dark at room temperature, absorbance was measured at 765 nm in the microplate reader. Quantification was performed using a calibration curve constructed from measurements of standard gallic acid at different concentrations (25-500µg/mL) and expressed as mg gallic acid equivalent per 100 g of honey

II.3.1.2. Flavonoid content

The determination of flavonoids in honey samples is based on the formation of a complex between Al^{+3} and flavonoids. The method of Topçu *et al.* (2007) is used with some modifications for the determination on a 96 well microplate.

Ten µL of 10% aluminum nitrate, 10 µL of 1M potassium acetate, and 130 µL of methanol were added to 50 µL of honey solution (100 mg/mL). The absorbance was measured at 415 nm in the microplate reader after 40 min of incubation in the dark at room temperature. Quercetin was used as a standard and results were expressed as mg quercetin equivalents per 100 g of honey. Both determinations were performed in triplicate and results were expressed as mean.

II.3.1.3. Antiradical activity evaluation

Spectrophotometric tests have been adopted to measure the antioxidant capacity of foods, the most popular being the 2,20-azino-bis-3-ethylbenzthiazoline-6-sulfonic acid (ABTS. +) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) tests.

II.3.1.3.1. DPPH radical scavenging assay

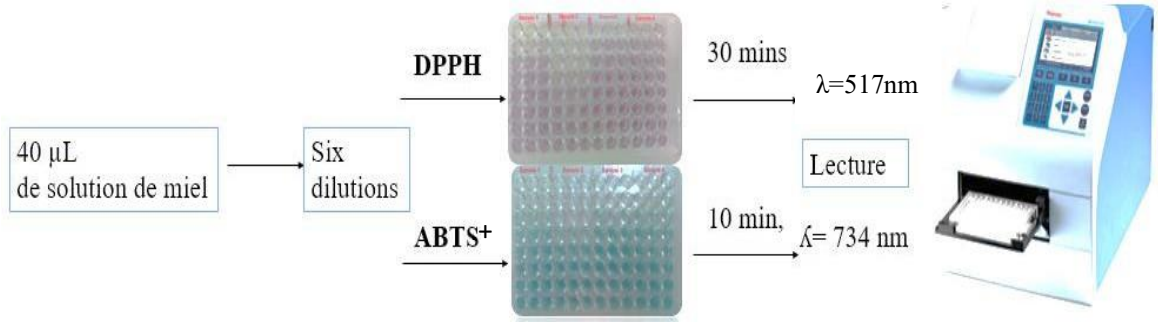
The DPPH (2,2-Diphenyl-1-Picrylhydrazyl) trapping test was determined by the spectrophotometric method, according to Blois (1958), with some modifications. One hundred and sixty µL of DPPH solution (with absorbance of 0.5 at 517 nm) was added to 40 µL of honey solution at different concentrations (from 100 mg/mL). The absorbance was measured at 517 nm. Ascorbic acid solution (0.003125-0.1 mg/mL) was

used as a positive control. Results were given as percent inhibition and 50% inhibition concentration (IC_{50}).

II.3.1.3.2.ABTS. +radical scavenging assay

The assay of the cationic radical ABTS .+ (2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) was determined by the spectrophotometric method of Re *et al*. (1999) with some modifications.

ABTS .+ solution was prepared by combining potassium persulfate K2S2O8, protected from light for 12 to 16 hours, (with an absorbance of 0.7 at 734 nm before use). One hundred sixty µL of ABTS .+ solution was added to 40 µL of honey solution. After 10 minutes, the absorbance was measured at 734 nm, the ABTS .+ activity was expressed as a percentage and as an inhibition concentration (IC_{50}). Ascorbic acid was used as a positive control.



DPPH/ $ABTS^{+}$ scavenging effect (%)= ($A_{Control}$ - A_{Sample} / $A_{Control}$)* 100

Figure 19: Representation of the measurement of antioxidant activity (Blois ,1985 ; Re *et al*., 1999).

II.3.2. *In vitro* enzymatic inhibition capacity of α-amylase

The α-amylase inhibition assay was performed according to Zengin *et al*. (2014) with few modifications, using iodine/potassium iodide (IKI). Fifty µL of (1U amylase solution) was added to 25 µL of honey solution at different concentrations (starting from 100 mg/mL) and incubated for 10 min at 37 °C, and then 50 µL of 0.1% starch was added into each sample solution and incubated for 10 min at 37 °C. After that, 25 µL of HCl (1M) was added to stop the enzymatic reaction, followed by the addition of

100 µL of IKI. The absorbance was read at 630 nm. Results were expressed as percent and inhibition concentration (IC_{50}) (Annexe 2.4).

A non-protein alpha amylase inhibitor (Acarbose) was used as a reference to compare activity. Experiments were performed in triplicate and results were expressed as mean value of inhibition percentages calculated with the following formula:

%INH=1-[(Ac-Ae)-(As-Ab)/(Ac-Ae)]

Ac = Absorbance [Starch + IKI + HCl + Vol of substitute honey solution+Vol of buffer enzyme]

Ae = Absorbance [Enzyme + Starch + IKI + HCL + Vol of S. honey solution]

As = Absorbance [Enzyme + honey solution + Starch + IKI + HCl]

Ab = Absorbance [honey solution + IKI + 125µL of Buffer]

II.3.3. Statistical analysis

The statistical software IBM SPSS 23.0 (IBM, Massachusetts, USA) and Statgraphics centurion V18 (The Plains, USA) were used for the multivariate analysis. First of all, a box plot for the biological properties studied concerning the different types of honey was made.

One-way analysis of variance (ANOVA) was used to determine whether there were statistically significant differences in antioxidant activities and α-amylase activity between sample groups. Differences between all pairs of groups were tested using the Bonferroni test.

The PCA was performed including the physicochemical variables, except for color in the Pfund scale and the pollen types most represented in the samples. The same variables were used for the cluster analysis. It was constructed using Ward's method which showed the distance between two clusters (A and B), as the increase in the sum of squares when they merge. Finally, a stepwise linear regression analysis was used to predict the biological properties studied. This is a method of regressing multiple variables by simultaneously eliminating irrelevant ones. The significance level was set at $\alpha=0.05$.

II.4. Non-targeted metabolomic approach analysis

On the basis of mass spectrometry (MS) and other matrices, the aim is to identify some metabolic components in honey samples, with the purpose of separating these samples according to their chemical composition and detecting one or more components considered as biological markers of each type of honey, on the basis of correlations and chemometric study.

Twenty-three samples of monofloral honeys were chosen for this analysis because of certain criteria, citing: The monoflorality confirmed by the polynomial study and of these samples, the exclusivity of these selected types of honey.The three types of honey studied were collected in areas characterized by their arid or semi-arid climate, close to the Saharan territory.

II.4.1. Characterization of the volatile fraction of honeys

II.4.1.2. Volatile compounds extraction

7.5 g of honey was placed in 50 mL vials with 7.5 g of sodium chloride (30%), continuous stirring with injection of a fibrous exponent (SPME 573264) into the headspace above the sample for 60 min at 50°C, and finally thermal desorption of the adsorbed substance in the injection port of the GC-MS analysis for 5 min at 250°C (Rodriguez-Flores *et al*., 2021).

II.4.1.3. Volatile compounds separation

Separation of compounds was performed on a DB-5MS column (30mx25mm i.D. thickness 0.25µm; J&W Scientific, Inc.). The internal temperature was programmed from 40°C to 170°C (3°C/min), from 170°C to 290°C (25°C/min), holding it at 290°C for 15 minutes. Helium was used as the carrier gas at a constant rate of 1 mL/min.The mass spectrum was used with anionization energy of 70 ev. The temperature of the transfer line and ionization source was 250 °C and 230 °C respectively. Xcalibur software was used to acquire the data. Compound identification was performed by comparing the results with those obtained for commercial standards and a library of MS compounds (NIST), which were confirmed by calculating retention indices (LRI) (Rodriguez-Flores *et al*., 2021)

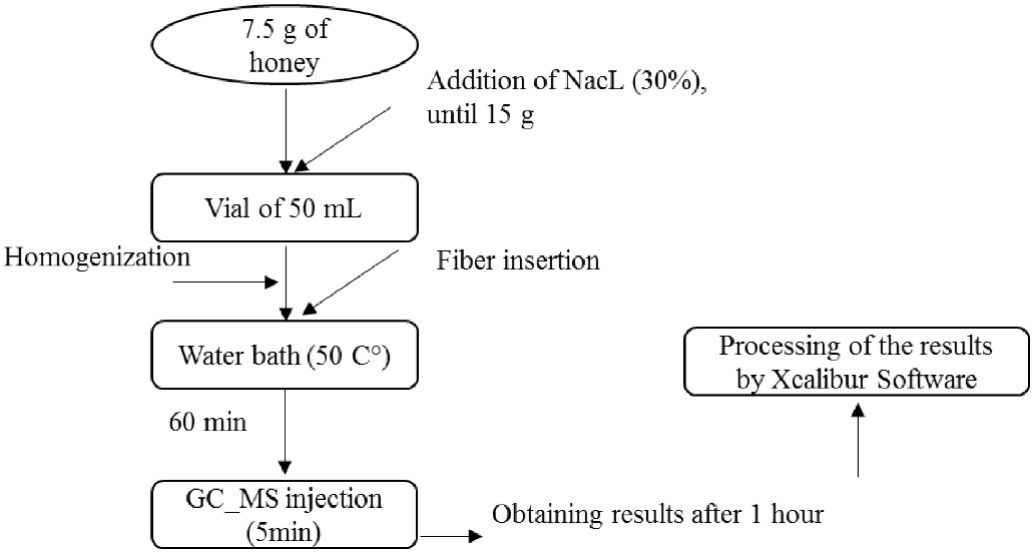


Figure 20: Volatile compounds extraction method (Rodriguez-Flores *et al*., 2021).

II.4.2. Statistical analysis

Data processing was performed using Microsoft Office. Statistical analyses were performed with XLSTAT (Addinsoft, New York, USA).

Principal component analysis (PCA) was performed. Differences between honey types were tested using a pairwise Mann-Whitney test. This is a fairly robust nonparametric test, useful when sample sizes are small and data do not have a normal distribution. All variables were entered into the analysis and the significance level was set at $\alpha<0.05$.

II.4.3. Sensorial analysis

The sensorial analyses of the different honey samples were carried out by a group of five tasters previously selected and trained according to international standards. The tests were carried out in an odorless room, protected from daylight and at room temperature as already described by Ghorab *et al*. (2021).

Tasters should follow the recommendations of the sensory evaluation, do not eat, smoke or take medication for at least one hour, stay away from noise and concentration during the test, rinse their mouths with plain unflavored water between each test, avoid cosmetics, perfumes etc…

The samples were presented to the panelists (tasters) as 20 mL in small transparent glasses, water was provided to rinse the mouth between samples.The panelists evaluated the honey samples of different origins for their global characteristics (visual, olfactory and gustatory) on a scale graduated on 10, in fact, for each attribute, the scale goes from the lowest to the highest note, followed by a questionnaire of grouping of the samples, as well as the global appreciation.

Descriptors of each of its perceptions (state, color, astringency and Spicy) were numerically evaluated by a previously given scale. A score of 1 for a liquid sample, 5 for a crystallizing sample and 10 for a sample in process of crystallization. For its color, if the sample is liquid, the assumed color range is as follows: White, Light amber, Amber, Dark amber and Dark. In the case of a crystallized sample, the range of colors to be discussed is: White, Straw, Gold, Orange and Brown. The proposed scale to characterize the color in both cases (liquid and crystallized) is: 2, 4, 6, 8, 10 respectively. Regarding the smell, savor and aroma, attributes are added to the choice with some details for each attribute, as mentioned in the tasting sheet (Annexe 4), noting its intensity by a score on a scale of 0 to 10 according to the accentuation of the characteristic.

A questionnaire was proposed to the distributors in order to know their opinion on the apparent differences between the samples according to their sensory characteristics.

The descriptors used for the evaluation can be found in the following table.

Table 5: Descriptors for sensorial analyses

	Descriptors	Points	
Estate	Liquid	1	
	In process	5	
	Crystalized	10	
Color	Liquid	Crystallized	
	White	White	2
	Light Amber	Straw	4
	Amber	Gold	6
	Dark Amber	Orange	8
	Dark	Brown	10
Smell	Fruity		
	Candy		
	Floral		
	Vegetal		
	Chemical		
	Animal		
	Degraded		
	Persistence	1-10	
Savor	Sweetness		
	Sourness		
	Saltiness		
	Bitterness		
	Persistence	1-10	
Aroma	Fruity		
	Candy		
	Floral		
	Vegetal		
	Chemical		
	Animal		
	Degraded		
	Persistence	1-10	
Astringency	yes	2	
	No	0	
Spicy	yes	2	
	No	0	

III. Results and discussion

III.1. Microscopic results

The pollen microscopic analyses provided information on the plants from which the honey comes and on the plant resources of the production area.

In Algeria, there are no reliable reference works or data banks of honey pollens, and similar works on monofloral honeys are still in process. Microscopic analysis has made it possible to count and identify the various pollen grains and other biotic elements present in the sediment.

III.1.1. Types of pollen identified

In the 59 honey samples collected during the study period, about 95 different pollen types belonging to 42 families were identified (Table 6).

The pollen types identified reflect the variety of vegetation in the various foraging areas. The two families Fabaceae and Asteraceae are the most dominant with a large number of types, followed by Apiaceae, Lamiaceae and Brassicaceae.

Table 6: Families and type of pollen identified.

Family	Pollen type	Family	Pollen type
Anacardiaceae	*Pistacia*	Ephedra	*Ephedra*
	Rhus	Ericaceace	*Erica*
Apiaceae	*Apium nudiflorum*t		*Arbutus*
	Bupleurum fruticosum t	Euphorbiaceae	*Crozophora tinctoria*
	Coriandrum sativum t		*Euphorbia* t
	Eryngium campestre t	Fabaceae	*Acacia*
	Ferula communis t		*Arachis hypogea*
	Foeniculum vulgare t		*Astragalus*
	Pimpinella anisum t		*Ceratonia siliqua*
	Other Apiaceae		*Genista*
Araliaceae	*Hedera helix* t		*Hedysarum*
Arecaceae	*Chamaerops humilis*		*Lotus* t
	Phoenix dactylifera		*Onobrychis*
Asparagaceae	*Urginea*		*Ononis natrix*
	Muscari		*Retama*
Asphodelaceae	*Asphodelus*		*Psoralea*
Asteraceae	*Anthemis* t		*Pisum sativum*
	Aster t		*Spartium junceum*
	Atractylis serratuloides		*Trifolium pratense* t
	Artemisia t		*Trifolium repens* t
	Carthamus lanatus		*Vicia*
	Centaurea t		Other Fabaceae
	Chrysanthemum t	Fagaceae	*Quercus*
	Echinops	Globulariaceae	*Globularia*
	Galactites tomentosus t	Lamiaceae	*Lavandula* t
	Launaea arborescens t		*Rosmarinus officinalis* t
	Scorzonera t		*Teucrium scorodonia* t
	Cichorium intybus t		*Thymus* **t**
	Otras Asteraceae		*Vitex*
Boraginaceae	*Borago officinalis*		Other Lamiaceae
	Echium	Lythraceae	*Lythrum*
	Phacelia		*Punica granatum*
Brassicaceae	*Brassica napus* t	Myrtaceae	*Eucalyptus*
	Eruca sativa t		*Myrtus*
	Raphanus t	Nitrariaceae	*Peganum harmala*
	Capsella t	Oleaceae	*Fraxinus* t
	Other Brassicaceae		*Olea europaea*
Buxaceae	*Buxus sempervirens*	Oxalicaceae	*Oxalis*
Capparaceae	*Capparis spinosa*	Papaveraceae	*Papaver rhoeas* t
Caryophyllaceae	*Paronychia argentea* t	Plantaginaceae	*Plantago*
	Other Caryophyllaceae	Plumbaginaceae	*Limonium*
Chenopodiaceae	*Chenopodium* t	Poaceae	Poaceae
Cistaceae	*Cistus*	Rhamnaceae	*Rhamnus* t
	Helianthemum		*Ziziphus lotus*
Convolvulaceae	*Convolvulus*	Rosaceae	*Crataegus* t
Crassulaceae	*Sedum*		*Prunus* t
	Opuntia ficus-indica		Other Rosaceae
Cucurbitaceae	*Citrullus*	Rutaceae	*Citrus*
	Cucurbita	Salicaceae	*Salix*
Cupressaceae	*Cupressus*	Smilacaeae	*Smilax*
Cyperaceae	*Carex*	Tamaricaceae	*Tamarix*
		Thymelaeaceae	*Thymelaea*

t: pollen type

III.1.2. Qualitative analysis

Table 7 represents the main pollen types identified in the honey samples according to the percentages of pollen representation (% Rep.), as well as their frequency classes, including: P: pollen present (<1% of the pollen spectrum), R: minority pollen (1-3% of the pollen spectrum), I: important pollen (3-15% of the pollen spectrum), A: secondary pollen (15-45% of the pollen spectrum), D: dominant pollen (>45% of the pollen spectrum). The families and pollen types are ordered according to the dominance of the identified pollen, thus six families were found in more than 50% of the samples, citing: Fabaceae, Brassicaceae, Myrtaceae, Papaveraceae, Oleaceae and Euphorbiaceae. The two families Asteraceae and Rhamnaceae were presented with a percentage of 49.2%. No pollen family appeared in all honey samples (100%). The maximum percentage of representation is 74.6% presented by the two families Fabaceae and Brassicaceae. The types *Genista, Brassica napus, Eruca sativa, Papaver rhoeas, Eucalyptus* and *Hedysarum* were identified in more than 50% of the samples.

The pollen that reached the dominant (D) category (>45%) in all samples were from the types of*: Genista, Retama, Capparis spinosa, Eruca sativa, Eucalyptus, Hedysarum, Spartium junceum, Atractylis serratuloides, Ziziphus lotus, Paronychia argentea,* and *Bupleurum fruticosum* respectively of which four were identified in more than 50% of the samples (Figure 20). Apart from these two types *Spartium junceum* and *Bupleurumfruticosum*, the pollen types already mentioned as dominant in some samples are themselves considered secondary (A) in other honey samples, in addition to *Pimpinellaanisum*, *Tamarix*, *Echium*, *Peganum harmala*, *Myrtus*, *Onobrychis*, *Foeniculum vulgare*, *Acacia* and *Globularia*. Other pollen types, apart from *Foeniculum vulgare, Acacia* and *Globularia* considered important (I) are: *Brassica napus*, *Papaver rhoeas*, *Olea europaea*, *Lotus*, *Ononis natrix*, *Centaurea*, *Chamaeropshumilis*, *Vitex*, *Helianthemum*, other Apiaceae, other Fabaceae, *Thymus*, *Euphorbia*, *Trifolium pratense*, *Trifolium repens*, *Astragalus*, *Buxus sempervirens*, *Erica*, *Rhus*, *Punica granatum*, *Phoenix dactylifer*a, *Crataegus*, *Quercus*, *Coriandrum sativum*, *Carex*, *Ferula communis, Artemisia, Citrus, Rhamnus, Pisum sativum* and *Arbutus*.The pollen types considered rare (R) are represented by the majority of the pollen types mentioned in the other samples.

The other pollen types hardly appear in the honey sediment. Most of them are included as pollen present (P) (Table 8).

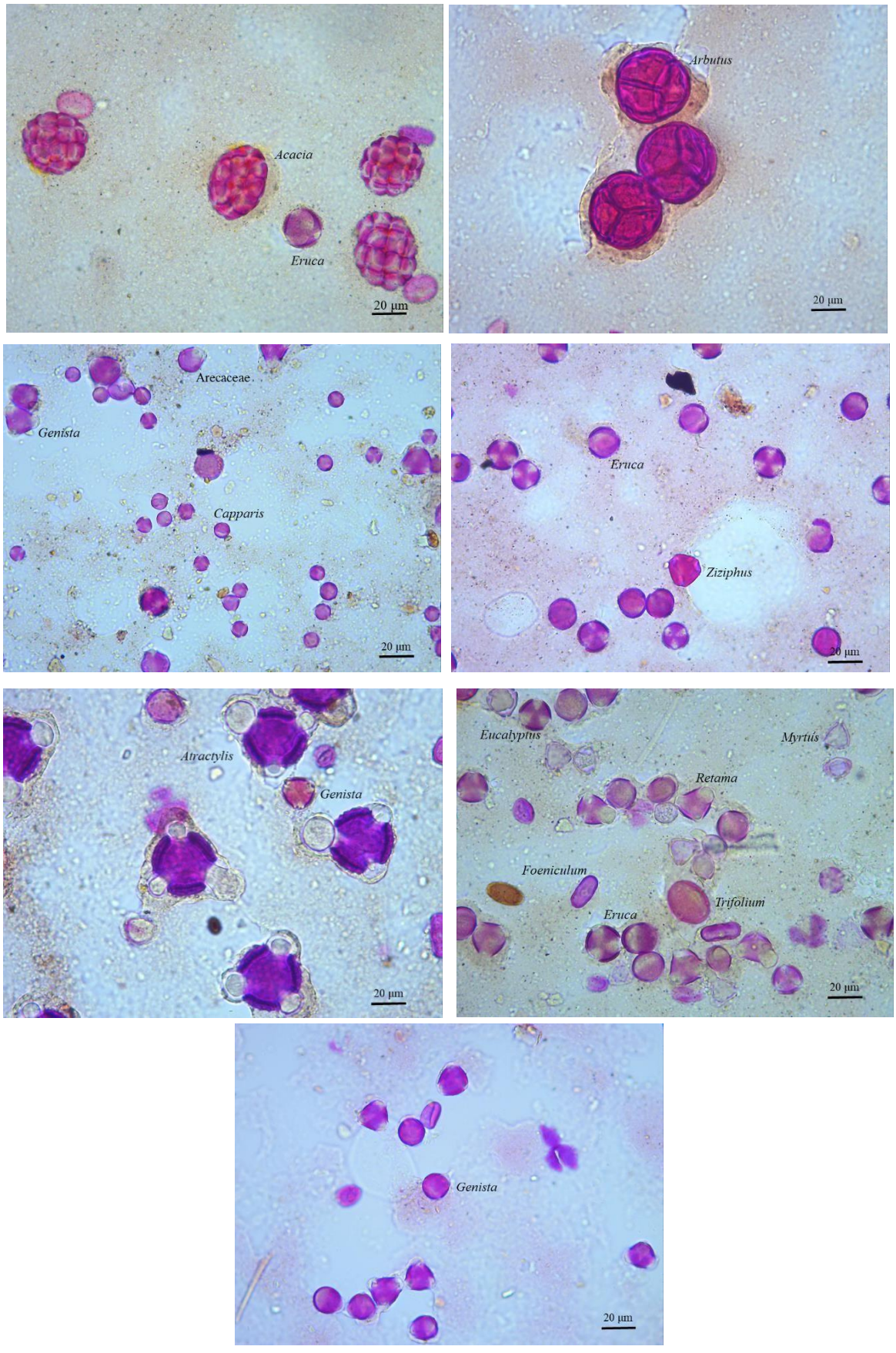


Figure 21: Main important pollen types in studied honey samples.

Table 7: Main pollen types identified in the samples. Percentage representation (% Rep.), and frequency classes.

Family	Pollen type	% Rep	P (0-1%)	R (1-3%)	I (3-15%)	A (15-45%)	D >45%
Fabaceae	*Genista*	74.6	7	8	12	8	9
Fabaceae	*Retama*	18.6	-	-	3	2	6
Capparaceae	*Capparis spinosa*	33.9	5	4	5	1	5
Brassicaceae	*Eruca sativa* t	69.5	8	9	15	5	4
Myrtaceae	*Eucalyptus*	62.7	17	4	9	4	3
Fabaceae	*Hedysarum*	54.2	16	4	5	4	3
Fabaceae	*Spartium junceum*	10.2	2	1	-	-	3
Asteraceae	*Atractylis serratuloides*	49.2	10	1	10	7	1
Rhamnaceae	*Ziziphus lotus*	49.2	9	2	14	3	1
Caryophyllaceae	*Paronychia argentea* t	16.9	3	1	4	1	1
Apiaceae	*Bupleurum fruticosum* t	16.9	8	1	-	-	1
Apiaceae	*Pimpinella anisum* t	42.4	11	4	6	4	-
Tamaricaceae	*Tamarix*	33.9	8	5	4	3	-
Boraginaceae	*Echium*	37.3	15	2	2	3	-
Nitrariaceae	*Peganum harmala*	42.4	11	5	7	2	-
Myrtaceae	*Myrtus*	8.5	2	-	2	1	-
Fabaceae	*Onobrychis*	18.6	6	3	1	1	-
Apiaceae	*Foeniculum vulgare* t	16.9	8	1	-	1	-
Fabaceae	*Acacia*	10.2	4	1	-	1	-
Globulariaceae	*Globularia*	5.1	1	1	-	1	-
Brassicaceae	*Brassica napus* t	74.6	30	6	8	-	-
Papaveraceae	*Papaver rhoeas* t	66.1	22	12	5	-	-
Oleaceae	*Olea europea*	62.7	22	10	5	-	-
Fabaceae	*Lotus* t	20.3	6	1	5	-	-
Fabaceae	*Ononis natrix*	27.1	9	3	4	-	-
Asteraceae	*Centaurea* t	42.4	19	2	4	-	-
Arecaceae	*Chamaerops humilis*	32.2	13	2	4	-	-
Lamiaceae	*Vitex*	11.9	2	1	4	-	-
Cistaceae	*Helianthemum*	28.8	8	6	3	-	-
Apiaceae	Other Apiaceae	20.3	8	1	3	-	-
Fabaceae	Other Fabaceae	20.3	8	1	3	-	-
Lamiaceae	*Thymus* **t**	15.3	5	1	3	-	-
Euphorbiaceae	*Euphorbia* t	57.6	18	14	2	-	-
Fabaceae	*Trifolium pratense* t	27.1	9	5	2	-	-
Fabaceae	*Trifolium repens* t	27.1	10	4	2	-	-
Fabaceae	*Astragalus*	10.2	2	2	2	-	-
Buxaceae	*Buxus sempervirens*	20.3	6	5	1	-	-
Ericaceace	*Erica*	27.1	13	2	1	-	-
Anacardiaceae	*Rhus*	20.3	9	2	1	-	-
Lythraceae	*Punica granatum*	20.3	9	2	1	-	-
Arecaceae	*Phoenix dactylifera*	10.2	3	2	1	-	-
Rosaceae	*Crataegus* t	10.2	3	2	1	-	-
Fagaceae	*Quercus*	27.1	14	1	1	-	-
Apiaceae	*Coriandrum sativum* t	22.0	11	1	1	-	-
Cyperaceae	*Carex*	22.0	11	1	1	-	-
Apiaceae	*Ferula communis* t	13.6	6	1	1	-	-
Asteraceae	*Artemisia* t	6.8	2	1	1	-	-
Rutaceae	*Citrus*	13.6	7	-	1	-	-
Rhamnaceae	*Rhamnus* t	5.1	2	-	1	-	-
Fabaceae	*Pisum sativum*	3.4	1	-	1	-	-
Ericaceace	*Arbutus*	1.7	-	-	1	-	-

Table 8: Pollen types less represented. Values below 3%.

Family	Pollen type	% Rep	P (0-1%)	R (1-3%)	I (3-15%)	A (15-45%)	D >45%
Apiaceae	*Eryngium campestre* t	22.0	8	5	0	0	0
Asteraceae	*Anthemis* t	40.7	20	4	0	0	0
Asteraceae	*Scorzonera t*	35.6	17	4	0	0	0
Asteraceae	Other Asteraceae	11.9	3	4	0	0	0
Poaceae	*Poaceae*	27.1	13	3	0	0	0
Chenopodiaceae	*Chenopodium* t	27.1	14	2	0	0	0
Brassicaceae	Other Brassicaceae	23.7	12	2	0	0	0
Caryophyllaceae	Other Caryophyllaceae	23.7	12	2	0	0	0
Brassicaceae	*Raphanus* t	15.3	7	2	0	0	0
Boraginaceae	*Phacelia*	6.8	2	2	0	0	0
Asteraceae	*Launaea arborescens* t	39.0	22	1	0	0	0
Asteraceae	*Aster* t	28.8	16	1	0	0	0
Asteraceae	*Galactites tomentosus* t	25.4	14	1	0	0	0
Oxalicaceae	*Oxalis*	18.6	10	1	0	0	0
Asteraceae	*Echinops*	13.6	7	1	0	0	0
Cistaceae	*Cistus*	13.6	7	1	0	0	0
Asteraceae	*Carthamus lanatus*	11.9	6	1	0	0	0
Asteraceae	*Chrysanthemum* t	11.9	6	1	0	0	0
Crassulaceae	*Sedum*	11.9	6	1	0	0	0
Oleaceae	*Fraxinus* t	11.9	6	1	0	0	0
Fabaceae	*Ceratonia siliqua*	8.5	4	1	0	0	0
Brassicaceae	*Capsella* t	5.1	2	1	0	0	0
Euphorbiaceae	*Crozophora tinctoria*	5.1	2	1	0	0	0
Lythraceae	*Lythrum*	5.1	2	1	0	0	0
Fabaceae	*Vicia*	3.4	1	1	0	0	0
Smilacaeae	*Smilax*	1.7	0	1	0	0	0
Apiaceae	*Apium nudiflorum* t	15.3	9	0	0	0	0
Asparagaceae	*Urginea*	13.6	8	0	0	0	0
Asparagaceae	*Muscari*	13.6	8	0	0	0	0
Cucurbitaceae	*Citrullus*	13.6	8	0	0	0	0
Plantaginaceae	*Plantago*	13.6	8	0	0	0	0
Salicaceae	*Salix*	13.6	8	0	0	0	0
Lamiaceae	*Teucrium scorodonia* t	11.9	7	0	0	0	0
Rosaceae	*Prunus* t	10.2	6	0	0	0	0
Cucurbitaceae	*Cucurbita*	8.5	5	0	0	0	0
Convolvulaceae	*Convolvulus*	6.8	4	0	0	0	0
Anacardiaceae	*Pistacia*	5.1	3	0	0	0	0
Fabaceae	*Arachis hypogea*	5.1	3	0	0	0	0
Fabaceae	*Psoralea*	5.1	3	0	0	0	0
Lamiaceae	Other Lamiaceae	5.1	3	0	0	0	0
Asteraceae	*Cichorium intybus* t	3.4	2	0	0	0	0
Boraginaceae	*Borago officinalis*	3.4	2	0	0	0	0
Crassulaceae	*Opuntia ficus-indica*	3.4	2	0	0	0	0
Ephedra	*Ephedra*	3.4	2	0	0	0	0
Thymelaeaceae	*Thymelaea*	3.4	2	0	0	0	0
Araliaceae	*Hedera helix* t	1.7	1	0	0	0	0
Asphodelaceae	*Asphodelus*	1.7	1	0	0	0	0
Cupressaceae	*Cupressus*	1.7	1	0	0	0	0
Lamiaceae	*Lavandula* t	1.7	1	0	0	0	0
Lamiaceae	*Rosmarinus officinalis* t	1.7	1	0	0	0	0
Plumbaginaceae	*Limonium*	1.7	1	0	0	0	0
Rosaceae	Other Rosaceae	1.7	1	0	0	0	0

III.1.3. Quantitative analyses of pollen

The general quantity of pollen in the present samples varies from 5000 pollen/g to 439000 pollen/g in the sample, with an average of 65159.5± 66211 and a range of 434000. These results show a notable difference in pollen content between the analyzed samples.

According to Maurizio's classification (1939), the majority of the present samples (63%) belong to class III, noted as pollen-rich honeys, followed by 15% of all samples belonging to class IV, considered as honeys very rich in pollen, then 19% belong to class V as honeys are extremely rich in pollen and finally 3% are as class II, considered as ordinary pollen honeys (Figure 21).

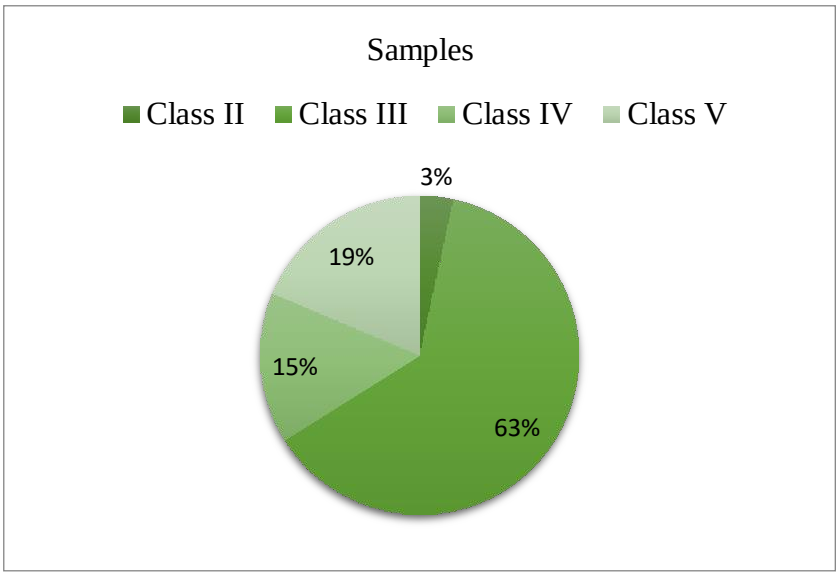


Figure 22: Honey samples classification according to Maurizio (1939).

III.2. Basic characterization for total honey samples

A basic characterization based on the microscopic study of the pollens existing in the honey samples as well as the quality parameters which confirms the good quality of use of these samples according to the International Honey Commission norms, revealed that all the studied honeys are in conformity with the traced standards, regarding the average number of pollen as already mentioned, as well as the water content 15%± 2.84, and their electrical conductivity 386.51 µs/cm, but with a large SD of 210.50 which means the significant difference between the values that range from 164.67 to 874.67.

The pH values are with an average of 4.0± 0.42, and an interval of (3.6-5.42), whose highest values exceed the norm of nectar honeys (4.5), which requires assumptions to be confirmed.

The levels of HMF reflecting the state of freshness of the samples are of an average of 6.72± 7.64 compared to a maximum standard of 45, of which some samples no longer contain this component while the maximum value being up to 29.15.Diastases also had an important mean value of 15.46± 8.44, of which a minimum value of 2.32 and maximum of 45.89 were noted.For its colors and according to the Pfund scale, the samples are from the extra white to the dark amber scale.

According to the standards of deviation, there are significant differences in the mineral content for all samples, which is not really the case for the carbohydrate composition.

Table 9: Overall results of basic characterization of the studied samples.

Parameter	M	SD	min	Max
Pk/1g	65159.5	66211	5000	439000
Moisture content %	15.74	2.48	12.23	22,57
Electrical conductivity (us/cm)	386.51	210.50	164.67	874.67
pH	4.07	0.42	3.60	5.42
Hmf (mg/kg)	6.72	7.64	0.00	29.15
Diastases	15.46	8.44	2.32	45.89
pfund scale (mm)	65	33.65	8	150
L	75.19	9.61	55.87	108.32
a*	1.25	5.00	-11.17	10.42
b*	22.21	6.95	-4.83	44.70
Na (mg/kg)	75.90	90.51	24	634
K (mg/kg)	982.89	811.05	174	3563
Ca (mg/kg)	67.68	19.20	37	122
Mg (mg/kg)	33.97	27.91	7	162
Fe (mg/kg)	-	-	<7	14
Mn (mg/kg)	-	-	<2.5	
Cu (mg/kg)	-	-	<2.5	
Zn (mg/kg)	-	-	<4.5	
Cd (mg/kg)	-	-	<1	
Pb (mg/kg)	-	-	<1	
P (mg/kg)	358.68	75.39	245	577
Trehalose %	-	-	-	
Glucose %	30.6	8.1	18.6	81.7
Fructose %	38.7	9.2	27.2	101.5
Saccharose %	1.2	2.5	0.0	10.7
Melecitose %	0.1	0.0	0.0	0.2
Turanose %	3.3	1.2	1.4	10.9
Maltose %	1.4	1.08	0.2	7.6

III.2.1. Chemometric evaluation considering the botanical origin and the general characteristic parameters

Cluster analysis was used to look for similarities between samples, and more specifically the typifiction of honey samples, using 34 variables: pH, EC, moisture, a*, L*, b*, Pfund, Na, Ca, Mg, P, PK, Glucose, Fructose, Turanose, Maltose, *Genista, Eruca sativa, Retama, Capparis spinosa, Eucalyptus, Hedysarum, Atractylis serratuloides, Ziziphus lotus, Spartium junceum, Pimpinella anisum, Paronychia argentea t, Peganum harmala, Tamarix, Echium, Papaver rhoeas, Brassica napus, Bupleurum fruticosum*and *Myrtus,* considered according to our knowledge as the most important variables and especially the parameters that can mention the difference between a type of honey to the other, on the one hand according to the codes of the samples and on the other hand according to the geographical origins (Figure 22).

As we already know, to form clusters, the procedure starts with each observation in a separate group. It then combines the two observations closest to each other to form a new group. After recalculating the distance between the clusters, the two closest clusters are combined. This process was repeated until there were only 3 groups left in this case.

According to the results given by the clustering as well as our knowledge, the honey samples can be grouped into 11 types, citing (*Acacia*=1) known as Mimouza honey as declared by the beekeeper, (*Arbutus unedo*=1) mentioned by the letters Am and it is a bitter honey type, (*Atractylis serratuloides*=14) mentioned by the letter (S) except S6 and S16 that were considered as *Genista* types, (*Bupleurum*=1), (*Capparis spinosa*=5) as discovered types from samples were mentioned by R' and M letters respectively, (*Eruca sativa*=5) mentioned by the letter (H) whose nomination by the beekeepers was confirmed, (Eucalyptus=4), this is the case of four of the nine samples declared by the beekeepers as Eucalyptus honeys, the other five are considered as polyfloral honeys, (*Genista*=9), (*Hedysarum*=1) a sample already assumed by the beekeeper to be *Retama*, (*Retama*=6) Retem honey, of which one sample contains a good amount of *Spartium* pollen which is considered a case of contamination, and polyfloral=12, although the samples collected since the beginning were all considered as monofloral honeys.

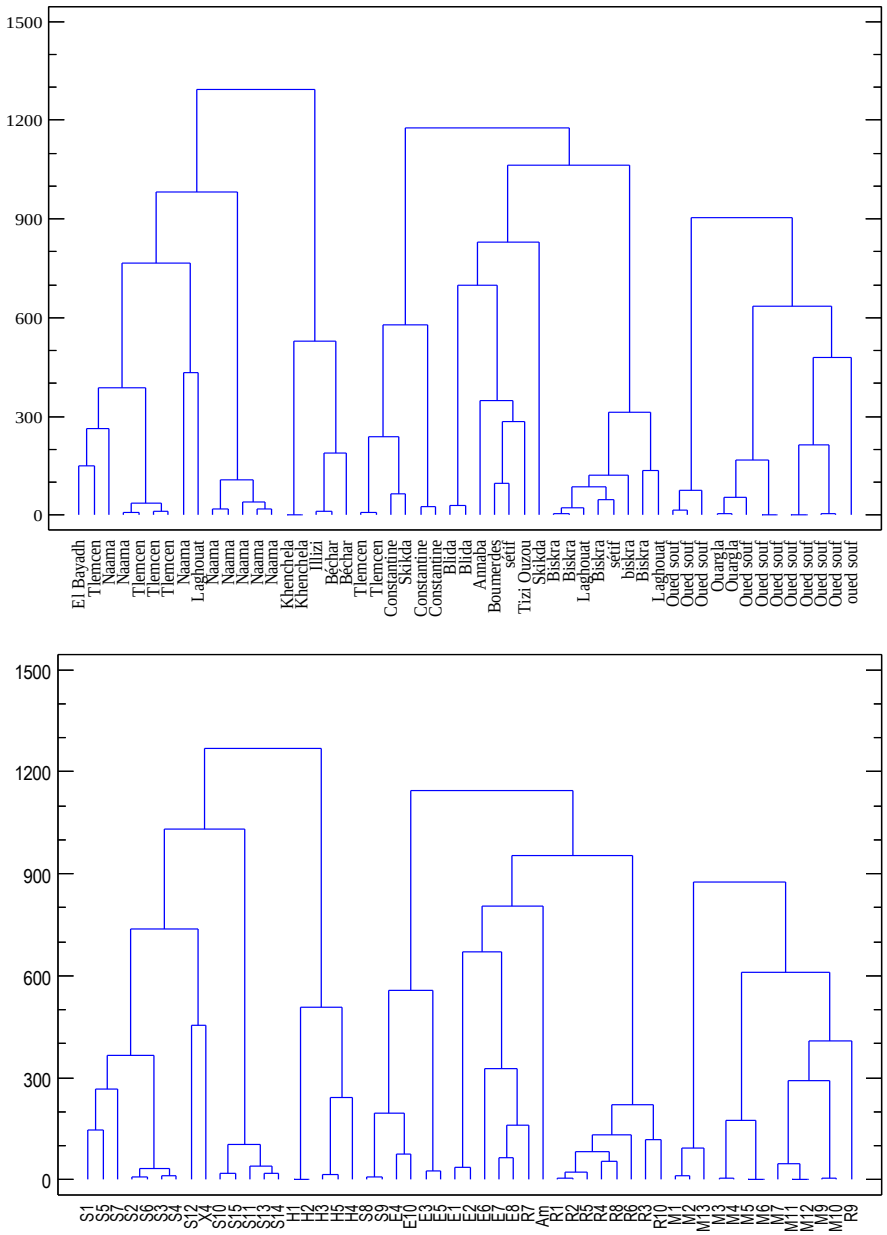


Figure 23: Clustering honey samples according to their botanical and geographical origins and their main characteristic parameters

For the grouping according to the geographical origin, it seems that the foraging and/or the harvest region influence strongly the distribution of the samples based on their measured parameters, it is mentioned in figure 23 that the close geographical regions are grouped together, it is the case of the regions of (El Bayadh, Tlemcen, Naama, Laghouat) which are regions of the north-west of Algeria whose climate is

The regions of Khenchela, Illizi and Bechar are semi-arid and arid regions located in the north-east for the first two regions and in the Saharan north-west. They are the origin of 5 samples of honey of *Eruca sativa* named locally (Harra) whose identical properties of the samples as well as the climatic proximity allowed grouping the regionsin the analytical scheme.

The Mediterranean coast regions as well as some Tell regions are close in the cluster, where the main species collected are Eucalyptus and multifloral honeys.

Other regions with arid climates are also grouped together, namely the regions of Biskra, Laghouat and Setif, which have been involved in the collection of *Retama* samples. The rest of the regions marked at the end of the cluster Oued Souf and Ouargla are two semi-arid north-western regions with a dry climate whose honeys come from some *Genista* and *Capparis*.

Some samples are not presented in the cluster as the case of *Acacia* honey (A), so they are far from being similar to the majority of the samples, as well as others considered as polyfloral.

The first group contains samples of honeys from *Atractylis* and *Eruca*, which are honeys from arid regions, with a light color and crystallized appearance. The second large group contains samples collected in the more humid regions of the Mediterranean coast, citing some polyfloral honeys and honeys of *Eucalyptus* and *Retama*. The last large group includes honeys from the region of Oued Souf and Ouargla are those of *Genista saharea* and *Capparis*.

A Principal Component Analysis was performed to study also the similarities as well as the differences between the different types of honey with the main physicochemical parameters, and then their geographical origins in order to group them (Figure 24). This is a dimensionality reduction method that transforms a large set of variables into a smaller set.

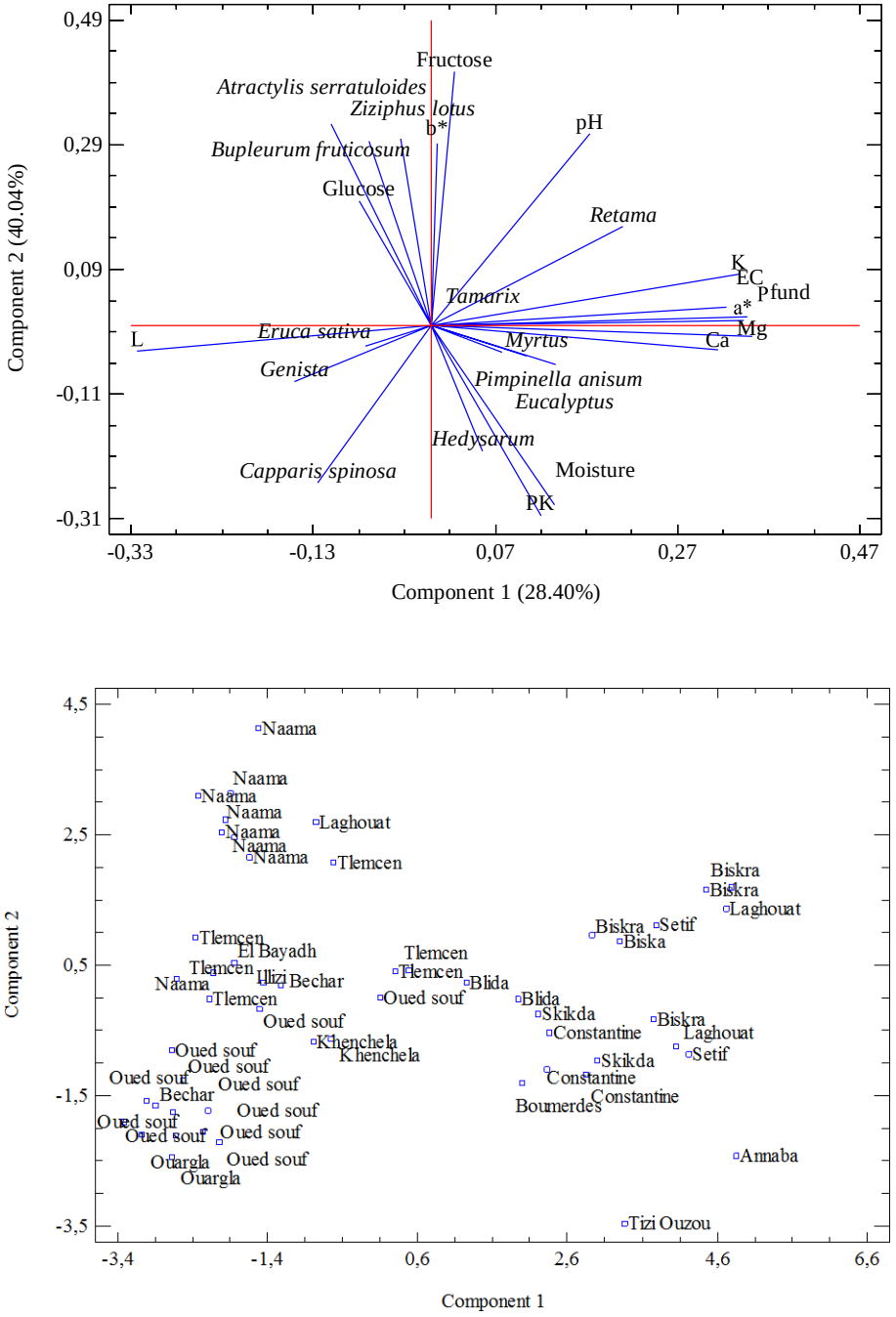


Figure 24: Principal component analysis (PCA) of the main pollen types and basic parameters of the studied honeys.

Twenty-five variables were selected for analysis: pH, EC, Moisture, K, a*, L*, b*, Pfund scale, Ca, Mg, PK, Glucose, Fructose, *Genista*, *Eruca sativa*, *Retama sphaerocarpa*, *Capparis spinose*, *Eucalyptus*, *Hedysarum*, *Atractylis serratuloides*, *Ziziphus lotus*, *Pimpinella anisum*, , *Tamarix*, *Bupleurum fruticosum* and *Myrtus*.

As is well known, the goal of the analysis is to obtain a small number of linear combinations of the variables present that explain most of the variability in the data. The correlation between the variables and the factors was represented in figure 24 whose first two components both account for 78.86 % of the variability.

In one hand, *Retama* pollen type is located near the parameters pH, EC, pfund scale, color coordinate a* and minerals. *Atractylis, Bupleurum* and *Ziziphus* pollen types are positively correlated with glucose and fructose contents and also with the b* color coordinate.*Eruca, Genista* and *Capparis* are positively correlated with L* color coordinate, and *Eucalyptus, Hedysarum, pimpinella* with *Myrtus* are located near to moisture and pollen content.On the other hand, geographical origins are grouped together according to the previous variables.

III.2.2. Discussion

Palynological analysis is used to know the botanical origin of honeys, and the interpretation of the pollen spectrum of each sample is a good starting tool for its characterization. Moreover, the Algerian territory is very heterogeneous, it is already composed of multiple structural units and each unit has different geographical and climatic characteristics and therefore a different vegetation cover.

As it was already mentioned in the results, the pollen families, none of them had the full frequency in relation to the number of samples, which can be explained by the variance of the geographical areas and their influence on the botanical coverage and then on the polinic spectrum of the honey samples.

Atractylis serratuloides honey samples, were collected in areas characterized by their arid or semi-arid climate near the Saharan territory. The Asteraceae are the most representative plants of the flora of arid regions (15%) with more than 352 species, among which *Atractylis* is very frequent, being mentioned 9 endemics (Le Houérou, 2001). More precisely, *A. serratuloides* has increased its distribution in recent years, as together with *P. harmala* and others such as *Nonea mucronata* or *Centaurea*, it occupies the degraded semi-arid steppes of northern Algeria. Today, these plants grow with other spontaneous plants in nitrogen enriched soils, near villages or water sources, so that other common herbaceous species like *Echium* or species of Apiaceae appear in the pollen spectra.

There are no scientific references dealing with the characteristics of this type of honey, it could be considered as an under-represented pollen type as a preliminary study in this case. It is known by its crystallized appearance, light color, called localy Sor, it is also widely produced and intended, according to the beekeepers, for the manufacture of pastries and yoghurts locally.

Retama honey samples with darker color, heavy appearance and increased odor, called localy honey of Retem, were obtained from *R. sphaerocarpa* of Fabaceae family, which is a Mediterranean plant well adapted to extreme drought conditions due to the development of molecular mechanisms allowing partial quiescence. This type of shrubby plant is abundant in steppes on deep soils and has an important ecological role in maintaining dunes and sandy soils, so that the valorization of honey production is important for the conservation of the ecosystem. The ecosystem is shared with other species of Fabaceae such as *Genista*, herbaceous plants and may also appear forest masses of *Eucalyptus*. The honey of this plant is poorly characterized, with the exception of a study on samples from the Spanish Mediterranean territory (Juan-Borrás *et al*., 2015). A sample of honey from a plant of the same family is detected as *Hedysarum*, considered as *Retama* by the beekeeper, which reinforces the importance of the pollen study.*Genista* and *Capparis* honeys, and despite their intense production, these types of honey are marketed indiscriminately, except in the Saharan region, where honey is generally quite limited, *G. saharae* honey being typical of this region.

The honey samples of *Eruca sativa*, localy named Harra honey, were obtained in the Sahara desert and a semi-arid East region. This plant is a cosmopolitan species used as food because of the health properties attributed to it (Alqasoumi, 2010). In Saharan areas, these plants are adapted to the particular ecology of the region and can occupy large areas of the territory and flower in a short period of time (Jafaar and Jafaar, 2019). This fact facilitates the collection of monofloral honeys and indeed in three samples the percentage of *Eruca* pollen grain was very high. On the contrary, two samples had a lower percentage of this pollen due to the presence of large amounts of *Peganum harmala* pollen localy named Harmel; those are samples from the arid south area of Khenchela. The beekeeping value of *P. harmala* is unknown, but as this plant is known to have a high alkaloid content, further research on the influence of this plant on honey composition is necessary (Kruzik *et al*., 2019).

Some pollen types were also found to be under-represented pollen grains. This is the case of *Acacia* pollen in the Mimouza honey and *Arbutus* pollen in the Lenj honey.

Most honey samples were collected in spring, with the exception of honey from Arbutus, an important plant for winter honey in the Mediterranean regions (Juric *et al*., 2020).

The introduction of *Eucalyptus* in the middle of the 19th century, allowed it to become one of the most important plants for honey production in the Tellian region, and one of the most produced honeys in many parts of the world. Regarding Acacia honey, its beekeeping value for nectar production is debated, but beekeepers have suggested that honey harvesting from this plant is possible. In addition, nectar secretion has been found in different *Acacia* species (Adgaba *et al*., 2017). Honey from *A. ehrenbergina*, *A. edgeworhi* from Yemen, *A. tortilis* from Oman and some *Acacia* species from Saudi Arabia have been mentioned in the scientific literature (Al-Mamary *et al*., 2002). Among the honey types already mentioned, only Eucalyptus honey is well known, the others are poorly studied and even some of them are described for the first time in the study.

III.3. Basic characterization of honey types

The honey types are separated, and a dissociated presentation is feasible. Each basic parameter in the following is presented by Box and whister plot by analysis of variance which is mainly intended to compare the means of the different levels, so it allows observing the dispersion and symmetry of the data set, and at the same time allowing us to compare the results.

III.3.1. Pollen number/1g (PK)

The total average of the pollen content / g in the present honeys is 65159.5±66211 with a minimum of 5000 and a maximum of 439000.

The honeys with the highest pollen content are respectively the *Hedysarum* sample with a value of 138250 followed by the *Capparis* type 129200± 26026.7, then the *Eucalyptus* type 100375± 38768.8, all three considered as class V honeys according to Maurizio's (1939) classification, also as extremely pollen-rich honeys. *Genista* pollen type honeys are for their globality in class IV with an average of 57656.3± 48426 with the samples considered as polyfloral.

Honeys of *Acacia*, *Arbutus*, *Atractylis*, *Eruca* and *Retama* are of class III, despite their differences in value (Table 10).

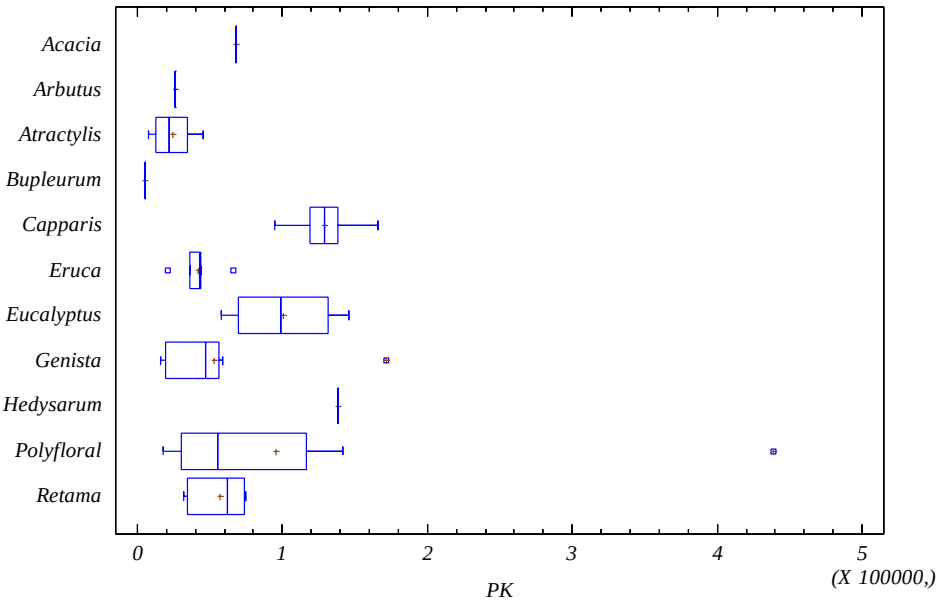


Figure 25: Box and Whisker Plot representation of pollen content/g of different honey types.

The *Beuplereum* honey sample is the least rich in pollen with a value of 5000 pollens/g, it is a class II sample.

Table 10: Descriptive analysis of the pollen content of the studied honeys.

Botanical Origin	*Count*	Mean	SD	Maurizo's class	Lower limit	Upper limit	Min	Max
Acacia	1	67750	-	III	-	-	-	-
Arbutus	1	26250		III	-	-	-	-
Atractylis	14	24392.9	12440.5	III	1112.15	47673.6	7750	45500
Bupleurum	1	5000	-	II	-	-	-	-
Capparis	5	129200	26026.7	V	90243.9	168156	94750	165750
Eruca	5	41900	16368.4	III	2943.93	80856.1	21000	66250
Eucalyptus	4	100375	38768.8	V	56820.8	143929	58250	145500
Genista	9	57656.3	48425.9	IV	26858.8	88453.7	18250	171500
Hedysarum	1	138250		V	-	-	-	-
Polyfloral	12	95208.3	115772	IV	70062.3	120354	17750	439000
Retama	6	56625	20425.3	III	21063.1	92186.9	32000	75000
Total	59	65159.5	66211	-	-	-	5000	439000

Globally, since the P-value = 0.0529 of the F-test is greater than or equal to 0.05, there is not a statistically significant difference between the mean pK from one level of botanical origin to another at the 95.0% confidence level. But 5 pairs show statistically significant differences at this confidence level that could be detected from table 11.

Table 11: Homogeneous and different groups for pollen content by botanical origin.

Level	Homogeneous Groups
Bupleurum	XXX
Atractylis	X
Arbutus	XXX
Eruca	XX
Genista	XX
Retama	XXX
Acacia	XXX
Polyfloral	XX
Eucalyptus	XX
Capparis	X
Hedysarum	XXX

III.3.2. Moisture content

The average moisture content of the analyzed honeys is presented in the table 12. Percentages between 12.23% and 22.57% are noted. The graph shows that the water content presents significant differences according to the types of honey. (Figure 26), as the general averages are not similar as can be seen in the table 12.

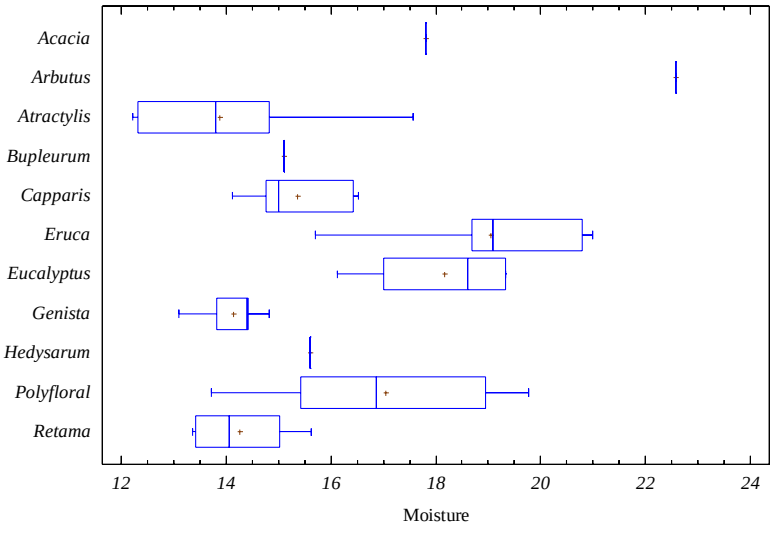


Figure 26: Box and Whisker Plot representation of the moisture content of different honey types.

The *Arbutus* honey sample is the wettest according to the results with a content of 22.57%, followed by an equally high percentage of samples of *Erica sativa* honey with

an average of 19.06% ± 2.13, whose minimum value is 15.7% and the maximum for a single sample from the region of Illizi of 21%. These two mentioned types are considered out of the water content standard according to IHC for honeys from non-tropical regions, the rest of the samples are below the threshold, whose average values are between 14.15% for *Atractylis* honeys and 18.17 for *Eucalyptus* honeys.

Table 12: Descriptive analysis of the moisture content of the studied honeys.

Botanical origin	Mean	SD	Lower limit	Upper limit	Min	Max
Acacia	17.8	-	-	-	-	-
Arbutus	22.57	-	-	-	-	-
Atractylis	13.90	1.82	13.29	14.50	12.23	17.57
Bupleurum	15.1	-	-	-	-	-
Capparis	15.37	1.06	14.35	16.40	14.13	16.53
Eruca	19.06	2.13	18.04	20.08	15.7	21
Eucalyptus	18.17	1.52	17.03	19.31	16.13	19.33
Genista	14.14	0.54	13.39	14.91	13.1	14.83
Hedysarum	15.6	-	-	-	-	-
Polyfloral	17.04	1.98	16.38	17.70	13.73	19.77
Retama	14.27	0.97	13.34	15.19	13.37	15.63
Total	15.72	2.48			12.23	22.57

A minimum value of all samples was noted for a sample of *Atractylis* as mentioned in the diagram with a value of 12.23%.The honeys of *Retama* and *Genista* are not far for their values with a percentage of moisture around 14%. *Capparis* and *Hedysarum* honeys are also close in terms of value.

According to the ANOVA test, since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the means of moisture from one level of botanical origin to another at the 95.0% confidence level, and 25 pairs have statistically significant differences at this confidence level. 5 homogeneous groups are identified using columns of Xs. Within each column, levels containing Xs form a group of means within which there are no statistically significant differences.

Table 13: Homogeneous and different groups for moisture by botanical origin.

Level	Homogeneous Groups
Atractylis	X
Genista	X
Retama	X
Bupleurum	XXX
Capparis	XX
Hedysarum	XXXX
Polyfloral	XX
Acacia	XXX
Eucalyptus	XX
Eruca	XX
Arbutus	X

III.3.3. Electrical conductivity

The results of the electrical conductivity parameter of the honey samples, derived from the mineral richness, are presented in the following diagram, with an average value of 377.97± 207.08 us/cm, a minimum of 164.67us/cm and a maximum of 874.67us/cm.

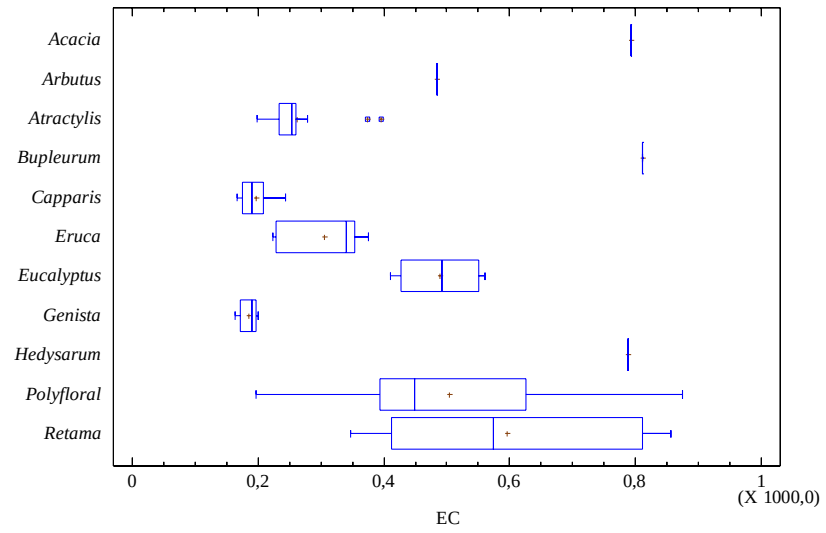


Figure 27: Box and Whisker Plot representation of the electrical conductivity of different honey types.

The highest average conductivity values are noted for the following three honey samples: *Bupleurum* 810.67 µs/cm, *Acacia* 792 µs/cm and *Hedysarum* 788.33 µs/cm. The lowest average values are noted for the types of *Genista* 185.80± 13.92 µs/cm,*Capparis* 197.10 ± 31.00 µs/cm, and *Atractylis* 262.39± 56.52 µs/cmhoney types, these are the three types that also have the lowest standard deviation, as the table 14 shows, with the presence of outliers for the *Atractylis* type (Figure 27).

Retama honeys have a high mean value of 595.39us/cm, but a very large standard deviation SD= 205.29, reflecting the large difference between samples in the same type. A similar remark for polyfloral honeys 503.518 ± 195.97 µs/cm.*Eruca* and *Eucalyptus* honeys are with average values (304.864 ± 72.79 µs/cm) and (489 ± 72.58 µs/cm) respectively.

Table 14: Descriptive analysis of the EC of the studied honeys.

Botanical Origin	Mean	SD	Lower limit	Upper limit	Min	Max
Acacia	792	-	-	-	-	-
Arbutus	483.33	-	-	-	-	-
Atractylis	262.39	56.52	215.95	308.83	199.67	396.33
Bupleurum	810.67	-	-	-	-	-
Capparis	197.07	31.00	119.35	274.78	166.67	244.33
Eruca	304.86	72.79	227.15	382.58	224.33	376.33
Eucalyptus	489	72.58	402.11	575.89	410.67	561.33
Genista	185.80	13.92	127.87	243.72	164.67	200.03
Hedysarum	788.33	-	-	-	-	-
Polyfloral	503.52	195.97	453.35	553.68	197.23	874.67
Retama	595.39	205.29	524.45	666.33	346.67	855.67
Total	377.97	207.08			164.67	874.67

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean ECs from one level of botanical origin to the other at the 95.0% confidence level. 32 pairs of the honey types these differences and 32 pairs were significantly different.

Table 15: Homogeneous and different groups for EC by botanical origin.

Level	Homogeneous Groups
Genista	X
Capparis	X
Atractylis	XX
Eruca	XX
Arbutus	XXX
Eucalyptus	X
Polyfloral	X
Retama	XX
Hedysarum	X
Acacia	X
Bupleurum	X

III.3.4. pH

The pH of the 59 samples ranged from values of 3.6 to 5.42, showing a mean value of 4.07, with a lower limit of the 95% mean of 4.73 and an upper limit of 5.05 (Table 16).

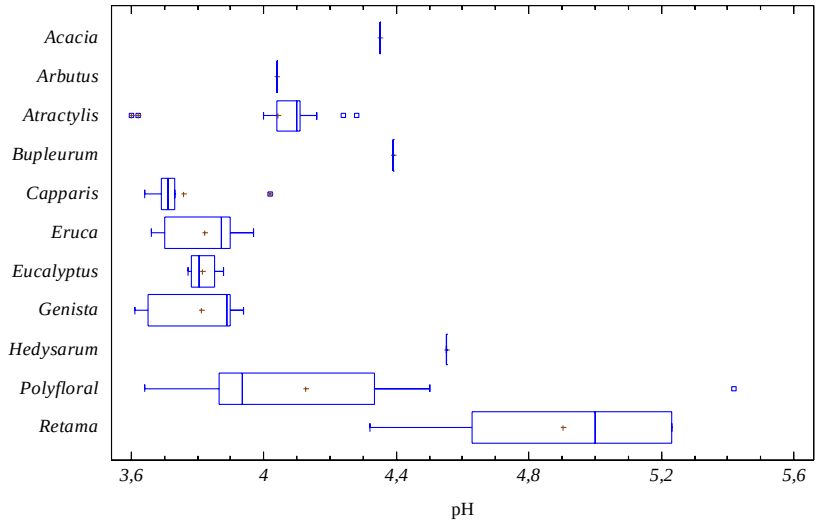


Figure 28: Box and Whisker Plot representation of the pH values of different honey types.

The minimum value was measured in a honey of *Atractylis* by 3.6 and the maximum value (5.23) in the honeys *Retama.*

The average pH values of the samples *of Capparis*, *Eruca*, *Eucalyptus* and *Genista* were between 3.76 and 3.82 (table 16), these values were the lowest, compared to the rest of the honey types.The *Retama* honey samples had the highest values and the highest average with a range of (4.2-5.23), thus these honeys with those of the multiflorals present the two broadest intervals.

Since the P-value of the F-test is less than 0.05, there is a statistically significant difference between the mean pH from one level of Botanical origin to another at the 95.0% confidence level.

Table 16: Descriptive analysis of the pH of the studied honeys.

Botanical origin	Mean	SD	Lower limit	Upper limit	Min	Max
Acacia	4.35	-	-	-	-	-
Arbutus	4.04	-	-	-	-	-
Atractylis	4.04	0.20	3.93	4.15	3.6	4.28
Bupleurum	4.39	-	-	-	-	-
Capparis	3.76	0.15	3.57	3.94	3.64	4.02
Eruca	3.82	0.13	3.63	4.01	3.66	3.97
Eucalyptus	3.82	0.05	3.60	4.02	3.77	3.88
Genista	3.81	0.14	3.67	3.95	3.61	3.94
Hedysarum	4.55	-	-	-	-	-
Polyfloral	4.13	0.48	4.01	4.25	3.64	5.42
Retama	4.90	0.38	4.73	5.07	4.32	5.23
Total	4.07	0.42			3.6	5.42

III.3.5. Hydroxylmethylfurfural (HMF) content

The HMF measured in the studied honey samples is considered low, with a total average value of 6.8 mg/kg, a lower value of 0 mg/kg for eight samples from *Atractylis*, *Eruca*, *Retama*, *Hedysarum* and polyfloral samples, and a higher value of 29.15 mg/kg for a sample of *Retama* type.

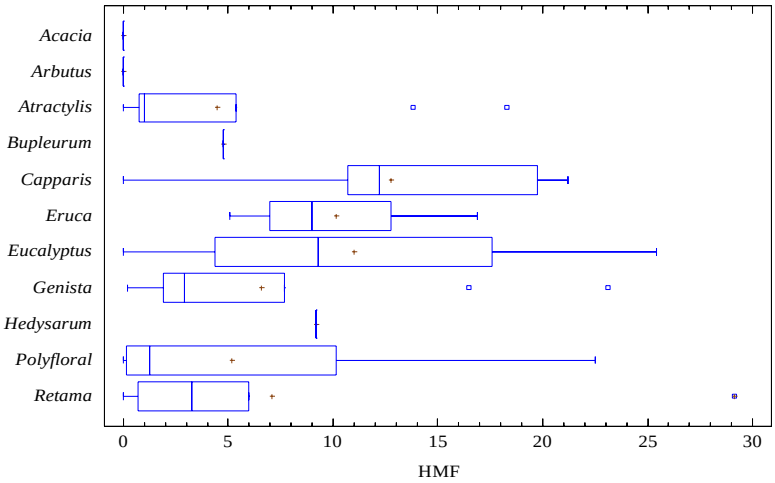


Figure 29: Box and Whisker Plot representation of the HMF content of different honey types.

The highest average HMF content corresponds to the *Capparis* honey samples (13.83± 7.6 mg/kg), followed by that obtained for the *Eucalyptus* (12.54± 3.3 mg/kg) and the polyfloral honey samples (9.53± 7.8). *Hedysarum*, *Arbutus* and *Atractylis* honeys have the lowest HMF content. It is obvious that some significant differences exist between several peers, but it is not necessary to mention them in this case.

Table 17: Descriptive analysis of hydroxymethylfurfural (mg/kg) of honey samples.

Botanical origin	Mean	Standard deviation	Lower limit	Upper limit	Min	Max
Acacia	4.8	-	-	-	-	-
Arbutus	0.2	-	-	-	-	-
Atractylis	3.29	5.69	0.06	6.02	0	18.3
Bupleurum	5.08	-	-	-	-	-
Capparis	13.83	7.60	9.27	18.39	3.99	21.18
Eruca	1.468	0.91	-3.09	6.03	0	2.44
Eucalyptus	12.54	3.30	7.44	17.64	9.0	16.89
Genista	6.50	7.57	3.10	9.90	0.33	23.11
Hedysarum	0	-	-	-	-	-
Polyfloral	9.53	7.87	6.59	12.48	0	25.4
Retama	7.34	11.34	3.18	11.50	0	29.15

III.3.6. Diastase Index

The average diastase content obtained for all samples was 15.43±8.45 ID.

The minimum and maximum values of this enzyme were 2.32 and 45.89 ID, measured in an *Arbutus* honey and *Hedysarum* honey, respectively.

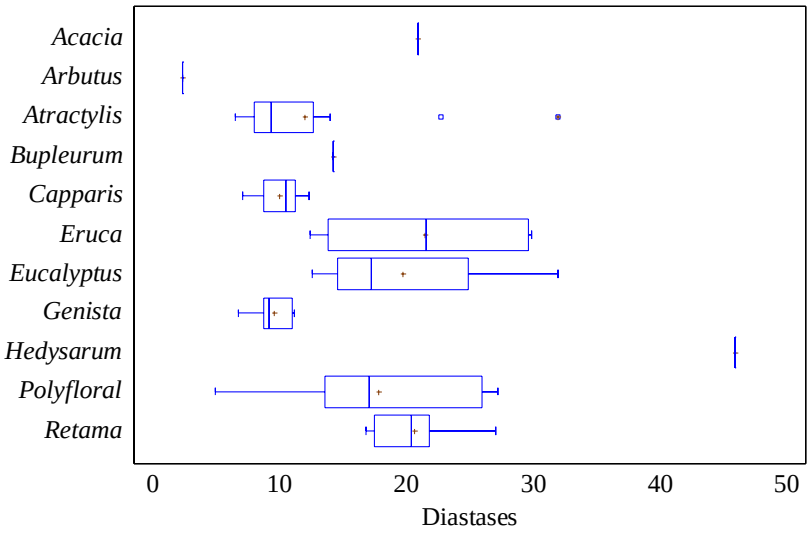


Figure 30: Box and Whisker Plot representation of the diastase content of different honey types.

Higher values were attributed to the *Hedysarum* sample followed by *Eruca*, *Acacia*, *Retama* and *Eucalyptus* honeys by the following average values: 45.89, 21.45 ± 8.31, 20.88, 20.65 ± 3.76, 19.72 ± 8.43 respectively.

The minimum values were presented by the sample of *Arbutus*, *Capparis* and *Genista* honeys with the following average values: 2.32, 9.99± 2.06, 9.58 respectively. As already mentioned for HMF, from the figure 30, it is obvious that significant differences exist between several peers, but it could be not necessary to mention them in this case.

Table 18: Descriptive analysis of diastase (ID) of the honey samples.

Botanical origin	Mean	SD	Lower limit	Upper limit	Min	Max
Acacia	20.88	-	-	-	-	-
Arbutus	2.32	-	-	-	-	-
Atractylis	11.97	7.13	9.57	14.36	6.51	31.91
Bupleurum	14.21	-	-	-	-	-
Capparis	9.99	2.06	5.98	13.99	7.12	12.31
Eruca	21.45	8.31	17.44	25.46	12.43	29.85
Eucalyptus	19.72	8.43	15.23	24.20	12.54	31.91
Genista	9.58	1.47	6.60	12.57	6.79	11.15
Hedysarum	45.89	-	-	-	-	-
Polyfloral	17.78	7.69	15.19	20.37	4.92	27.23
Retama	20.65	3.76	16.99	24.31	16.83	27.04
Total	15.43	8.45			2.32	45.89

III.3.7. Color estimation

The color of honey is a primary sensory characteristic that determines consumer choice. During the collection of the present samples, an apparent difference in the intensity of their colors was observed. The two methods used (Pfund's scale and CIEL*a*b*), gave results that allowed the samples to be classified correctly.

III.3.7.1. Color by the Pfund scale method

The studied honey types from different parts of Algeria show colors ranging from white to dark amber. In Pfund scale measurement the color is ranged from 8 mm to a maximum of 150 mm (Table 20).

A statistically significant difference between the mean Pfund scale from one level of botanical origin to another at the 95.0% confidence level. Also 33 type pairs, indicating that these pairs show statistically significant differences at the 95.0% confidence level.

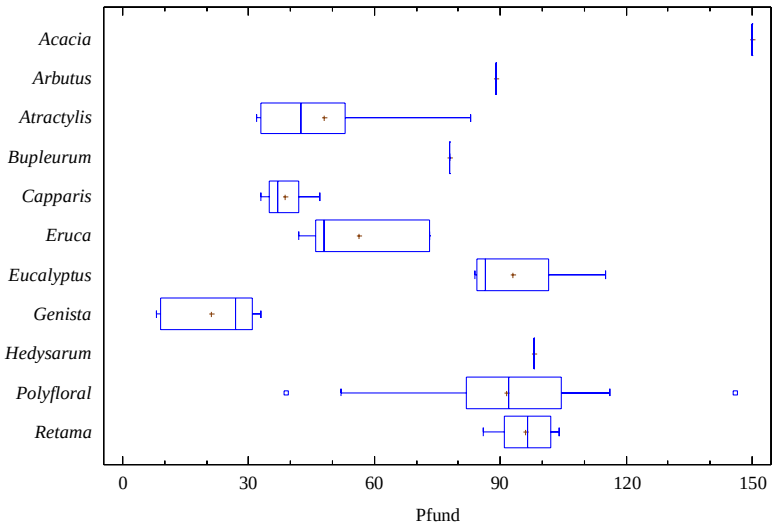


Figure 31: Box and Whisker Plot representation of the pfund scale of different honey types.

Table 19: Homogeneous and different groups for Pfund scale by botanical origin.

Level	Homogeneous Groups
Genista	X
Capparis	XX
Atractylis	XX
Eruca	XXX
Bupleurum	XXX
Arbutus	XX
Polyfloral	X
Eucalyptus	X
Retama	X
Hedysarum	X
Acacia	X

The darkest types of honey are those between amber and dark amber colors, they are presented by *Acacia, Hedysarum, Retama, Eucalyptus*, polyfloral and *Arbutus*, whose average value is between 89 and 150 mm.

The honeys of *Retama* of amber scales color have a mean value of 96± 6.72 with a range of 86 to 104, whose 95% confidence interval for the mean was between 85.71 and 106.29 mm.Those of *Eucalyptus* are not far from these values with a mean value of 93± 14.76 ranging from 84 to 115, and the 95% confidence interval for the mean was between 80.40 and 105.60 mm.The lightest honeys are *Genista, Capparis* and *Atracylis* types with average values of: 21± 11.76, 38.8± 5.67 and 47.93± 16.25 respectively, with White and extra light amber color scales.

The 95% confidence interval for the means of these three types mentioned was between 12.60 and 29.40 mm, 27.53 and 50.07 and 41.20 and 54.66 respectively.

Table 20: Descriptive analysis of pfund content of the honey samples.

Botanical origin	Mean	SD	Scale	Lower limit	Upper limit	Min	Max
Acacia	150	-	Dark amber	-	-	-	-
Arbutus	89		Amber	-	-	-	-
Atractylis	47.93	16.25	Extra light amber	41.20	54.66	32	83
Bupleurum	78	-	Light amber	-	-	-	-
Capparis	38.8	5.67	Extra light amber	27.53	50.07	33	47
Eruca	56.4	15.31	Light amber	45.13	67.67	42	73
Eucalyptus	93	14.76	Amber	80.40	105.60	84	115
Genista	21	11.76	White	12.60	29.40	8	33
Hedysarum	98	-	Amber	-	-	-	-
Polyfloral	91.33	27.94	Amber	84.10	98.61	39	146
Retama	96	6.72	Amber	85.71	106.29	86	104
Total	64.32	33.90				8	150

III.3.7.2. Color according to the CIEL*a*b* method

The results of the CIEL*a*b* coordinates showed that the value of brightness L* (L=0, black, and L=100, coloreless) had a general average of 75.2± 9.6, with a range between 55.9 and 108. Considered as the most luminous honey types those of *Genista* 84.4± 4.1 and *Eruca* 84.0± 14.1, and those with a lower value of lumunosity are *Hedysarum* 61.4, *Acacia* 61.9, *Retama* 65.5±1.8 and *Eucalyptus* 66.5±3.9.

Regarding the coordinate a*, where the positive values reflect a reddish color while the negative values reflec the greenish one, the most reddish honeys are those of *Hedysarum, Acacia, Bupleurum* and *Retama* types, while the types with greenish nuances are those of *Genista, Capparis, Eruca* and *Atractylis*.

For the blue/yellow b* coordinate (b >0, yellow, and b <0, blue), the yellowish honey types were *Bupleurum, Atractylis, Hedysarum, Retama,* and *Capparis*, with mean values of 44.7, 25.1 ± 6.3, 22.2, 21.8 ± 2.6, and 21.1 ± 0.6.

Table 21: Descriptive analysis of CIEL*a*b* coordinates of the honey samples.

Botanical origin	Mean			Lower limit			Upper limit			Min			Max		
	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*	L*	a*	b*
Acacia	61.9	8.84	17.2	-	-	-	-	-	-	-	-	-	-	-	-
Arbutus	71.6	3.98	21.2	-	-	-	-	-	-	-	-	-	-	-	-
Atractylis	78.6± 4.9	-1.51± 1.9	25.1± 6.3	76.0	-2.61	22.8	81.1	- 0.42	27. 5	71. 2	-3.09	17.0	803	3.78	43
Bupleurum	71,2	7.85	44.7	-	-	-	-	-	-	-	-	-	-	-	-
Capparis	81.3± 0.9	-2.90± 0.9	21.1± 0.6	77.2	-4.70	17.1	85.5	- 1.04	25. 0	80. 2	-3.99	20.4	82	-1.94	22
Eruca	84.0± 14.1	-2.19± 5.5	18.5± 13.1	79.8	-4.02	14.5	88.2	- 0.36	22. 4	73. 7	-11.17	4.8	108	2.6	26
Eucalyptus	66.5± 3.9	5.6± 1.6	20.1± 2.0	61.8	3.55	15.7	71.2	7.65	24. 5	62. 1	3.3	17.9	72	6.93	23
Genista	84.4± 4.1	-3.42± 1.5	17.7± 4.2	81.3	-4.78	14.8	87.6	- 2.06	20. 7	80. 0	5.0	10.7	90	-1.28	25
Hedysarum	61.4	10.4	22.2	-	-	-	-	-	-	-	-	-	-	-	-
Polyfloral	68.8± 8.5	4.5± 4.2	22.6± 6.3	66.1	3.33	20.1	71.5	5.69	25. 1	55. 9	-5.03	11.5	86	9.73	34
Retama	65.5± 1.8	7.2± 1.3	21.8± 2.6	61.6	5.52	18.2	69.3	8.86	25. 4	63. 6	6.2	20.0	68	9.59	27
Total	75.2± 9.6	1.2± 5.0	22.0± 6.9							55. 9	-11.17	4.8	108	10.42	45

The representation figures inserted below, along with Fisher's Least Significant Difference table, show the significant differences between the values of each color coordinate between several pairs of honey type.

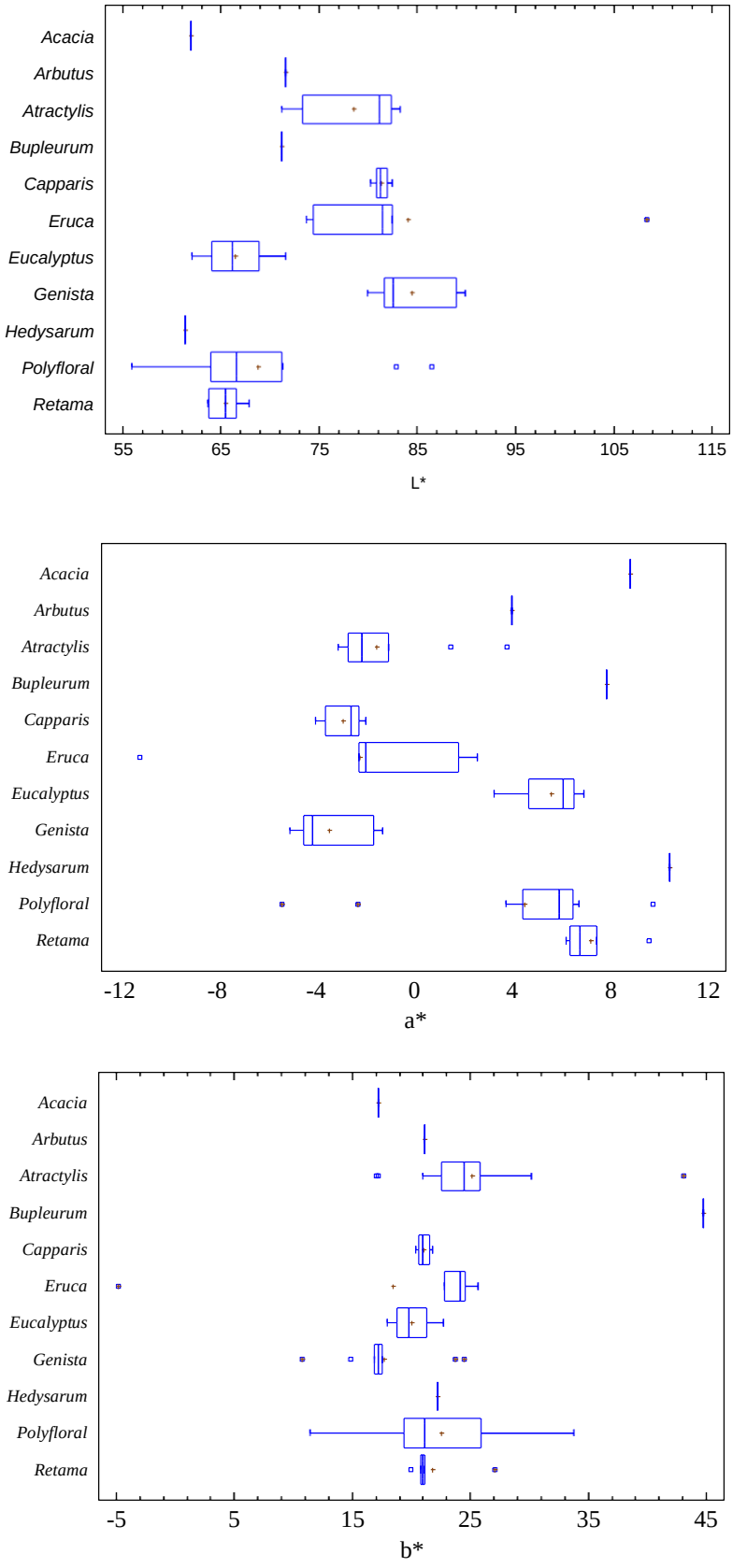


Figure 32: Box and Whisker Plot representation of the CIEL*a*b* coordinates different honey types.

Table 22: Homogeneous and different groups for CIEL*a*b* coordinates by botanical origin.

Level	L* Homogeneous Groups	a*Homogeneous Groups	b*Homogeneous Groups
Hedysarum	X	X	XX
Acacia	X	X	X
Retama	X	XX	X
Eucalyptus	X	XX	XX
Polyfloral	X	XX	XX
Bupleurum	XXX	X	XX
Arbutus	XXX	X	XX
Atractylis	X	X	XX
Capparis	XX	X	XX
Eruca	XX	X	X
Genista	X	X	X

As can be seen in the PCA (Figure 33), the trend coordinate (a*) between red and green is related in this case for the present honeys studied to the pfund scale and the main mineral composition. The lightness coordinate (L*) and the yellow and blue trending coordinate (b*) are not affected by the proportionality.

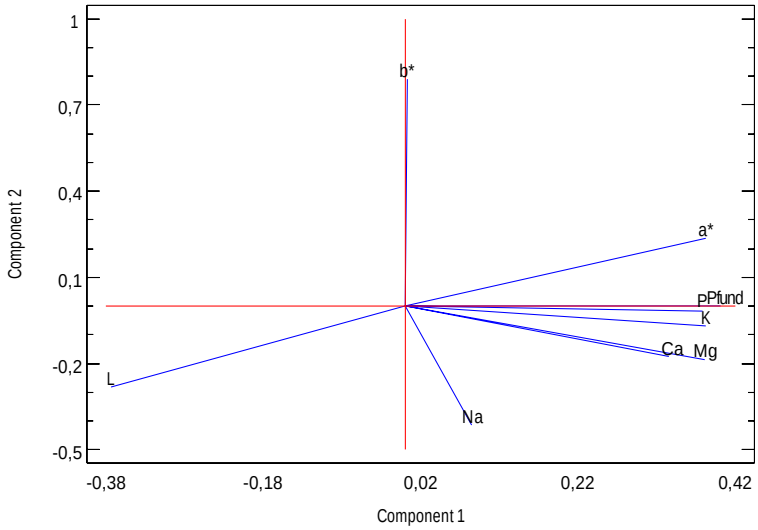


Figure 33: Principal component analysis (PCA) for the main color components the studied honeys.

III.3.8. Mineral content

In order to study the content of mineral elements of nutritional interest in honey samples, the following elements were quantified: Na, K, Ca, Mg and P.

According to the results K^+ is the most abundant mineral, compared to the other minerals identified in the honeys, with an average content of 986.76± 817.59 mg/kg (Table25). The data on this mineral showed a wide variation, from a minimum value of 174 to a maximum value of 3563 mg/kg. The highest values of K were found mainly in the acacia honey sample and Retama honeys by values of 3563 and 2284.83± 274.4 respectively.

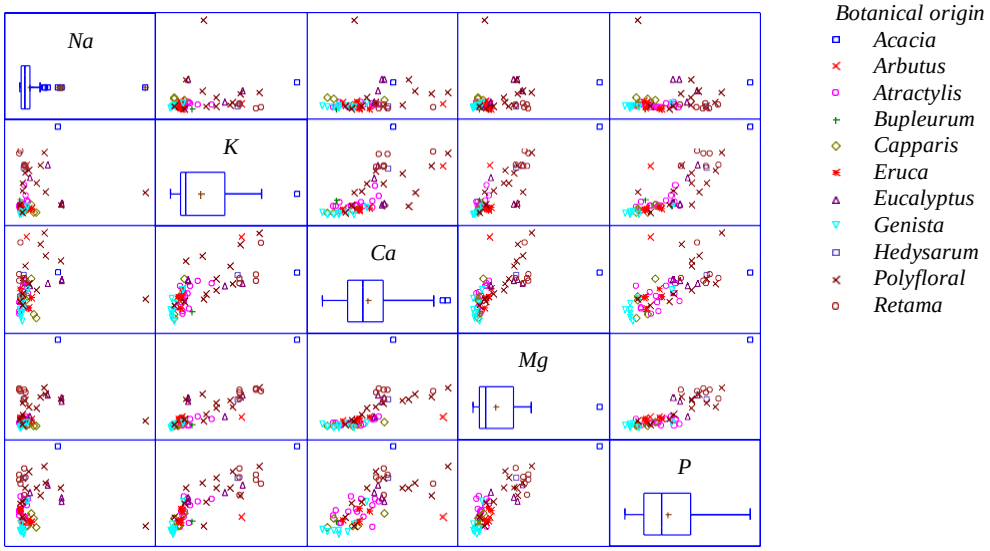


Figure 34: Representation of average mineral content for each type of honey.

Table 23: 95.0% confidence intervals of the main minerals.

Total content	Lower limit	Upper limit
Na	52.28	100.27
K	771.7	1201.7
Ca	62.82	72.97
Mg	26.69	41.49
P	338.46	378.43

In addition, statistically, the K content of the honeys showed significant differences in the average content with the rest of the honeys analyzed ($p<0.05$).

The lowest concentrations for the whole samples were noted for Mg with a total average of 34.09± 28.14. *Acacia* and *Retama* honeys were also the richest in this

mineral with mean values of 162 and 70.5± 6.2 respectively. Statistically in addition to K, Mg is significantly different at P.

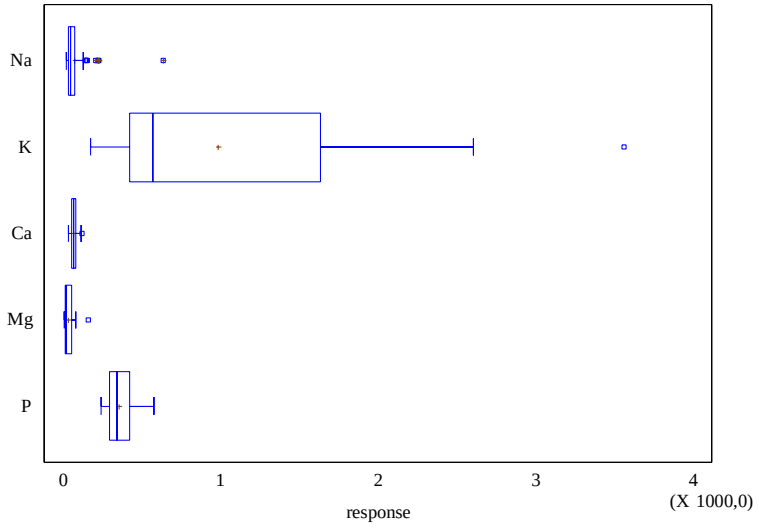


Figure 35: Representation of the average content of minerals in all honey samples.

Table 24: Homogeneous and different groups of minerals by botanical origin.

	Homogeneous Groups
Mg	X
Ca	X
Na	X
P	X
K	X

Table 25: Descriptive analysis of the content of some minerals (mg/kg) of the studied honey types.

Samples M±SD (Interval)	Na mg/kg	K mg/kg	Ca mg/kg	Mg mg/kg	P mg/kg
Acacia	207	3563	85	162	577
Arbutus	54	2071	118	27	312
Atractylis	44± 15.20 (28-70)	617.36± 235.10 (342-1274)	60.29± 10.22 (45-76)	17.36± 4.92 (11-27)	335.93± 36.35 (277-390)
Bupleurum	25	751	47	15	300
Capparis	78.6± 22.15 (53-105)	365.40± 123.07 (253 -549)	56.8± 15 (41-79)	14,6± 2,6 (13-19)	294.6± 21.7 (277-327)
Eruca	46.2± 25.76 (25 -79)	421.2± 36.68 (361-460)	60.8± 6.46 (51-69)	24.4± 3.05 (20-28)	315.6± 22.6 (296-344)
Eucalyptus	171.75±73.96 (73 -229)	1258.25± 743.33 (630-2120)	75.75± 2.99 (72-79)	53.75± 16.99 (29-67)	402.5± 27.7 (374-438)
Genista	38.75± 11.71 (26 -62)	249.88± 105.62 (174-488)	49.88± 9.78 (37-67)	11± 3.80 (7-17)	275.5± 40,1 (245-371)
Hedysarum	42	1979	82	59	461
Polyfloral	135.33± 168.03 (24-634)	1295.92± 675.31 (269-2562)	80.83± 22.64 (53-122)	45.83	405. 4± 65.8 (280-501)
Retama	39.17± 12.35 (26 -55)	2284.83± 274.4 (2027-2608)	83.67± 14.15 (74-112)	70.5± 6.2 (59-76)	451.2± 34.2 (393-494)
Total	76.28± 91.26 (24-634)	986.76± 817.59 (174-3563)	67.9± 19.3 (37-122)	34.09± 28.14 (7-162)	358.45± 76.02 (245-577)

III.3.9. Sugar content

Six sugars were identified in the analyzed honeys: Glucose, Fructose, Melicitose, Turanose, Maltose and Saccharose. They constitute the main component of honey. They are the main component of honey. The average total content is 72.67± 6.13%, with a range between 47.53 and 83.77%. The glucose and fructose sugars are the main sugars of the honey, always exceeding 50% of the total sugars of this food in pure state.

Table 26: Descriptive analysis of the content of some sugars (%) of the studied honey types.

Samples M± SD (Interval)	Glucose (%)	Fructose (%)	(F/G)	Saccharose (%)	Melecitose (%)	Turanose (%)	Maltose (%)
Acacia	29.0	36.6	1.26	0.0006	0.03	3.0	1.4
Arbutus	29.7	37.8	1.27	0	0.04	1.9	0.7
Atractylis	32.27± 2.89 (25.4-37.0)	39.81± 3.34 (29.6-42.5)	1.24	0.47± 0.34 (0.1-0.9)	0.05± 0.05 (0.0004-0.16)	3.2± 0.39 (2.4-3.6)	1.56±0.50 (0.8-2.2)
Bupleurum	37.0	40.0	1.08	0.11	0.04	2.0	1.5
Capparis	28.3± 2.14 (24.8-30.6)	35.32± 1.33 (33.5-36.5)	1.26	0.38± 0.21 (0.12-0.6)	0.10± 0.03 (0.06-0.13)	3.08± 0.34 (2.7-3.6)	1.12± 0.40 (0.7-1.6)
Eruca	36.0± 2.20 (33.7-39.0)	38.36± 1.83 (36.6-41.2)	1.07	0.10± 0.10 (0.002-0.25)	0.08± 0.05 (0.02-0.12)	2.72± 0.25 (2.3-2.9)	0.42± 0.13 (0.2-0.5)
Eucalyptus	31.02± 0.94 (29.7-31.7)	38.67± 1.99 (37.1-40.9)	1.25	0.253± 0.12 (0.112-0.4)	0.10± 0.01 (0.09-0.11)	2.93± 0.49 (2.3-3.4)	1.18± 0.21 (1.0-1.4)
Genista	26.5± 4.18 (22.7-35.7)	34.38± 3.27 (30.7-39.5)	1.31	4.76± 4.13 (0.6-10.7)	0.05± 0.03 (0-0.10)	2.97± 0.62 (2.2-3.9)	2.21± 0.19 (1.9-2.5)
Hedysarum	28.6	37.0	1.29	0.1	0.03	3.1	0.5
Polyfloral	27.92± 3.64 (18.6-32.2)	36.78± 3.29 (27.2-40.1)	1.34	0.09± 0.15 (0.001-0.5)	0.07± 0.05 (0.01-0.12)	3.38± 1.05 (1.4-5.4)	1.04± 0.52 (0.2-2.0)
Retama	27.22± 1.60 (24.7-29.4)	38.3± 0.42 (37.6-38.8)	1.41	0.22± 0.13 (0.1-0.4)	0.04± 0.01 (0.02-0.05)	3.6± 0.24 (3.2-3.9)	1.0± 0.43 (0.3-1.5)
Total	29.8± 4.10 (18.6-39.0)	37.48± 3.22 (27.2-42.5)	1.27	0.96± 2.27 (0.001-10.7)	0.06± 0.04 (0-0.16)	3.12± 0.66 (1.4-5.4)	1.30± 0.63 (0.2-2.5)

The predominant sugar Glucose in the honeys studied is with an average content of 29.8± 4.10 % (table 26). This sugar varied between a minimum of 18.6% quantified in a polyfloral sample and a maximum of 39.0% quantified in a sample of *Eruca*.

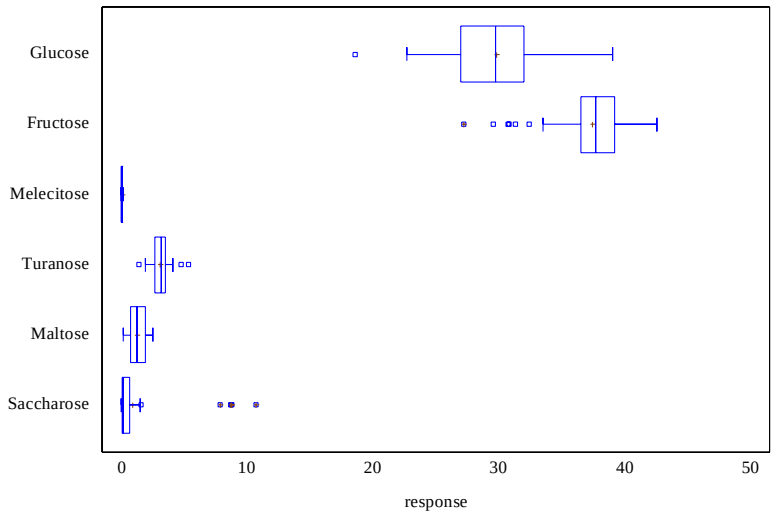


Figure 36: Representation of the average content of sugars in all honey samples.

Table 27: Homogeneous and different groups of sugars by botanical origin.

	Homogeneous Groups
Melecitose	X
Saccharose	XX
Maltose	X
Turanose	X
Glucose	X
Fructose	X

Fructose content also varied significantly, as shown in (Figure 36), with a higher average value than glucose, 37.48± 3.22%. The maximum value 42.5%, was quantified in a honey of *Atractylis* type, and the minimum value 27.2% was for a polyfloral sample (Table 26).

Analyzing the data obtained for these two main sugars, it is found that there is a significant difference in their content in the 11 types of honey. The highest average of glucose concentration is marked for *Bupleurum* 37.0% followed by *Eruca* honeys 36.0 ± 2.20 %, then *Atractylis* 32.27 ± 2.89 % and *Eucalyptus* 31.02 ± 0.94 %. Samples with the lowest glucose content were those of *Genista* 26.5± 4.18% and *Retama* 27.22± 1.60 %. Regarding the fructose content, it is observed that the minimum average concentration was noted for *Genista* samples 34.38 ± 3.27%, and the maximum for *Bupleurum* sample followed by *Atractylis* 39.81± 3.34 and *Eucalyptus* 38.67± 1.99. The lowest values of F/G ratio are for the honeys of *Eruca sativa,* and *Bupleurum,* and the highest ones are for the honeys of *Retama sphaerocarpa* and *Genista saharae*,

Of the identified sugars, turanose is the third most abundant, and is present in all samples studied (Figure 37), with an average content of 3.12± 0.66 (Table 26). The range of values for this sugar varies from a minimum of 1.4% to a maximum of 5.4%, with the presence of what are statistically called outliers. *Retama*, *Atractylis* and *Capparis* honeys show the highest values of this sugar.

Maltose was the next most detected sugar with an overall average content of 1.30± 0.63, *Genista* honeys had the highest content while minimum values were found for *Eruca*, *Hedysarum* and *Arbutus* honeys. Sucrose could be detected in the majority of samples, with an average content of 0.96± 2.27%, the maximum value being for *Genista* honeys with an overall average of 4.76± 4.13 and a value as high as 10.7%.

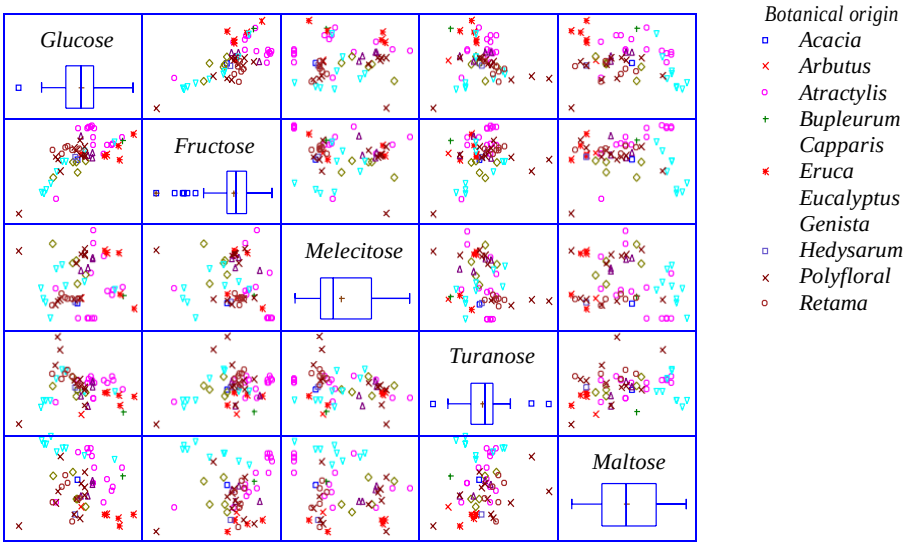


Figure 37: Representation of average sugars types content for each type of honey.

Table 28: 95.0 percent LSD intervals.

	Lower limit	Upper limit
Glucose	27.63	30.54
Fructose	36.63	38.32
Melecitose	0.05	0.08
Turanose	2.95	3.30
Maltose	1.13	1.47
Saccharose	0.36	1.55

Statistical differences and indifferences are presented in the tables for the minority sugars as well.

III.3.10. Discussion

The marketed honeys intended for consumption and especially the labelled ones, must be in conformity with the quality standard of honey. And as the quality is influenced by some parameters such as the polllen and chemical composition, the color and others, the samples must be destined to the preliminary evaluation.

In view of the obtained results, the good quality of use of the analyzed honey samples can be confirmed.

The first parameter to be evaluated is the presence of plant pollen in the honey samples. It can be influenced by the floral diversity in the foraging regions as well as the richness of the honey plants. The extraction method can also play a role in the pollen content of the honeys.

Water content of honeys is related to the botanical origin, the geographical origin, the climatic conditions, the season of the year, the humidity of the nectar and the degree of maturation of the hive, and as honey is a very hygroscopic product, its content can undergo variations during storage, causing an increase in the amount of water in the upper layers. It is a parameter that conditions the crystallization, and indirectly the fermentation of honey by yeasts (Bogdanov *et al*., 1999). The range of water content in all the studied samples is considered wider compared to the one found by Ghorab *et al*. (2021) for honey samples from an Algerian Mediterranean region (16.4%-19.8%) as well as the one found by Guerzou *et al*. (2021) for honeys from semi-arid regions of Algeria (14%-18.8%). And since the honeys currently studied are of Mediterranean origin for some samples and of arid origin for others, the width of the range of moisture content values may make sense. Honey types harvested from arid regions had minimal water content compared to those harvested from humid regions, which may be explained by the nature of the climate and possibly by altitude. Surprisingly, for a honey produced in arid areas, honeys from *Eruca sativa*, had a high average, which may be explained by early harvesting, but also by inadequate management practice, or perhaps by the nature of the plant itself, as *Eruca sativa* honeys have never been studied for their water content.

Considering that honey is a food of vegetal origin processed by bees, some parameters such as acidity and electrical conductivity reflect the characteristics of the vegetation and the territory. Acquarone *et al*. (2007), on honeys from different regions

of Argentina, observed that variations in pH and electrical conductivity of honeys depended on the soil characteristics of the region, and used these two parameters as geographical markers of honeys. Escuredo (2012) noted the same observation for honeys from Galicia, with considerably low pH values (average value of 4.3). For the present samples of totally different floral and territorial origins, we can assume that these parameters can also be floral markers, and instead of saying that *Retama* honeys have a higher average pH than nectar honeys or that it is a nectar/honey mixture, we can assume that the botanical origin is another exception in this case, as it is known for *Ziziphus* honeys.

Among the physicochemical parameters analyzed, the electrical conductivity (EC) provides us with useful information to differentiate honeys of different floral origins, presenting higher values in honeydew honeys (Bogdanov *et al.,* 1999). Contrary to the pH, it is observed that honeys of the same botanical origin have not so similar electrical conductivities due to their different geographical origins; this is well marked in the high values of the EC. This parameter depends mainly on the content of organic acids, proteins and some complex sugars, so its close relationship with the mineral content is known, and by this last when can strengthen the influence of geographical origin explained by the difference in composition.

Diastase activity and HMF content are parameters used mainly for the evaluation of the freshness of honey. Their perturbations are usually related to each sample separately, which does not allow them to be used as botanical or geographical markers.

HMF is generated in honey mainly due to the presence of simple sugars such as glucose and fructose and some acidic substances; it is formed by the Maillard reaction as well as during caramelization (Krishnan *et al*., 2021). The value of the diastase can vary significantly depending on the honey sample but also on the honey type under strict reservation conditions, the current legislation establishes a minimum value of 8 on the scale of Schade.

For lower diastase values, the honey can be considered in general as heated, except for honeys with low enzyme content which are allowed at a minimum of 3 ID, provided the HMF value is less than 15 mg/kg. The diastase content was less than 8 ID in only 9 samples, which at the same time had a very low HMF content, less than 15 mg/kg, except for two cases: a sample of *Capparis* from the arid region of Ouergla with

an HMF of 16.5 while its ID is 7.11, in which case the sample may have been slightly heated or the sample may have been influenced by the temperature of the region.

The *Arbutus* sample from the Mediterranean region with almost zero HMF had an ID of 2.32, which may be due to the intense accumulation of nectar by the bee that forced it to accelerate trophalaxy and storage in the hives with minimal addition of diastase content.

In the rest of the honeys, the HMF content remained at values below 40 mg/kg, and in most samples at values close to zero. Therefore, these two parameters indicate a high degree of freshness of the analyzed honeys. Achouri *et al.* (2019) on honey from northwest Algeria and by Winkler method found HMF values between 5.9± 1.9 and 47.1±1.9 mg/kg, they also cited that several authors report high HMF values in some Algerian honey samples. Three studies conducted in central and eastern Algeria by Makhloufi *et al*. (2010), Draiaia *et al*. (2014) and Mouhoubi-Tafinine *et al*. (2016) show that several samples have high levels up to 1380 mg/kg. Values between 2.84 and 117.7 mg/kg were found by Guerzou *et al.* (2021) on honeys from semi-arid regions of Algeria.

With respect to color, the lightest honey samples were the most greenish (Figure PCA). On the coordinate (b*), the values were always positive and coincided with the intensity of the yellow color. A similar result was found by Al-Farsi *et al*. (2018) on honeys from Oman, where *Acacia* honeys were the darkest type compared to Sidr and multifloral honeys. The *Retama* honey type has so far only been characterized by Juan-Borras *et al.* (2015), whose reported color margin was the same as ours. *Arbutus* honey production has been reported in some Mediterranean regions such as Sardinia and Corsica (Yang *et al*., 2014; Petretto *et al*., 2015) and amber color was the most common.

The color of *Eucalyptus* honeys produced in Spain was described as amber, which is consistent with the classification of our samples (Juan-Borras *et al*., 2015; Escuredo *et al*., 2019), but *Eucalyptus* honeys from Ecuador were considered extra light amber (Valdes-silverio *et al*., 2018), these apparent differences may be due to the influence of biogeographic region and the eucalyptus species that contribute to honey production.

Clearest type of honey was *Genista,* followed by *Capparis, Atractylis* and *Eruca*, the last three types not being described in the scientific literature, neither for their color nor for any other parameter.

It is well observed that the samples show differences in color intensity depending on their type. It has already been confirmed by many studies that the botanical origin is the main factor affecting the color intensity, which depends on the composition of the honey in pigments such as flavonoids, phenols, carotenoids and mineral content, as well as on the storage conditions (Al-Farsi *et al*., 2018). In spite of their low concentration in honey, the minerals are of great help for the characterization, because of their association with some of the parameters of honey, they are present in honey come almost exclusively from the nectar, an assumption that can be revised by the fact that one of our results shows a difference in EC for a type of honey from different geographical origins. K was the main mineral that was detected, followed by P, then Na, then Ca and finally Mg. Similar results were found by Ghorab *et al*. (2021) on Algerian Mediterranean honeys. Fe, Mn, Cu, Zn, Cd, Pb were always below the detection limit. It is well known that darker honeys have higher mineral content (Escuredo et al., 2013), thus *Acacia, Arbutus* and *Retama* samples have the highest values. The sodium content is so related to the distance to the sea, and then, the closer the samples are to the sea, the higher the Na content, this is seen in our results. Minerals also influence the taste, since they are present as salts and excite the taste pupils of the salty papillae.

The high content of sugars in honey makes it an important energy food, because they are the main constituents, representing about 95% of the dry matter, and they also constitute a good protective medium for other rather important minimal components such as fragile enzymes (Farrow, 1981).

Most of the simple sugars are not found in the nectar but are formed during the ripening and storage of the honey, so different types of honey generally contain the same sugars, but in varying amounts, their percentage being related to the flora and, to a lesser extent, to the climate and geographical origin, but studies have revealed that the type and concentration of sugars are the most effective in indicating the difference between monofloral honeys (Cucu *et al.,* 2021). Then for most types of honey, a higher concentration of sugars is represented by fructose (Miguel *et al*., 2017).

One of the quality criteria of honey is the sucrose content, which must be less than 5 g/100g, with exceptions in some monofloral honeys (lavender, acacia, etc.). This criterion is related to a possible fraud, because an inadequate feeding of the hives in sugar syrups can be responsible for the falsification of honeys. But in the case of the Algerian honeys studied, none exceeded the concentration of 5 g/100g.

The monosaccharides, fructose and glucose were the most studied because they are the main carbohydrates in honey. Their ratio (F/G) is very important because it is one of the parameters describing the crystallization of the honey sample. Glucose is proportional to the crystallization, so the higher the ratio (F/G), the more the honey can keep its liquid state and vice versa.

In our case, honeys of *Eruca*, visually considered as crystallized at room temperature, they had the lowest (F/G) ratio, followed by the honey sample of *Bupleurum* and then the ones of *Atractylis* with moderation. The latter type is also crystallized at room temperature and it is assumed that other factors are responsible, including the minimum water content. *Retama* and *Genista* (Merkh) samples had the highest ratio (F/G), meaning that both types are far from being crystallized at room temperature and retain their fluid-liquid form.

III.4. Biological capacities measurement

The samples selected for the present analyses have already been characterized as follows: *Acacia* sample (Mimouza), *Arbutus* sample (Lenj), four *Atractylis serratuloides* (Sor) honey samples, four honeys from *Capparis spinosa* (Kebbar), two honeys from *Eruca sativa* (Harra), four honeys from *Eucalyptus*, six honeys from *Genista saharae* (Merkh), six honey samples from *Retama sphaerocarpa* (Retem) and six polyfloral honeys.

III.4.1. Phenolic compounds content

Values of total phenolic content varied from an average value of 43.6 mg GAE/100g to 181.7 mg GAE/100g (Table 29). Honey samples from *Capparis*, *Eruca*, and *Genista* had minor content while samples from *Arbutus*, *Acacia*, and polyfloral had the highest amount.

Flavonoid content varied from 1.2 mg EQ/100 for an *Eruca* honey sample to 5.5 mg EQ/100g the *Arbutus* one, this latter as well as the *Acacia* honey sample and polyfloral samples had the highest content, while the *Eruca, Atractylis* and *Genista* honeys had the lowest content.

III.4.2. *In vitro* antioxidant activities

The radical scavenging activity (RSA), considering the inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) by the honey solution, varied from an average percentage of 29.7% for *Genista* samples to a percentage of 68.5% for *Retama* samples. Some types of honey had RSA values above 50%. These are *Acacia* honeys, *Arbutus* honeys, some *Eucalyptus* honeys, polyfloral honeys and *Retama* honeys. However, desert and steppe samples like *Atractylis*, *Capparis*, *Eruca* and *G. saharae* had the lowest values.

Something similar occurs with ABTS .+ inhibition. The samples of *Acacia*, *Arbutus*, *Eucalyptus*, polyfloral and *Retama* had percentages above 50% while the samples of *Actractylis*, *Capparis*, *Eruca* and *Genista* had inhibition percentages below 50%. The lowest value was for *Atractylis* samples with a mean value of 25.3% and the highest value was for *Arbutus* honey with 89.1% as mean value. The statistically significant differences were for the *Retama* samples with the other groups, the polyfloral with the *Atractylis*, *Capparis*, *Eruca* and *Genista* samples, and finally the *Eruca* and *Retama* honeys with the *Eucalyptus* samples. The inhibition of ABTS .+ favoured a better discrimination of the antioxidant activity between the honeys. Moreover, the groups were more homogeneous than the values obtained for the DPPH radical scavenging activity. The lowest values were found for *Atractylis*, *Capparis* and *Genista* samples which showed significant differences with *Eucalyptus*, Polyfloral and *Retama* samples. *Eruca* samples showed significant differences with *Atractylis* and *Genista* samples but also with *Eucalyptus*, polyfloral and *Retama* honeys while *Retama* showed higher values with significant differences with all groups except polyfloral samples.

The sample that showed good activity with the lowest median inhibitory concentration (IC_{50}) regarding the DPPH assay was the *Arbutus* honey sample (2.9 mg/mL) followed by the average of all *Retama* honey samples (average value of 12.04

mg/mL). The lowest percentages were noticed for *Atractylis, Capparis, Genista* and *Eruca* honeys for both activities (Table 29).

III.4.3. Alpha amylase

A preliminary evaluation of the anti-diabetic activity was performed by an α-amylase inhibition test, and the results were expressed as percentage inhibition and IC_{50}. The *Arbutus* sample showed the best α-amylase inhibition activity, being the only sample that reached 50% inhibition activity. This sample showed slightly higher levels of flavonoids and polyphenols than the other samples. It is also remarkable that the *Eruca* samples showed quite high inhibition of the α-amylase enzyme (46.8%), despite their low phenol content and even their limited antioxidant power. Regarding the differences between the sample groups, *Atractylis, Capparis, Eucalyptus, Genista* and polyfloral honeys showed similar and statistically different values than *Eruca* and *Retama* samples. Similarly, the α-amylase activity was statistically different for *Eruca* and *Retama* honeys.

Table 29: Descriptive analyse of antioxidant activities and α-amylase inhibition for the different types of honey.

Parameter	*Acacia* (n=1)	*Arbutus* (n=1)	*Atractylis* (n=4)	*Capparis* (n=4)	*Eucalyptus* (n=4)	*Eruca* (n=2)	*Genista* (n=6)	*Retama* (n=6)	Polyfloral (n=6)
Phenolic content (mg/100g)	163.8 ±1.8	181.7 ± 1.9	60.9 ±10.0	43.6 ±2.3	139.0 ±14.5	43.9 ±1.6	44.7 ±8.3	116.9 ±6.7	140.2 ±34.2
Flavonoid content (mg/100g)	5.3 ±0.9	5.5 ±0.0	1.4±1.1	2.1 ±2.0	4.5 ±12.6	1.2 ±1.1	1.8 ±3.6	4.9 ±4.2	4.7 ±13.5
RSA (%) IC_{50} (mg/mL)	65.0±1.62 13.4±0.65	58.3±1.94 2.9±2.09	34.6±7.3 NI	29.7±4.3 NI	44.9±10 16.2±9.7	25.4±0.8 NI	29.7±5.4 NI	68.5±3 12.4±0.7	54.1±9.2 14.2±7.4
ABTS .+ (%) IC_{50}(mg/mL)	85.1±1.83 3.5±0.09	89.1±1.49 1.8±0.04	25.3±3.2 NI	40.6±4.6 NI	69.2±1 11.6±1.6	45.5±0.6 NI	32.5±7.8 NI	85.2±1.8 7.3±2.8	80.2±8.9 9±1.4
α-amylase (%) IC_{50}(mg/mL)	37.0±2.09 NI	53.0±1.01 3.6±0.99	10.8±1.8 NI	10.9±4.8 NI	13.2±1 NI	46.8±1.2 NI	10.4±4.3 NI	41.9±2.8 NI	12.4±1.4 NI

NI: Not identified

III.4.4. Chemometric evaluation considering botanical and geographical origin

Principal component analysis (PCA) was performed to investigate the relationships between variables and samples. The procedure extracts eight components that explain 91% of the variance in the samples. The first two components explain 47% of the variance in the data and the variables with the most weight are CIE (L*, a*b*) and phenol content in component 1 and α-amylase and Apiaceae pollen in component 2.

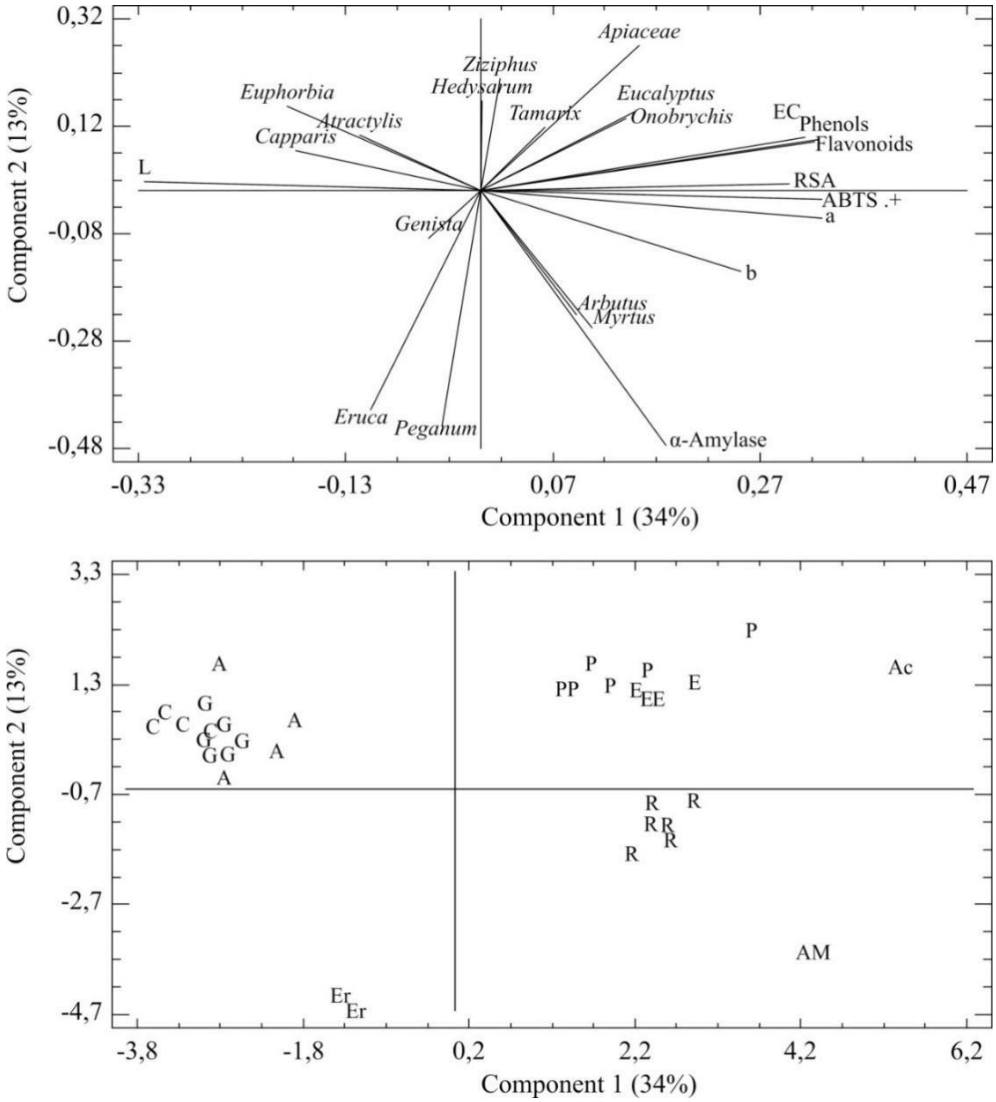


Figure 38: Principal Component Analysis (PCA) of the different measured parameters of the studied honeys. Honey types: A: *Atractylis*, Ac: *Acacia*, AM: *Arbutus*, C: *Capparis*, E: *Eucalyptus*, Er: *Eruca*, G: *Genista*, P: Polyfloral, R: *Retama.*

It is important to note that CIElab and other physicochemical values were added to the analysis because of their link to antioxidant effects of honeys according to the literature.

The projection on a plane of the variables introduced in the analysis could be seen in figure 38. On the right hand side, flavonoid and phenol contents are located close to electrical conductivity, RSA and ABTS .+ inhibition. This indicates the proximity and positive correlation between them. The higher the L-value, the lower the biological activity of the honey samples.

On the other hand, α-amylase inhibition was located in the same way as *Arbutus* and *Myrtus* pollen, showing the previously mentioned higher activity of this sample.

The projection of the cases considering both components showed the samples clearly grouped with respect to their botanical origin. On the right, in the positive quadrant, the samples of polyfloral (P) and *Eucalyptus* (E) are close, these two groups of honey having very similar properties. *Acacia* honey (Ac) is not far behind. The *Retama* (R) samples are located together in the negative quadrant, as well as the *Arbutus* (AM) sample. The latter sample had the highest phenolic content and α-amylase activity, as mentioned. On the left, the clearest samples with the lowest inhibition values of the studied parameters were introduced: *Capparis* (C), *Genista* (G) and *Atractylis* (A). Finally, we note the position of the two *Eruca sativa* samples, clearly separated from each other, at the bottom of the figure. This position is due to their inhibition of α-amylase, one of the most potent.

Another way to search for similarities among samples is using cluster analysis. Two separated clusters have been obtained (Figure 39). The samples on the left (first cluster) are samples obtained from the Tellian and Steppe regions. There are three sub-groups: The *Arbutus* honey (clearly differentiated), the *Retama* samples, and other groups with polyfloral and *Eucalyptus* evidencing the closeness among these samples. One of the polyfloral samples is grouped with the Acacia sample due to their proximity in some physicochemical properties such as color, electrical conductivity, and polyphenol content. The second cluster includes the samples from the aridest areas. *Eruca* honey was separated from *Genista* and *Capparis* samples. Finally, *Atractylis* samples were differentiated in a single subgroup. The most important variables for

clustering samples were color, electrical conductivity, polyphenol, and flavonoid content as well as RSA, ABTS .+ inhibition, and a-amylase inhibition.

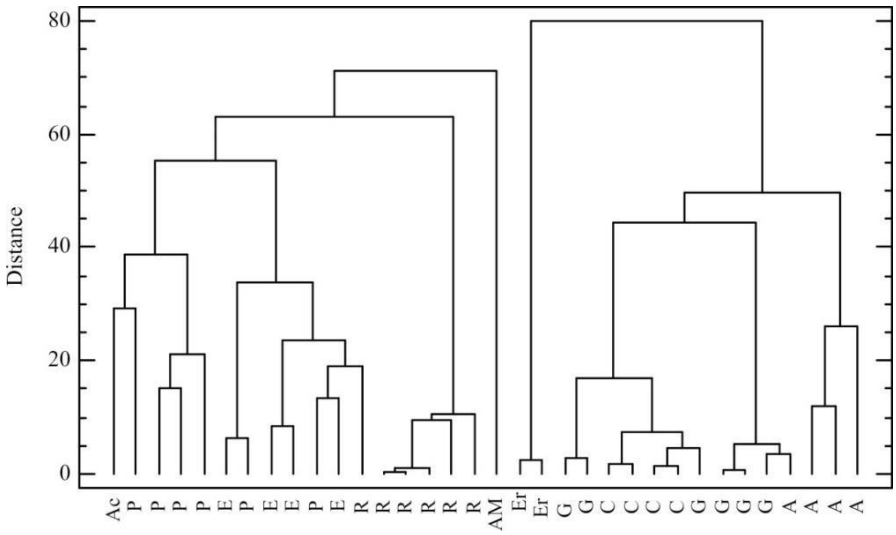


Figure 39: Analyse des clusters des échantillons de miel. Types de miel : A : *Atractylis*, Ac : *Acacia*, AM : *Arbutus*, C : *Capparis*, E : *Eucalyptus*, Er : *Eruca*, G : *Genista*, P : Polyfloral, R : *Retama*.

Finally, a multiple linear regression analysis was applied to predict the value of some dependent variables as RSA, ABTS .+ inhibition and α-amylase inhibition. The best obtained models were showed in Table 30. For RSA, the best model predicted 71.5% of the variance of the data using as independent variables total flavonoid content and coordinate a*. For ABTS .+ inhibition an excellent model explaining 91.1% of the variation of the data using the same independent variables as before (flavonoids content and coordinates a*) was found.

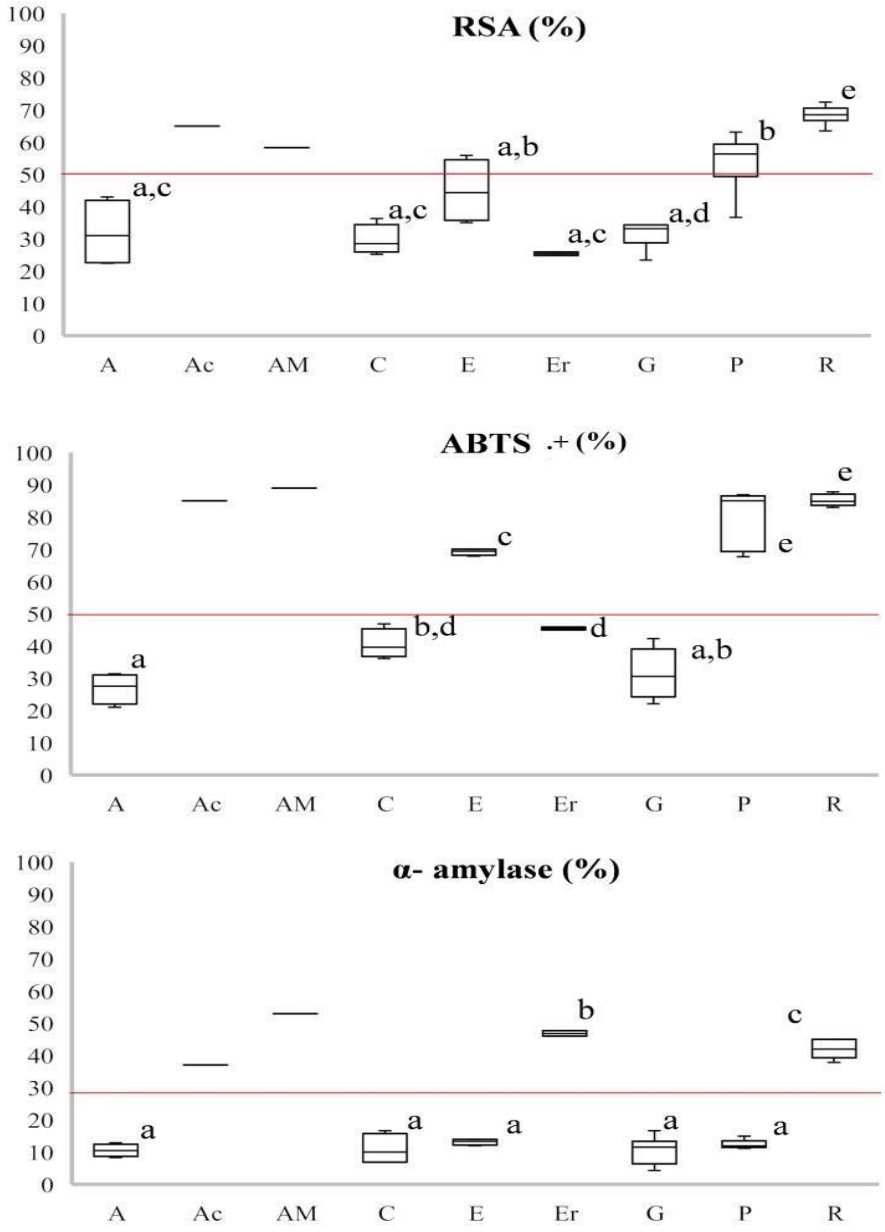


Figure 40: Box plot diagrams of antioxidant activities and α-amylase inhibition. Honey types: A: *Atractylis*, Ac: *Acacia*, AM: *Arbutus*, C: *Capparis*, E: *Eucalyptus*, Er: *Eruca*, G: *Genista*, P: Polyfloral, R: *Retama*. Groups not sharing a letter are significantly different (p-value ≤0.05).

Table 30: Different multiple linear regression models considering as dependent variable the RSA, ABTS .+ inhibition and α- amylase inhibition.

Dependent	**R**	**R^2**	**Ajusted R^2**	**Std Error of the estimate**	**F**	**Sig.**
RSA	0.845	0.715	0.706	9.013	56.03	0.000
ABTS .+	0.954	0.911	0.905	7.617	158.00	0.000
α- amylase	0.889	0.791	0.762	7.436	27.38	0.000
		Understandardized coefficients		**Standardized Coefficients**	**T**	**Sig.**
Dependent	**Predictors**	**B**	**Std error**	**Beta**		
RSA	Constant	24.649	3.622		6.806	0.000
	Flavonoids	0.521	0.119	0.552	4.392	0.000
	a*	1.257	0.401	0.394	3.136	0.004
ABTS .+	Constant	26.702	3.458		7.722	0.000
	Flavonoids	0.817	0.113	0.582	7.220	0.000
	a*	2.077	0.383	0.438	5.429	0.000
α- amylase	Constant	10.322	1.696		6.085	0.000
	Retama	0.261	0.053	0.471	4.910	0.000
	Arbutus	3.283	0.689	0.414	4.765	0.000
	Eruca sativa	0.540	0.109	0.428	4.964	0.000
	a*	1.047	0.284	0.358	3.692	0.001

III.4.5. Discussion

Regarding the phytochemical composition, high content of phenols in *Arbutus* honey (Lenj) was mentioned also by Otmani *et al*. (2019) from a nearby region with almost the same climatic conditions. It can be noticed that the phenolic contents were very significant in the present samples and higher than those found by Zaidi *et al*. (2019) on thirty one samples of selected honeys from Algeria (14.5 to 99.6 mg GAE/100g), but lower than those noted by Ouchemoukh *et al*. (2017) (from 90 to 318 mg GAE/100g) on thirty five honey samples from north Algeria, while the flavonoid content are so close to our results.

The antioxidant activity is the capacity of honey to slow the oxidative reactions within in vitro and/ or in vivo reactions. According to Salgueiro *et al*. (2014), previous studies have reported that the 2,2-diphenyl-1-picrylhydrazyl (DPPH) and 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS.+) tests are among the valid methods for determining the antioxidant properties of food. Arbutus honey had the highest inhibition for the DPPH assay with a value close to those found by Otmani *et al*. (2019). The darkest honeys were the samples with the highest percentages of antioxidant activity and the highest electrical conductivity and phenolic content.

The α-amylase inhibition assay results indicated that not only polyphenols and flavonoids are responsible for the inhibition of the enzyme (α-amylase) and probably other components may also affect. In this context, Krishnasree and Ukkuru (2017) assumed that phytochemicals, without mentioning their nature (polyphenols, carotenoids or other), could be competitive inhibitors of the α-amylase enzyme after their experiments on honeys from various origins, and that's a proposition we shared as well. They also declared that the use of honey in cases of diabetes remains a myth. On the other hand, Zaidi *et al*. (2019) tested the capacity of phenolic extracts of Algerian honeys on α-glucosidase inhibition in a work on antidiabetic activity, while considering it as a possible alternative to synthetic molecules for the treatment of diabetes. Therefore, further research is needed to confirm this hypothesis in the sense of a reversible enzyme competition.

Chemometric tools confirmed the relation among flavonoids content and the biological properties studied as well as showed better results for ABTS .+ method. The relationships among flavonoids and color of honeys was pointed before (Al-Mamary *et al*., 2002). Concerning α-amylase inhibition, the best regression model explained 79.1% of the data variation, where the independent variables used were the pollen parameters *Retama, Arbutus*, *Eruca sativa* and the coordinate a*. This means that *Arbutus* honey produced in the Algerian Tell had high α-amylase inhibition, but also some desert honey as those from *Eruca sativa* or *Retama* can be distinguished by this property. However, further studies on these honey types are necessary to confirm these results.

So far we can evaluate the types of honeys, we assume that the honey of *Arbutus* is a honey with strong biological characteristics plus its quality well verified in this case, the honeys of *Retama* are also a new type to strengthen the studies that we have seen its properties and its exclusivity, Then the honeys of Atractylis, given their intense production according to the beekeepers and their particular composition, can be the subject of further research, as well as the honey of *Eruca,* which is still a honey to be verified, especially for its glycemic index, because it contains both a high content of low ratio (F/G) and a significant inhibition of alpha amylase.

These last three types were chosen for the evaluation of their volatile and sensory profile.

III.5. Volatile fraction results

A total number of 67 volatile compounds were identified in the 23 samples for the three selected types of honey. The relative concentration (%) of the most important components for each type of monofloral honey is presented in the following table (31), as well as the number of samples, and the linear retention index calculated on the basis of the standard mixture of alkanes (LRIc). The identified compounds were classified into different chemical categories: acids, alcohols, aldehydes, alkanes, aromatic alcohols, benzene derivatives, chromene derivatives, esters, furans, ketones, nitrile, nitrogen compounds, phenols, sulphur compounds, terpenes and others.

The most dominant compounds are benzene derivatives with 12 compounds and aldehydes with 11 compounds, of which the most important in terms of abundance (total frequency) are: Benzene acetaldehyde (95.65%), benzaldehyde (91.32%) and 1,2-benzene dicarboxylic acid, 2-methylpropylbutyl ester (52.5%) for the first category and decanal (91.3%) with nonanal (86.95%) for the second. This is followed by ketones and terpenes with 9 and 8 volatile compounds respectively such as 2-buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl) (43.47%) and 2-naphthalene methanol, decahydro-α,α,4a trimethyl-8-methylene-, [2R-(2α,4aα,8a,8aβ)] (47.8%) respectively. Other compounds belonging to other chemical categories were also identified such as 6 alcoholic compounds, 4 phenolics, 2 acids and 2 sulphur compounds and others.

With regard to each type of honey, there were different types of volatile compounds between the honey types as well as common compounds between them. In addition, the number of volatile compounds varied considerably between honey types, with a higher number of compounds in *Atractylis* honey and a lower number in *Retama* honey. *Atractylis* honey samples showed the greatest variety and amount of compounds with a total of 48 volatile compounds.

The *E. sativa* and *Retama* honey samples presented 30 and 19 volatile compounds respectively. Eight of the total organic compounds identified were present in all three types of honey (2-buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl), methyl salicylate, phenylethyl alcohol, thymol, benzaldehyde, benzene-acetaldehyde, decanal and nonanal).

In addition, the following 9 compounds were present in both *Atractylis* and *Retama* honeys: Oxime-, methoxy- phenyl, 2,6,6-trimethyl-2 cyclohexene-1,4-dione, p-menth-1-en-8-ol, benzoic acid, 4-hydroxy-3,5-dimethoxy-,hydrazide, α-linalool, 2-

cyclohexen-1-one, 3,5,5-trimethyl, benzoic acid, 3,5-dimethoxy-,methyl ester, lilac aldehyde D and lilac aldehyde.

Eruca samples shared 5 compounds with *Atractylis* honey; (benzene, 1,2-dimethoxy, benzyl alcohol, 1,2-benzenedicarboxylic acid, 2-methylpropyl butyl ester, α-methyl-α-[4-methyl-3-pentenyl] oxirane menthanol and safranal). No volatile compounds were shared only by *Eruca* and *Retama* honey, presenting in common those that also appear in *Atractylis* honey (2-buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl), methyl salicylate, phenylethyl alcohol, thymol, benzaldehyde, benzeneacetaldehyde, decanal and nonanal).

The average relative concentration (%) of the common compounds presented in the different monofloral honeys was compared using a pairwise Mann Whitney test (α value ≤0.05). Statistically significant differences were marked with letters in table 31.

Four compounds (benzene, 1,2-dimethoxy, decanal, nonanal and phenylethyl alcohol) were significantly different between *Eruca* and *Atractylis* honeys (marked with letter b). Benzene, 1,2-dimethoxy and phenylethyl alcohol showed higher concentrations in *Eruca* honey (3.1% and 2.7%, respectively) than in *Atractylis* honey (0.9% and 1.2%). While decanal and nonanal had higher percentages in *Atractylis* samples (8.8% and 18.0% respectively) than in *Eruca* samples (3.3% and 4.5%).

The letter d denotes significant differences between the 2 compounds shared by *Retama* and *Atractylis* honey. *Retama* honey showed higher concentration values of the common compounds lilac aldehyde (11.5%) and lilac aldehyde D (13.3%) than *Atractylis* honey. No significant difference was found between the values of the common compounds of *Eruca* and *Retama* honeys.

Table 31: Volatile compounds identified in honey samples

Volatile Compounds	RT	RI[c]	ID	*Eruca sativa*		*Retama*		*Atractylis*	
				M (SD)	N	M (SD)	N	M (SD)	N
3-Ethyl-2-pentanol	8.5	879	RI[1]					2,0(0.1)	2
Oxime-, methoxy- phenyl	9.2	899	RI[2]			3.9(2.9)	2	1,6(0.7)	6
Benzaldehyde	11.2	947	RI, MS	1(5.4)	5	2.8 (1.9)	5	3,8(1.5)	11
2-Furancarboxaldehyde, 5- methyl	11.3	948	RI, MS					0,9(0.3)	9
Dimethyl Trisulfide	11.6	958	RI, MS	13.9(12.7)	5				
5-Hepten-2-one,6-methyl	12.3	975	RI, MS					0,8(0.3)	8
Octanal	13.1	993	RI, MS					0,4(0.1)	6
1-Hexanol,2-ethyl	14.5	1025	RI, MS					0,6(0.2)	3
Benzyl Alcohol	14.6	1027	RI, MS	4.1(2.7)	4			1,1(0.6)	7
Benzeneacetaldehyde	15.1	1037	RI, MS	24.3(28.1)	5	8.0(2.4)	7	5,5(2.9)	10
Acetophenone	16.1	1060	RI, MS	2.0(0.6)	3				
Linalool oxide (fr,1)	16.2	1061	RI, MS					2,4(2.2)	7
α-Methyl-α-[4-methyl-3-pentenyl]oxirane menthanol	16.5	1068	RI, MS	1.1(0.4)	4			2,3(1.7)	5
Phenol, 2-methoxy	17.1	1081	RI, MS					7,2(1.3)	7
α-Linalool	17.5	1090	RI[3]			2.5(2.5)	2	5,6(1.9)	6
1,6-Octadien-3-ol,3,7- dimethyl	17.7	1094	RI, MS					7,1(1.2)	6
1,5,7-Octatrien-3-ol,3,7-dimethyl	17.8	1095	RI, MS					17,4(6.1)	2
Nonanal	18.1	1102	RI, MS	4.5(0.7)**b**	5	29.0(20.0)	6	18,0(6.3)**b**	9
Phenylethyl Alcohol	18.3	1107	RI, MS	2.7(0.6)**b**	5	3.8(1.9)	3	1,2(0.3)**b**	10
2-Cyclohexen-1-one, 3,5,5-trimethyl	18.7	1114	MS			4.1(2.2)	2	6,1(3.9)	4
Benzyl methyl ketone	19.1	1124	RI, MS	1.1(0.5)	3				
1-Cyclohexene-1 carboxaldehyde,5,5-dimethyl-3-oxo	19.5	1132	MS					2,7(1.5)	5
Unknown (C8H7N)	19.7	1135		8.4(1.6)	5				
2-Hydroxy-3,5,5-trimethyl-cyclohex-2-enone	19.7	1136	RI, MS					3,8(0.1)	2
2,6,6-Trimethyl-2-cyclohexene-1,4-dione	20.1	1145	RI, MS			14.3(7.3)	2	1,8(0.1)	3
Benzene, 1,2-dimethoxy	20.1	1145	RI, MS	3.1(2.8)**b**	5			0,9(0.1)**b**	3
Lilac aldehyde	20.3	1149	RI[4]			11.5(8.0)**d**	6	9,4(12.9)**d**	3
Benzene,1-ethenyl-4-methoxy	20.3	1149	RI, MS					6,1(0.7)	3
Lilac aldehyde D	20.7	1158	RI[4]			13.3(5.7)**d**	4	8,5(9.2)**d**	2
p-Menth-1-en-8-ol	22.3	1191	RI, MS			6.4(7.2)	3	1,8(0.7)	2
Safranal	22.3	1192	RI, MS	1.4(0.3)	2			5,4(0.3)	3
Methyl Salicylate	22.4	1195	RI, MS	2.3(0.5)	2	3.3(2.0)	2	1,0(0.04)	3
Pentanenitrile, 5-(methylthio)	22.6	1198	RI, MS	1.2(0.1)	3				
Benzofuran, 4,7-dimethyl	22.8	1202	MS					3,1(0.5)	3
Decanal	22.9	1204	RI, MS	3.3(2.3)**b**	5	21.7(18.0)	6	8,8(3.1)**b**	10
3,Cyclohexene-1-acetaldehyde, α,4-dimethyl	23.1	1210	RI, MS					1,1(0.8)	5
Tetrasulfide, Dimethyl	23.2	1211	RI, MS	11.9(1.7)	3				
Furan ,3-phenyl	23.5	1219	RI, MS	1.0(0.2)	2				
Benzenepropanenitrile	24.4	1237	RI, MS	0.5(0.1)	2				
Benzeneacetic acid, ethyl ester	24.7	1244	RI, MS	0.2(0.1)	5				
Benzaldehyde, 4-methoxy	24.9	1249	RI, MS					14,2(3.2)	5
Benzeneacetic acid	25.6	1263	RI, MS	0.3(0.2)	3				
Benzylidenemalonaldehyde	25.8	1268	MS			1.4(1.1)	2		
1H-Indene-4-carboxaldehyde,2,3-dihydro	25.9	1271	MS	0.9(0.2)	2				
Nonanoic acid	26.5	1283	RI, MS	0.2(0.1)	4				
Phenol, 2-methyl-5 (1-methylethyl)	26.5	1284	RI, MS					4,0(1.1)	3

Table 31

Volatile Compounds	RT	RI[c]	ID	*Eruca sativa*		*Retama*		*Atractylis*	
				M (SD)	N	M (SD)	N	M (SD)	N
Thymol	26.7	1288	RI, MS	1.6(1.3)	3	9.9(3.4)	3	3,0(2.4)	7
2H-1-Benzopyran,3,5,6,8a- tetrahydro-2,5,5,8a-tetramethyl-,trans	27.8	1312	RI, MS	0.3(0.2)	5				
2,6,10,10-Tetramethyl-1-oxa-spiro[4,5]dec-6-ene	27.9	1314	RI, MS			7.6(6.2)	3		
Naphtalene,1,2-dihydro-1,5,8- trimethyl	29.0	1341	RI, MS					3,5(1.9)	4
Eugenol	29.6	1353	RI, MS					2,2(0.2)	5
Benzene,4-ethenyl-1,2-dimethoxy	30.2	1367	RI, MS					2,8(0.2)	3
n-Decanoic acid	30.6	1377	RI, MS	0.7(0.5)	5				
2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)	30.9	1384	RI, MS	8.8(1.6)	2	3.9(2.7)	3	0,7(0.1)	5
2-Pyrrolidinethione,1-methyl-	32.9	1431	MS	12.7(2.0)	3				
5,9-Undecadien-2-one, 6,10-dimethyl-,(E)	33.6	1448	RI, MS					1,2(1.2)	6
1,3,7,7-Tetramethyl-9-oxo-2-oxabicyclo[4,4,0]dec-5-ene	34.9	1479	RI, MS					1,4(1.0)	3
1,6,10-Dodecatrien-3-ol,3,7,11-Trimethyl-, (E)	37.8	1553	RI, MS					5,8(3.4)	6
Benzoic acid, 3,5-dimethoxy-,methyl ester	38.6	1571	RI, MS			19.3(0.2)	2	7,7(3.1)	6
Agarospirol	40.4	1619	RI[5]					2,4(1.3)	5
2-Naphtalene methanol, decahydro-α,α,4a- trimethyl- 8- methylene-, [2R-(2α, 4aα,8a,8aβ)]	41.4	1644	RI, MS					5,1(5.8)	11
α-Bisabolol	42.6	1676	RI, MS					0,8(0.6)	9
Heptadecane	43.5	1701	RI[6]	1.1(0.2)	4				
Benzoic acid, 4-hydroxy-3,5-dimethoxy-,hydrazide	44.7	1772	RI[7]			6.0(5.3)	2	3,4(1.7)	6
Octanoic acid, octyl ester	44.9	1785	RI, MS	1.6(1.0)	2				
2-Ethylhexyl salicylate	45.1	1796	RI, MS					2,0(2.3)	7
1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester	45.9	1869	RI, MS	1.3(0.3)	5			2,3(0.9)	7

RT/Retention time; **Mean**: indicates relative area (peak area relative to the total peak area in %); **SD**: Standard deviation; **RI[c]**, linear retention index determined on a ZB-5MSi column relative to a series of n-alkanes (C7–C40); **ID**:Identification by theoretical RI in the NIST Chemistry Web Library and by MS, constituent identified by comparison of mass spectra. RI not identified in the NIST library were compared to the following sources: [46]: Miyazawa *et al*, 2008; [47]: Niu *et al*., 2016; [48]: Radulović *et al*.,2013; [49]: Ciotlaus *et al*., 2020; [50]: Abd Majid *et al*., 2018;[51]: Alissandrakis *et al*., 2007; [52]: Jerković *et al*., 2011.
Letters(a, **b**, **c)** explain the significant differences (α-value < 0.05) between honey types by Mann-Whitney test. a: explain the differences between *Eruca sativa* and *Retama* honey; b: explain the differences between *Eruca sativa* and *Atractylis* honey and c: explain the differences between *Retama* and *Atractylis* honey.

Some volatile compounds were related to the type of honey and some of them had a high frequency, being present in all samples of the group. *Atractylis* honey had 26 unique volatile compounds,*E. sativa* honey had 17 volatile compounds while *Retama* honey had only 2 specific volatile compounds (Table 31). A representative chromatogram of each type of monofloral honey is presented in figure 41.

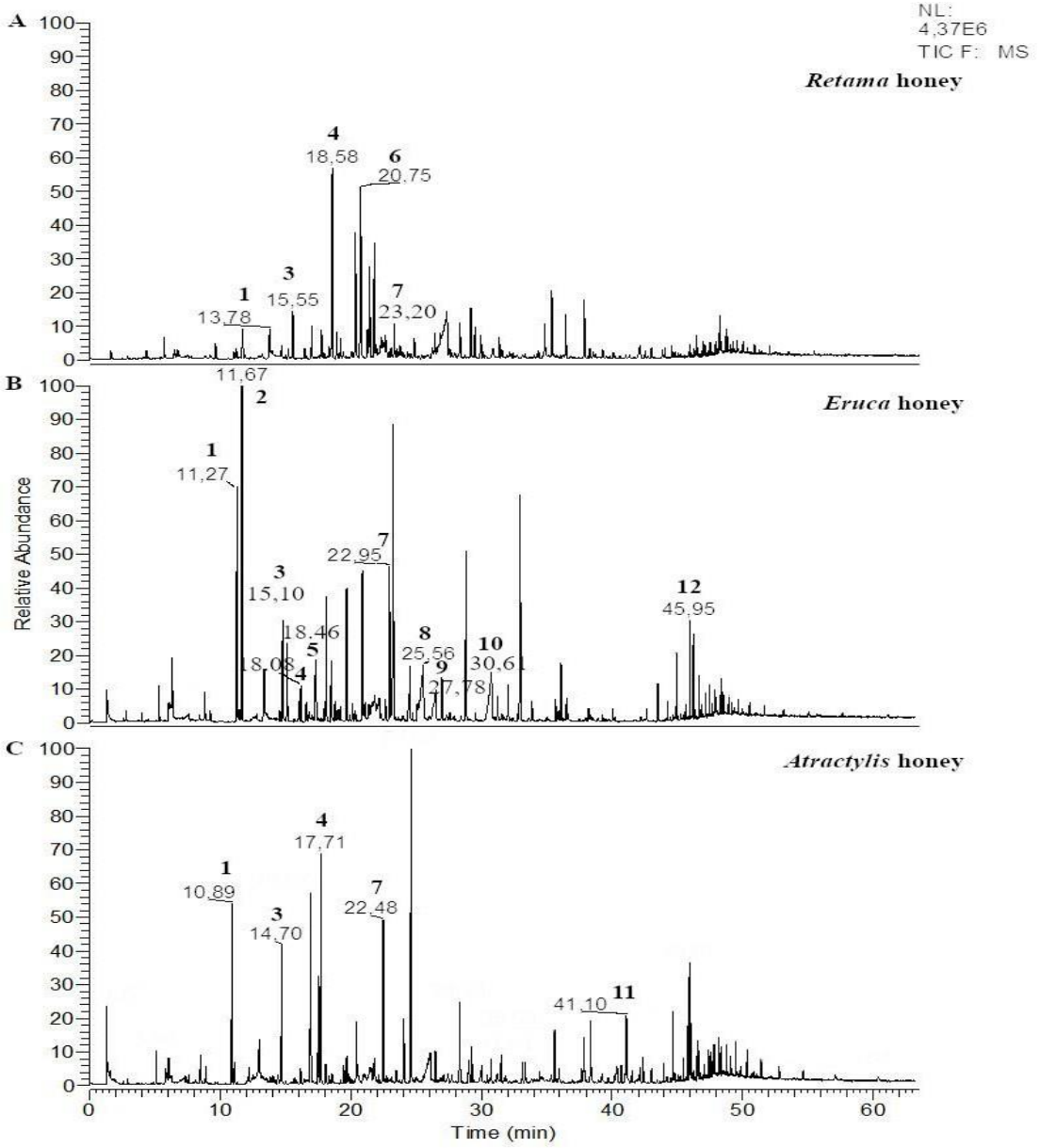


Figure 41: Chromatogrammes GC-MS des trois types de miel A: *Retama sphaerocarpa*; B: *Eruca sativa*; C: *Atractylis serratuloides.* 1. Benzaldehyde; 2. Dimethyl trisulfide; 3. Benzeneacetaldehyde; 4. Nonanal; 5. Phenylethylalcohol; 6. Lilac aldehyde; 7. Decanal; 8. Benzenacetic acid, ethyl ester; 9. 2H-1-Benzopyran,3,5,6,8a-tetrahydro-2,5,5,8a-tetramethyl-,trans; 10. N-Decanoic acid; 11.2-Naphtalene methanol, decahydro-α,α,4a- trimethyl- 8- methylene-, [2R-(2α, 4aα, 8a,8aβ)]; 12. 1,2-Benzenedicarboxylic acid, butyl 2-methylpropyl ester.

III.5.1. *Atractylis serratuloides* honey volatiles

As already mentioned, *Atractylis* honey presents the greatest amount and variety of volatile compounds, but only a few of them were identified in a large number of samples, as well as others with a high concentration (Table 31, Figure 41). The volatile compounds: 1,6,10-dodecatrien-3-ol,3,7,11-trimethyl-, (E) with a relative concentration of 5.8%, 1,6-octadien-3-ol,3,7-dimethyl with 7.1% and phenol, 2-methoxy with 7.2%, stand out for their high concentration in the *Atractylis* honey samples. Other compounds with a high relative concentration and frequency in this honey are 1,5,7-octatrien-3-ol,3,7-dimethyl with 17.4% and a frequency of 18.18%, benzene,1 ethenyl-4-methoxy with 6.1% concentration and a frequency of 27.27%, and benzaldehyde, 4-methoxy with a concentration of 14.2% and a frequency of 45.45%.

The following compounds stand out for their presence in more than 50% of the samples of this type of honey: 2-naphthalene methanol, decahydro-α,α,4a-trimethyl-8-methylene-, [2r (2α,4aα,8a,8aβ)] with a concentration of 5.1% but present in all samples of *Atractylis* honey, 2 ethylhexyl salicylate 2% with a frequency of 63.6%, 2-furancarboxaldehyde, 5-methyl, 5,9 undecadien-2-one, 6,10-dimethyl-,(E), 5-hepten-2 one,6-methyl, linalool oxide (fr,1), octanal and α-bisabolol are also with significant presence in the samples. Although presented as a common compound among the honeys studied, nonanal presents a high concentration of 18% and was present in 9 samples of *Atractylis* honey.

As can be seen in (Annexe 5.1), there are similarities and differences in the different profiles of *Atractylis* honey samples, the four samples (S2, S3, S4 and S5) are almost identical, overall they do not differ much from S1. Samples S11, S13 and S14 are also almost identical, while S8 is the most different from the rest of the profiles.

The differences and similarities are a function of the concentrations of the compounds and also of their nature expressed by the retention times (Rt).

III.5.2. *Eruca sativa* honey volatiles

Benzene acetaldehyde and 2-buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl) were the most important compounds in *Eruca* honey samples, however an unknown compound and dimethyl trisulphide were also identified in high values and frequency. 2-Pyrrolidinethione, 1-methyl- and tetrasulphide, dimethyl, were present with high values and finally 2H-1-benzopyran, 3,5,6,8a-tetrahydro-2,5,5,8a-tetramethyl-, trans, benzene acetic acid, ethyl ester and n-decanoic acid did not have high values but were present in all the *Eruca* honey samples. According to (Annexe 5.2), the first two samples from arid region of Khenchela are identical, the third one is different, with some compounds with close retention time or sometimes the same, but the last two from the Bechar region are also identical.

III.5.3. *Retama sphaerocarpa* honey volatiles

Nonanal, decanal, benzoic acid, 3,5-dimethoxy-, methyl ester, 2,6,6-trimethyl-2-cyclohexene-1,4-dione, lilac aldehyde D, lilac aldehyde are the most concentrated and frequent compounds in *Retama* honey, but 2,6,10,10-tetramethyl-1-oxa-spiro [4,5] dec-6-ene could also be labelled for the same reasons, as well as for its exclusivity to this type of honey.

The samples which are of various regional origins (Biskra, Setif and Laghouat) present differences quite remarkable that the appearances, the first 4 samples originating from Biskra are not too different, but it is the case compared to the others (Annexe 5.3).

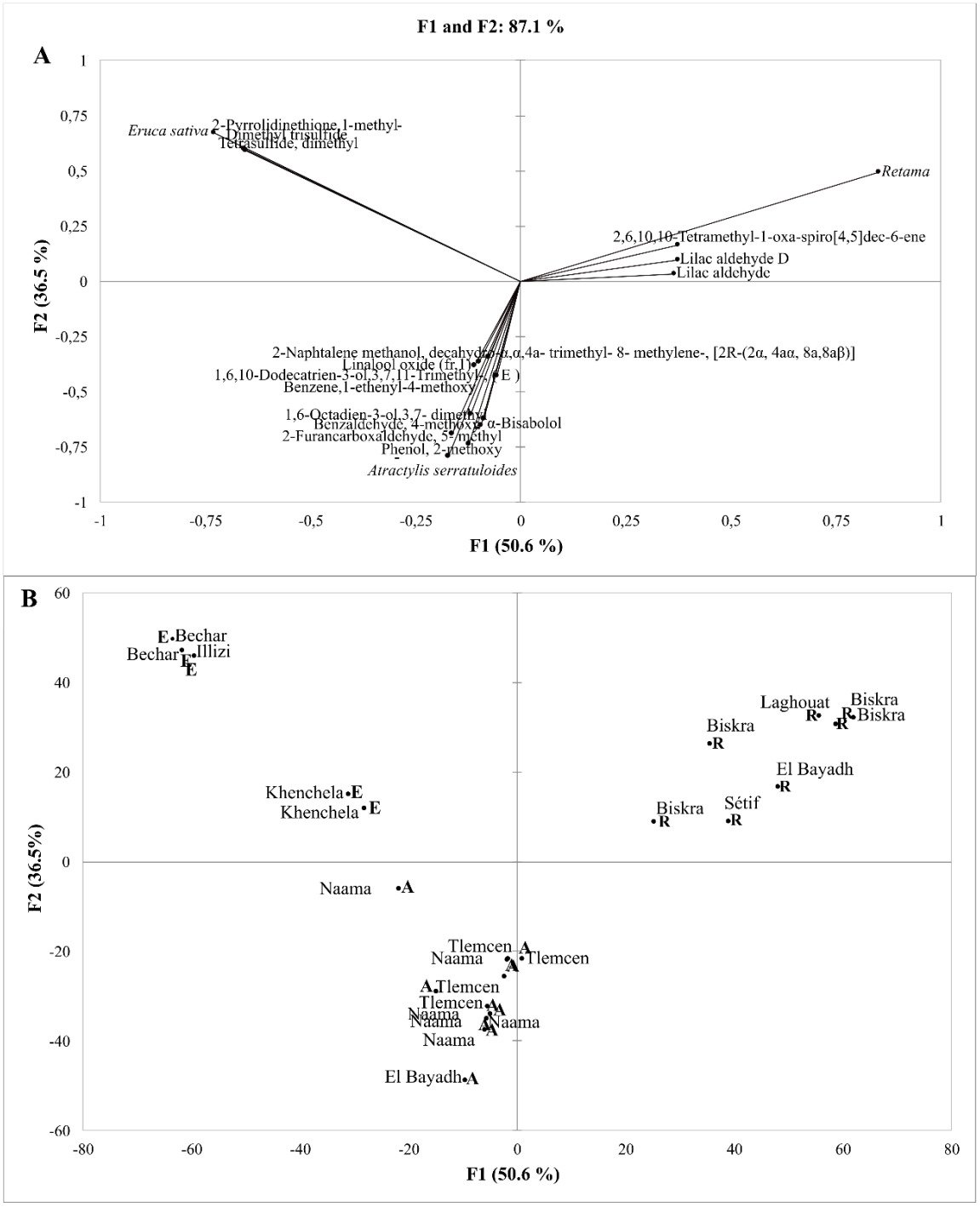


Figure 42: Principal component analysis of the first two factors (F1 and F2) (A) as a function of volatile organic compounds obtained by HS-SPME methods and (B) as a function of their geographical origin.

III.5.4. Relationship between botanical and geographical origin and volatile profile

Principal component analysis (PCA) was performed to investigate the relationships between the different pollen types, volatile compounds and geographical origins of the samples. Nineteen variables were selected for analysis: (*E. sativa, R. sphaerocarpa, A. serratuloides*, 2-furancarboxaldehyde, 5-methyl, dimethyl trisulphide ; 2-naphthalene methanol, decahydro-α,α,4a- trimethyl- 8- methylene-, [2r-(2α, 4aα, 8a,8aβ)], α-bisabolol, lilac aldehyde, lilac aldehyde D, benzaldehyde, 4-methoxy, benzene, 1-isocyano-2-methyl-, linalool oxide (fr,1), tetrasulphide, dimethyl, 1,6-octadiene-3-ol,3,7-dimethyl, 1,6,10-dodecatrien-3-ol, 3,7,11-trimethyl-, (E), 2-pyrrolidinethione,1-methyl-, 2,6,10,10-tetramethyl-1-oxa-spiro[4,5]dec-6-ene, benzene,1-ethenyl-4-methoxy and phenol, 2-methoxy), which are the three dominant pollen types and the most representative chemical compounds detected by GC/MS considering the honey type.

Figure 44 shows the graphical representation of the first two components, which both represent 87.1% of the variability. The correlation between the variables and the factors has been represented in Figure 42A. As can be seen, the pollen type *Atractylis* is located close to the volatile compounds 1,6,10-dodecatrien-3-ol,3,7,11-Trimethyl-, (E), 1,6-octadien-3-ol,3,7-dimethyl, 2-furancarboxaldehyde, 5-methyl, 2-naphthalene methanol, decahydro-α,α,4a-trimethyl-8-methylene-, [2R-(2α, 4aα, 8a,8aβ)], benzaldehyde, 4-methoxy, benzene, 1-ethenyl-4-methoxy, linalool oxide (fr,1), phenol, 2-methoxy and α-bisabolol. Thus, the correlation coefficients between these variables are high. Similarly, the type of *E. sativa* pollen, located in the upper left quadrant, was strongly positively correlated with 2-pyrrolidinethione, 1-methyl-, dimethyl trisulphide, dimethyl tetrasulphide and with a compound that could not be identified (unknown) but is present at a high frequency in the samples and at high concentrations. This could be a compound with the following chemical formula: C8H7N. The volatile compounds lilac aldehyde, lilac aldehyde D, and 2,6,10,10-tetramethyl-1-oxa-spiro [4,5]dec-6-ene also correlate strongly with the *Retama* type.

When the point factors were projected, the samples were well separated according to their botanical origin. In Figure 42B, samples of each type of honey are shown indicating the region where they were produced as well. The *Atractylis* honeys are located very close in the lower part of the figure. It is worth mentioning that despite the significant variation of *Atractylis* pollen in the samples, all samples appeared grouped

together. As previously identified, the samples correspond to the geographical origins of El Bayadh, Tlemcen and Naama.

On the right, the samples from *Retama* are grouped together. All samples were grouped in the same way according to their locality of origin: Biskra, Setif and Laghouat. However, the *Eruca* samples are represented in two groups related to the geographical origin and also to the *Eruca* pollen type content. Two samples are a bit distant and come from a semi-arid region (Khenchela) having as secondary pollen *P. harmala* which could play a role in the volatile composition of the honey. The rest of the samples came from two typical Saharan regions (Illizi and Bechar) and in all these samples the percentage of *Eruca* was above 85%.

III.5.5. Discussion

More than 600 volatile compounds have been identified in honey, belonging to different biosynthetic pathways (Karabagias *et al*., 2014). Monofloral honeys were particularly studied in search of a common volatile fingerprint that facilitates the distinction of one type of honey from another (Da Costa *et al*., 2018). The main chemical group was benzene derivatives, such as benzaldehyde or benzene-acetaldehyde, considered to be the dominant volatile compounds in many monofloral honeys worldwide (Ruisinger and Schieberle, 2012; Jercovic, 2013; Yang *et al*., 2014; Machado *et al*., 2020).

Benzene compounds such as benzaldehyde, benzene-acetaldehyde or benzyl alcohol stand out in the studied honey. Similarly, they are found in other honeys of Algerian origin (Neggad *et al*., 2019). Ketones, aldehydes, alcohols and terpenes are also categories with a significant number of volatile components in the present results. Thus, in this study the presence of some important aldehydes such as decanal or nonanal was highlighted. These compounds commonly found in honey samples, but also in insects and plants, have been mentioned as important organic compounds in the interaction between plants and pollinators (Neggad *et al*., 2019; Bojke *et al*., 2020).

Although many studies have focused on the volatile profile of monofloral honeys (Sesta *et al*., 2008; Bianchi *et al*., 2011; Tette *et al*., 2017; Karabagias *et al*., 2020). Experimental results have shown that some of the compounds may also be present in honeys of other botanical origins (Bianchi *et al*., 2011). The volatile profile of the three types of honey has in common compounds such as: Benzaldehyde,

benzeneacetaldehyde, nonanal, phenylethyl alcohol, decanal, safranal and others, although they are honeys obtained mainly from plants of different botanical families.

The three types of honey chosen for this study were collected, as already mentioned, in areas characterised by their arid or semi-arid climate near the Saharan territory.

The Asteraceae are the most representative plants of the flora of arid regions (15%) with more than 352 species, among which *Atractylis* is very frequent (Le Houérou, 2001).

More specifically, the plant *A. serratuloides* has increased its distribution in recent years, as *P. harmala* and others such as *Nonea mucronata* or *Centaurea* occupy degraded semi-arid steppes in northern Algeria. Today, these plants grow together with other spontaneous plants on nitrogen enriched lands, near villages or water sources, so that other common herbaceous species like *Echium* or species of Apiaceae appear in the pollen spectra. There are no scientific references dealing with the characteristics of this type of honey but they are known for their crystallized appearance, light color, very sweet taste and vanilla smell.

This monofloral honey presents the greatest diversity of pollen types in their pollen spectra and, consequently, a great variability in volatile compounds that make this honey unique, but also difficult to compare with other monofloral honeys. Other unifloral honeys of the same family (Asteraceae), such as sunflower honey, had as volatile markers compounds such as α-pinene or 3-methyl-2-butanol (Svečnjak *et al*., 2019). None of these compounds were found in the *Atractylis* honey samples of this study, however, it is worth mentioning the presence of 2-naphthalene methanol, decahydro-α,α,4a- trimethyl-8-methylene-, [2R-(2α,4aα,8aβ)], also known as β-eudesmol, which was found exclusively in *Atractylis* honey. This compound has previously been reported as an important volatile in extracts from the rhizome of *Atractylodes lancea*, a plant of the same family (Asteraceae, daisy subfamily), and has shown a positive effect in improving intestinal motility in mice (Yamahara *et al*., 1990). It also exhibits various pharmacological activities, including anticancer activity against cholangiocarcinoma (Tshering *et al*., 2021).

Some of the compounds that have been identified as important in this type of honey have been identified in honey from arid and semi-arid areas or in extracts of plants of the same family. For example, phenol, 2-methoxy, identified in Sudanese honey is another compound that can be found in this honey (Tahir *et al*., 2021).

Linalool (1,6-Octadien-3-ol, 3,7-dimethyl) and some derivatives like linalool oxide (fr, 1), were present in this type of honey. These compounds associated with nectar (Soria *et al*., 2009), showed activity against renal carcinoma as linalool (Zapata *et al*., 2014) in *Lippia alba* (Verbenaceae) or excellent biological activities in *Achillea ligustica* (Asteraceae) (Maggi *et al*., 2009).

1,6,10-dodecatrien-3-ol,3,7,11-Trimethyl-, (E) (nerolidol) a sesquiterpene alcohol used as an anti-tumor, analgesic, anti-bacterial, anti-inflammatory, sedative and fungicide was found as a phytocompound of *Lactuca runcinata* (Asteraceae).

These compounds associate *Atractylis* with the medicinal use of this plant in Algeria and confer a healthy value to honey derived from the nectar of this genus.

Another compound found only in the *Atractylis* type of honey but with a lower frequency was α-bisabolol. Although there is little information on volatiles in Asteraceae honeys, this compound was determined as one of the main volatile compounds in Cuban *Turbina corymbosa* honey (Ceballos *et al*., 2010) and in *Acacia* capped honey (Vyviurska *et al*., 2016).

Finally, hotrienol compounds, germacrene D, cis-linalool oxide, trans-linalool oxide, epoxylinalool, nerolidol, benzene acetaldehyde and p-cymene-8-ol were mentioned as markers in other Asteraceae honeys such as *Solidago virgaurea* honeys (Jasicka-Misiak *et al*., 2018).

In the case of *Retama* honeys, which were obtained from *R. sphaerocarpa*, which is a Mediterranean plant well adapted to extreme drought conditions due to the development of molecular mechanisms allowing partial quiescence.

This type of shrubby plant is abundant in steppes on deep soils and has an important ecological role in maintaining dunes and sandy soils, so that the valorisation of honey production is important for the conservation of the ecosystem. The ecosystem is shared with other Fabaceae species such as *Genista*, herbaceous plants and may also appear *Eucalyptus* forest masses. For this reason, one sample had *Eucalyptus* as secondary pollen, showing the importance of this honey source worldwide.

To our knowledge, there is no information on the volatile compounds of *Retama* honey in the scientific literature. However, other Fabaceae honeys have been studied with regard to these organic compounds. For example, nonanal was identified as a volatile compound in *Anthyllis hermanniae* (Fabaceae) honey from Corsica, while it was represented in high concentrations in the present *Retama* honey samples from Algeria, as well as lilac aldehyde, although the concentrations were lower in *Retama*

honey (Yang *et al*., 2014). Similarly, related studies on the volatile profile of *Trifolium* honey (another plant of the Fabaceae family) showed that nonanal and lilac aldehyde were representative volatile compounds along with decanal (Jerković *et al*., 2016; Machado *et al*., 2020).

Lilac aldehyde A, B, C and D has also been found in black locust (*Robinia*) honey from Italy (Aronne *et al*., 2014). However, different isomers of this compound have been reported for other monofloral honeys such as Citrus and Thymus honeys (Castro-Vázquez *et al*., 2007; Špánik *et al*., 2014; Karabagias *et al*., 2020).

The honey samples of *E. sativa* were obtained in the Sahara desert. This plant is a cosmopolitan species used as food due to the health properties attributed to it (Alqasoumi, 2010). In Saharan areas, these plants are adapted to the particular ecology of the region and can occupy large areas of the territory and flower in a short time (Jafaar and Jafaar, 2019).

This fact facilitates the collection of monofloral honeys and indeed, in three samples the percentage of *Eruca* pollen grains was very high. On the contrary, two samples had a lower percentage of this pollen due to the presence of large amounts of *P. harmala* pollen. The beekeeping value of *P. harmala* is unknown, but as this plant is known to have a high alkaloid content, further research is needed on the influence of this plant on honey composition. Regarding the volatile profile, some of the compounds detected in this study can be found in honeys of similar botanical origin. This is the case of Czech rapeseed honey (Brassicaceae) (Kružík *et al*., 2019) which presents aldehydic compounds such as benzaldehyde or decanal. The presence of sulphur compounds could be related to the fact that plants of the Brassicaceae family contain glucosinolates formed mainly of sulphur and nitrogen, which are responsible for the pungent taste and smell of these vegetables (Bell *et al*., 2017).

The amount of these glucosinolates in Brassicaceae plants was related to biotic and abiotic stresses, which are common in the arid areas where the samples were produced. Furthermore, dimethyl trisulphide was identified among sulphides in *E. sativa* flower oils (Blažević and Mastelić, 2008). Finally, safranal was mentioned as a common volatile compound present in honey of Brassicaceae (Makowicz *et al*., 2019).

The results showed the presence of common volatile compounds in honeys of the same botanical origin, although from different regions. On the other hand, many compounds that define the volatile profile of these three types of honeys were only observed in these honeys. These observed differences may be due to the particularity of

these samples, both in terms of their geographical origin and the fact that they come from specific areas with characteristic flora.

The volatile profile provided by this study concerning *Retama, Eruca* and *Atractylis* honeys, can contribute to the characterization and valorization of this beekeeping product in Algeria. In addition to contributing to the establishment of a regulation that moderates, monitors and promotes quality control of the product, which is currently lacking in this region and which honey producers must respect.

III.6. Sensorial analysis

After having results of the tasters, the honey samples were grouped again according to their types and divised in three different profiles as mentioned in the figure 43.

We note that the tasters have analyzed other samples with the last 23 ones used for the volatile profiles, so they can be considered as controls in the classification requested in the questionnary.

Color and aroma as main attributes were selected to schematize the profile of each type of honey. Differences in color intensity and sweetness are clear in the three profiles. In addition to the sensory profiles, a table with numerical values has been inserted for each type of honey in order to present more characteristics already detailed in the part material and methods.

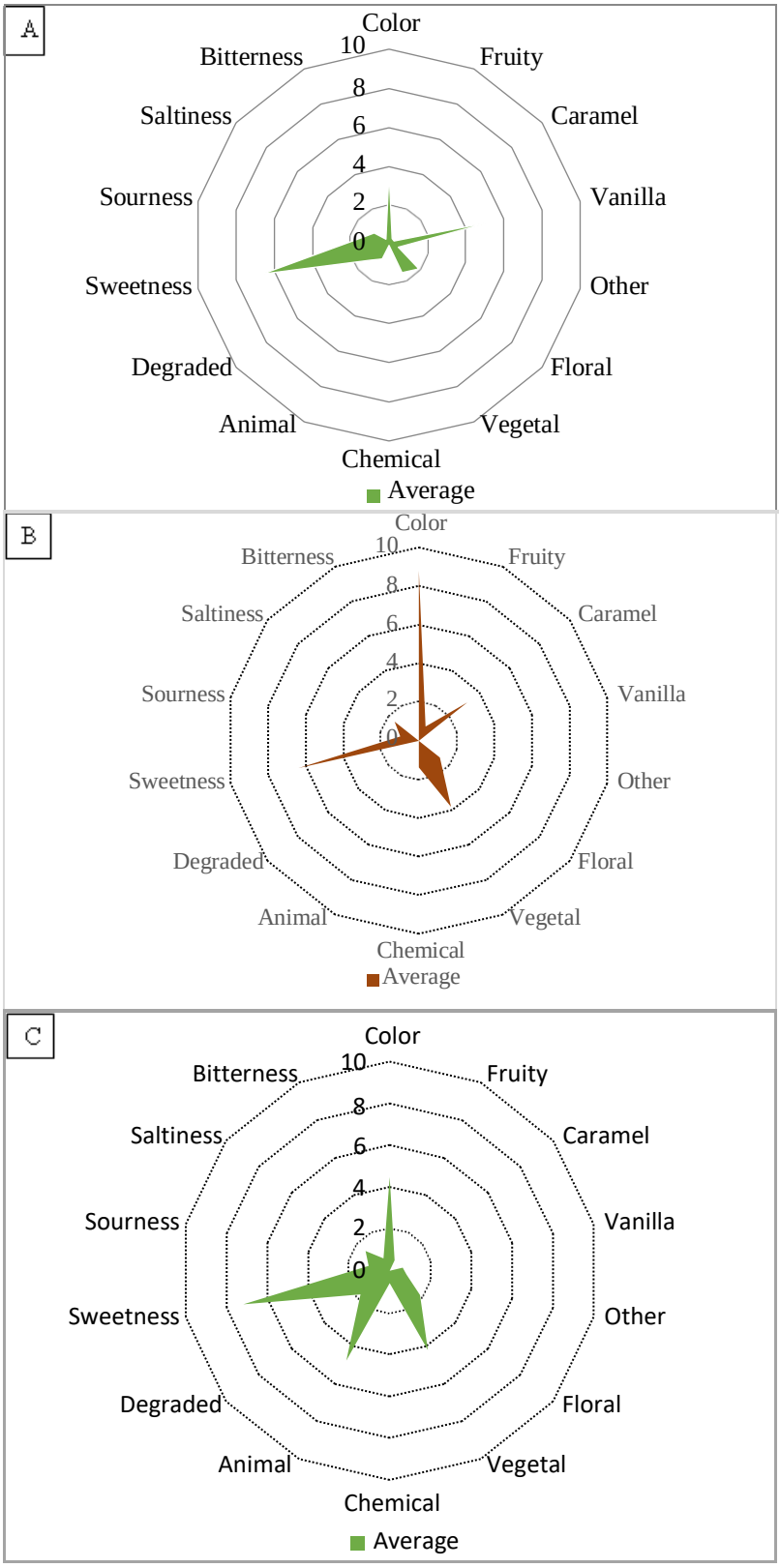


Figure 43: Sensory profiles of the three types of honey: A: *Atractylis serratuloides*, B: *Retama* sphaerocarpa and C: *Eruca sativa.*

III.6.1. *Atractylis serratuloides* honey sensorial profile

The samples of *Atractylis* honeys previously randomly described as clear crystallized honeys with candy and sweet smell had the results described in the table by the average of the professional tasters' scores:

Table 32: Sensorial characteristics of *Atractylis serratuloides* honey samples.

	S. Code	S1	S10	S11	S13	S14	S2	S3	S4	S5	S7	S8	M
Visual	Estate	10	10	10	10	10	10	10	10	10	10	10	10
	Color	2	10	2.8	3.6	3.5	2.8	3.6	4	6	6.4	8	3.71
Smell	Fruity	0	10	0.6	1	0	0	0	0	0	1.4	3.2	0.38
	Caramel	0	10	1.6	0	0	0	1.2	0	0.4	0	0	0.32
	Vanilla	9	10	2.8	5.2	8	5.8	5.4	7.2	6	0	1.4	5.36
	Other	0	10	0.6	0.6	0	0	1.2	1.4	0	0	1.6	0.48
	Floral	0	10	1.2	1.2	1.75	3	1.2	2.6	3.8	2.8	3	1.935
	Vegetal	0	10	1.2	2.4	0	1.8	1.2	2.8	2.4	2.6	6	1.56
	Chemical	0	10	0	0	0	0	0	0	0	0	0	0
	Animal	0	10	2.8	1.2	0	0	0	0	0	2.8	1.4	0.8
	Degraded	0	10	0	0	0	1.8	1	2.4	1.4	3.4	1.8	1.26
	Persistance Smell	6	10	3.4	5	4.25	4.2	3.8	5	5	3.4	5	4.305
Savor	Sweetness	0	10	6.6	6.6	7.75	7.8	8	7.6	7.2	7	7.8	6.635
	Sourness	7	10	1	0.6	0.5	1.2	0.75	1.4	1	1.2	1.8	1.545
	Saltiness	0	10	1	1	0.5	1.4	1	1.4	1	1.4	2.8	0.99
	Bitterness	0	10	0.4	0	0	0.2	0.5	0.6	1	0.4	0.4	0.35
	Persistance	3	10	4	4.75	4.25	5	5	4.33	5	2.44	5.33	5.01
Aroma	Fruity	0	10	3.8	2.4	0	1.2	1	2	0	2.6	2.6	1.52
	Caramel	0	10	1.6	2.4	0.75	0	0	0	2	2.6	1.4	1.075
	Vanille	5	10	2.6	4.4	4.75	4.6	4.8	4.4	5.5	0	0	3.845
	Other	0	10	1.4	0.8	0	0	2.8	1	0.75	1.8	1.4	1.035
	Floral	0	10	0	0	0	2.8	1.2	3.6	3.25	2	3.6	1.285
	Vegetal	5	10	0	1.6	0	1.8	0	3.4	1.5	3	3.4	1.85
	Chemical	0	10	0	0	0	0	0	0	0	0	0	0
	Animal	0	10	0.6	0	0	0	0	0	0	0	1.8	0.12
	Degraded	0	10	0	0	0	0	0	0	0	0	0.4	0
	Astringency	0	10	0.5	0	0	0.5	1	0.5	0	0	1	0.25
	Spicy	0	10	0	0	0	0	0	0	0	0	0.5	0

Tasters agreed on the crystallized state of all *Atractylis* honey samples, this could be due to the low water content for this type of honey. Sugar content also as already mentioned has an influence on crystallization. The odor had several opinions as that of sweet-vanilla. The samples were rated as sweet for their savor as well as for their aroma which they had assumed as vanilla or white chocolate with floral traces.

III.6.2. *Retama sphaerocarpa* honey sensorial profile

Samples of *Retama* honey of dark color and accentuated smell, noted by the tasters as viscous honey for some samples and others in progress or more fluid, of color between dark amber and dark, a smell between caramel and vegetable, and of sweet savor with traces of saltiness. For its aromas the samples have supposed as with vegetable aromas and a little floral.

Table 33: Sensorial caracteristics of *Retama sphaerocarpa* honey samples.

	S. Code	R1	R2	R3	R4	R5	R6	R8	M
Visual	Estate	1	1	1	1	5	5	1	2.14
	Color	7.6	7.6	8.4	8	8.4	8.4	9.2	8.8
Smell	Fruity	1	2.6	1.4	1.2	2.4	3	2	0.8
	Caramel	3.4	3	2.8	3.8	3.2	0.8	3.6	3.2
	Vanilla	0	0	0	0	0	1.6	0	0
	Other	0	0	0	0	0	0	0	0
	Floral	2.8	1.4	1.4	0	0.8	1.4	1.2	1.4
	Vegetal	4.4	5.4	3	4	2.4	4	2.4	3.8
	Chemical	0	0	0	0	0	0	2	1.4
	Animal	2	1.2	1	1.4	2	0	0	0
	Degraded	1.6	0	1.8	0	1.6	2.8	0	0
	Persistance smell	3	4	4.8	4.6	4.4	4.2	4	4.4
Savor	Sweetness	6.4	6.6	6.6	6.2	6.8	6.6	6.4	6.4
	Sourness	1.6	1.4	2.2	1	0.8	1.2	1.2	1
	Saltiness	2.4	2.2	2.8	2.6	0.8	2.2	1.4	1.6
	Bitterness	0.6	1	0.8	1	0.6	0	0.6	0
	Persistance savor	3.6	3.75	4.5	4.67	4.5	4.25	4.75	4.25
	Fruity	0.6	3	1.2	1.2	1.4	2.8	2.6	2.4
	Caramel	4.2	4.6	4.2	2.8	4.6	3.8	3.4	4.4
	Vanille	0	0	0	0	0	1.2	0	0
	Other	0	0	0	2	1.4	0	0	0
Aroma	Floral	0.2	0	0	1.2	1.4	1.4	2.6	1.4
	Vegetal	1	3	3	2.8	3.2	2.6	2.6	2.6
	Chemical	0	0	0	0	0	0	0	0
	Animal	0	1.4	0.4	0.4	0.4	0	0.4	0
	Degraded	0	0	0	0	0	0	0.4	0
	Astringency	0.5	0.5	1	1	0.5	0.5	1	0.5
	Spicy	0.5	1	1	1	1	1	0	0

III.6.3. *Eruca sativa* honey sensorial profile

Eruca sativa, this type of honey also obtained in crystallized form and of clear color, noted by the whole of the tasters as crystallized honey, with color between straw and gold, of an animal smell with vegetable and floral traces with important persistence, the sweet savor for this type of honey was the most important compared to the other previous types.

Table 34: Sensorial characteristics of *Eruca sativa* honey samples.

	S. code	H1	H2	H3	H4	H5	M
Visual	Estate	10	10	10	10	10	10
	Color	4.8	5.2	4.4	4	4	4.48
Smell	Fruity	1.6	1.2	0	0	0	0.56
	Caramel	0	0	0	0	0	0
	Vanilla	0	1.6	1.6	0	0	0.64
	Other	2.4	1.6	0	0	0	0.8
	Floral	3.4	1.2	2.2	1	1.4	1.84
	Vegetal	5.4	5.4	3.8	3.8	2.6	4.2
	Chemical	0	0	1.6	0	1.4	0.6
	Animal	2.8	2.6	4.8	6.6	7	4.76
	Degraded	1.4	1	2.2	1.2	3.2	1.8
	Persistance Smell	5.2	4.6	5.8	5	6	5.32
Savor	Sweetness	7	7	7.75	7.5	6.75	7.18
	Sourness	1.6	1.5	0.25	0.5	1.25	1.05
	Saltiness	2.2	1.4	1	1	1.75	1.5
	Bitterness	0.4	0.6	1.25	0.5	0.67	0.67
	Persistance savor	5.5	4	4.33	4.67	4	4.5
Aroma	Fruity	2.6	1.6	2.2	1.2	1.4	1.8
	Caramel	0	1.2	0	1.2	0	0.48
	Vanille	0	0	3	0	0	0.6
	Other	3.6	2	1.4	0	0	1.4
	Floral	3.2	1.4	1.4	1.2	1.4	1.72
	Vegetal	0	3.6	3	3.2	3	2.56
	Chemical	0	0	0	0	0	0
	Animal	0.4	0	2.4	4	4.2	2.2
	Degraded	0	0	0	1.4	1.4	0.56
	Astringency	1	1	1.5	1	0.5	1
	Spicy	1	0	1	1	0.5	0.7

The aroma was shared between the plant and the animal, some of which marked the mineral property as another attribute.

III.6.4. Questionary summary

After the tasters' comments, it appears that *Atractylis* honeys are well separated from the rest of the samples described by vanilla or white chocolate for all perceptions, followed by the group of *Retama* honeys that can be mixed with the other groups for some samples, as well as *Eruca* samples were grouped. 3/5 of the tasters preferred *Atractylis* honeys for their candy taste, smell and aroma similar to white chocolate. 1/5 preferred *Retama* honeys as less sweet, caramelized and a bit salty, 1/4 preferred both, *Retama* honeys as less sweet but with a strong smell and *Atractylis* honeys as vanilla, but super sweet.

The *Eruca* honeys were not the best for the tasters present because of a strong metallic and animal aroma according to them.

Conclusion and outlook

The main honey families in the present samples collected in different regions of Algeria are Fabaceae, Asteraceae, Apiaceae, Lamiaceae and Brassicaceae. None of these pollen families appeared in all honey samples, due to different geographical origins.

The dominant pollen types in the honeys were *Genista, Retama, Capparis spinosa, Eruca sativa, Eucalyptus, Hedysarum, Spartium junceum, Atractylis serratuloides, Ziziphus lotus, Paronychia argentea,* and *Bupleurum fruticosum* respectively.

The pollen richness of the honeys is included mainly in class III of Maurizio's classification, between 2000 and 50000 pollen grains/g of honey.

Some samples were considered under-representative due to the presence of pollens of poliniferous plants in the sample of honeys, others are known to be monofloral and others like the one of *Atractylis* and since no data bank is available, it was accepted as under-representative.

Some basic parameters of honey characterization are useful with pollen results for its classification according to floral and geographical origin.

Significant differences were found between some samples, pH, humidity, electrical conductivity and color were the main parameters that could differentiate the samples.

Eleven classes of honey could be extracted in this study: *Acacia, Arbutus, Atractylis, Bupleurum, Capparis, Eruca, Eucalyptus, Genista, Hedysarum, Retama* and polyfloral.

Among the honey samples, some declared as monofloral by their beekeepers and sometimes labeled honeys, turned out to be other types and others turned out to be polyfloral.

For their valorization in terms of biological activities, the darker honey samples presented a good antioxidant activity with a high phenolic content, while the lighter ones, in spite of their lower content in antioxidant compounds, could be good sugar substitute for diabetics because they contribute to slow down the degradation of the starch in honey by the supposed effect of enzymatic inhibition, especially if it presents a low glycemic index, which it is imperative to measure especially after the elimination of a high percentage in the concerned samples of *Eruca sativa* and then a study of

confirmation by clinical trials is still necessary. On the other hand, it is strongly confirmed that the botanical origin influences the composition and the biological activity of honey.

The three types of honeys chosen at the end for further research are *Atractylis serratuloides*, *Retama sphaerocarpa* and *Eruca sativa*, which are the types of honeys typically considered as new in the shared literature. The results showed the main volatile profile and pollen characteristics of these honey types produced in the arid and semi-arid regions of Algeria. These honey types share common volatile components, but they also exhibit unique botanical type compounds that can be considered as chromatographic fingerprints. The botanical origin as well as the HS-SPME chromatographic profile of the honey revealed a large number of identified compounds from different classes. The most promising were: **2-naphthalene methanol, decahydro-α,α,4a- trimethyl-8-methylene-, [2R-(2α, 4aα, 8aβ)]**or**(β-eudesmol**) 1,6,10-dodecatrien-3-ol,3,7,11-Trimethyl-, (E) (β-Nerolidol), 1,6-Octadien-3-ol,3,7-dimethyl (linalool) and phenol, 2-methoxy as typical compounds for *Atractylis* honey. The first mentioned was the most important, it was found in all samples of *Atractylis* honey, and even in samples that had percentages of less than 10% plant pollen.which requires more research on the pollen of this honey considered under-representative, and at what minimum level it can be set.

Lilac aldehyde and lilac aldehyde D as the most important in terms of availability for *Retama* honey, for the present results, we do not consider them as exclusive for this type of honey. **Dimethyl trisulfide** was an exclusive compound for *Eruca* honeys.

Any variation in the characteristics of the honey mentioned or measured in this study or even others, can influence its organoleptic properties and distinguish it from another, which was confirmed by different sensory profiles for each honey type.

The labeling and declaration of the monoflorality of honey without passing by the step of melissopalynology is a common error strongly practiced by the beekeepers. This step itself considered as a basis in the study of honey is not sufficient if it is not confirmed by another set of analyses.

In modern science, the chemical platforms of detection of molecules have made a big step to the researcher in the traceability of his product.

The honey especially remains a product too complicated at the level of its composition, whose analyses require a good study of all the conditions of its manufacture.

The present work which approached this problem seems interesting, the following points are recommanded:

-Increasing the number of the monofloral honey samples to be analyzed.

-Starting by the melissopalynological analyses during the collection period.

-Using other volatile extraction methods (Exp: ultrasonic method) with two different solvents and compare the three profiles.

-Tring to link between the sensory profile with the volatile one, as well asilustrating by the olfactory gas chromatography (GC/O) results.

-Linking the *in vitro* tests to others *in vivo*.

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Annexe 1 : Different pollen types percentages in honey samples

A.V= Apicultural value. N= nectariferous plant. P= polliniferous plant

1.1.Samples marked as (S)

Family	Pollen type	apicultural value	S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16
Acanthaceae	*Acanthus molle*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Amaryllidaceae	*Allium*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Anacardiaceae	*Anacardium t*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Anacardiaceae	*Pistacia*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Anacardiaceae	*Rhus*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.1	1.4	1.2	0.0
Apiaceae	*Apium*	*N*	0.7	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.3	0.0	0.8	0.0
Apiaceae	*Bupleurum*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.2	0.0	0.2	1.1	0.0	0.4	0.7	0.0	0.0
Apiaceae	*Coriandrum*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.9	0.3	0.0	0.0
Apiaceae	*Eryngium campestre* t	*N*	1.8	0.0	0.0	0.2	1.1	0.0	0.7	2.2	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Apiaceae	*Ferula communis* t	*N*	11.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.2	0.0
Apiaceae	*Foeniculum* vulgare t	*N*	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Apiaceae	Other Apiaceae	*N*	0.0	0.0	0.0	0.0	0.2	0.0	12.9	13.6	11.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Apiaceae	*Pimpinella* anisum t	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.1	19.7	20.9	0.2	1.1	0.0	0.0	0.1	0.0	0.0
Arecaceae	*Chamaerops*	*P*	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.5	0.8	0.3	0.4	0.3	0.0	0.0
Arecaceae	*Phoenix dactylifera*	*P*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Asparagaceae	*Urginea*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Asparagaceae	*Muscari*	*N*	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	*Anthemis t*	*N*	0.4	0.0	0.9	1.2	0.4	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.2	0.0
Asteraceae	*Aster t*	*N*	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.1	0.4	0.2	0.0
Asteraceae	*Atractylis serratuloides*	*N*	64.1	6.2	14.8	6.5	45.0	0.9	17.6	10.1	8.4	40.7	28.9	6.7	31.4	34.5	34.6	3.6
Asteraceae	*Carthamus*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	2.5	0.0	0.4	0.0	0.2	0.0	0.0	0.1	0.2	0.0
Asteraceae	Centaurea t	N	0.7	0.0	0.2	0.0	0.4	0.0	0.0	3.6	5.9	1.1	0.6	0.0	0.3	0.4	0.5	0.0
Asteraceae	*Echinops*	*N*	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.1	0.1	0.3	0.0
Asteraceae	*Galactites t*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	*Launaea*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.6	0.3	0.2	0.3	0.1	0.2	0.0
Asteraceae	*Scorzonera t*	*N*	0.0	0.0	0.0	0.5	1.1	0.0	0.0	0.8	0.0	0.3	0.5	0.0	0.3	1.5	0.5	0.0

Asteraceae	*Otros* Asteraceae	*N*	0.0	0.0	0.3	0.0	0.0	2.3	1.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Boraginaceae	*Echium*	*N*	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	18.2	15.4	0.5	16.4	13.2	14.9	0.0
Brassicaceae	*Brassica napus* t	*N*	0.0	0.0	0.0	0.5	1.1	0.4	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.7	0.0	0.0
Brassicaceae	Other Brassicaceae	N	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Brassicaceae	*Eruca sativa* t	*N*	4.2	0.0	0.2	0.0	16.2	0.0	31.5	0.2	0.4	2.1	2.7	0.2	1.5	2.2	1.8	0.0
Brassicaceae	*Sinapis alba t*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	2.2	1.4	0.2	1.0	2.0	0.0	0.1	0.4	0.2	0.0
Brassicaceae	*Raphanus*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.6	0.7	0.5	0.5	0.0	0.9	0.0	0.9	0.0
Brassicaceae	*Capsella*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Buxaceae	*Buxus* sempervirens	*P*	0.0	0.0	0.2	0.0	0.0	0.2	0.0	0.0	0.0	1.6	2.1	0.0	2.4	3.3	2.3	0.0
Capparaceae	*Capparis* spinosa	*N*	0.0	0.0	0.0	0.2	0.0	0.0	1.5	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Caryophyllaceae	*Other*	*N*	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.2	0.2
Caryophyllaceae	*Paronychia*	*P*	0.2	0.0	0.0	0.0	0.9	0.0	0.0	0.0	0.0	0.0	9.0	2.9	14.9	14.7	12.6	0.0
Chenopodiaceae	*Chenopodium* t	N	0.0	0.0	0.2	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.1	0.1	0.2	0.0
Cistaceae	*Cistus*	*P*	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
Cistaceae	*Helianthemum*	*P*	0.4	3.8	0.0	0.0	0.0	1.9	0.4	1.2	0.0	1.7	0.6	0.2	0.7	1.1	0.6	4.7
Convolvulaceae	*Convolvulus*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Crassulaceae	*Sedum*	*N*	0.0	0.0	0.0	0.0	0.0	1.1	0.1	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cucurbitaceae	Cucurbita	N	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.2
Cyperaceae	*Carex*	*N*	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ericaceace	*Erica*	*N*	0.0	0.0	0.0	0.0	0.0	0.4	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0
Euphorbiaceae	*Euphorbia t*	*N*	0.2	0.0	0.0	0.0	0.9	0.0	1.7	1.6	1.5	0.2	0.0	0.0	0.3	0.1	0.0	0.0
Fabaceae	*Acacia*	*P*	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Ceratonia* siliqua	*P*	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.6	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Genista t*	*N*	2.9	88.6	76.6	85.6	20.5	90.6	0.0	9.7	5.1	2.7	1.4	0.0	0.4	3.4	5.3	89.9
Fabaceae	*Hedysarum* coronarium	*N*	0.4	0.0	0.2	0.0	0.0	0.0	0.0	0.0	1.1	1.0	0.8	0.0	0.9	0.6	0.0	0.0
Fabaceae	*Lotus t*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Onobrychis*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Ononis* natrix	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.2	2.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	Other Fabaceae	N	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Fabaceae	*Retama*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.5	0.0	0.0	0.0	0.0
Fabaceae	*Spartium junceum* t	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Trifolium pratense t*	*N*	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.3	0.0	0.0	0.0
Fabaceae	*Trifolium repens t*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.1	0.3	0.8	0.0
Fagaceae	*Quercus*	*P*	0.2	0.4	0.2	0.0	0.0	0.0	0.4	0.0	0.0	0.2	0.0	0.0	0.1	0.3	0.2	0.0

Lamiaceae	*Other* Lamiaceae	*N*	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lamiaceae	*Teucrium scorodonia* t	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lamiaceae	*Thymus t*	*N*	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.2	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.7
Lamiaceae	*Vitex*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.1	5.3	0.0	10.2	1.4	4.0	0.0
Lythraceae	*Punica* granatum	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Myrtaceae	*Eucalyptus*	*N*	0.2	0.0	0.0	0.2	0.4	0.0	0.7	11.6	12.5	4.3	3.5	0.3	3.7	3.3	3.5	0.0
Nitrariaceae	*Peganum* harmala	*N*	0.0	0.0	0.0	0.0	0.0	0.0	11.3	0.0	3.5	2.4	0.3	0.3	1.0	1.0	1.5	0.0
Oleaceae	*Fraxinus t*	*N*	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oleaceae	*Olea* europaea	*P*	0.2	0.0	0.3	0.0	0.2	0.0	1.1	1.0	0.0	0.3	0.0	2.7	0.0	0.1	1.1	0.0
Oxalicaceae	*Oxalis*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.3	0.0	0.4	0.0	0.0	0.0
Papaveraceae	*Papaver rhoeas t*	*P*	1.1	0.2	0.7	2.4	2.8	0.0	3.3	1.0	2.9	0.0	0.0	0.0	0.0	1.2	0.6	0.5
Plantaginaceae	Plantago	P	0.0	0.0	0.0	0.0	0.0	0.2	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Poaceae	*Poaceae*	*P*	0.0	0.0	0.7	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
Rhamnaceae	*Ziziphus lotus*	*N*	8.3	0.0	3.5	2.2	5.8	0.2	6.5	8.3	14.3	8.1	9.3	78.3	5.2	8.0	7.0	0.0
Salicaceae	*Salix*	*N*	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0
Tamaricaceae	*Tamarix pq?*	*P*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tamaricaceae	Tamarix gr?	P	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	8.4	0.0	4.8	1.8	3.0	0.0
Others	Others	N	0.2	0.4	0.9	0.2	0.0	0.7	1.0	1.6	2.2	1.3	1.7	0.3	0.3	0.8	0.8	0.2
	sum		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

1.2. Samples marked as (E) A and Am

Family	Pollen type	apicultural value	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	A1	AM1
Anacardiaceae	*Anacardium t*	*N*	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0	0.0	0.0
Anacardiaceae	*Pistacia*	*N*	0.0	0.0	0.2	0.0	0.3	0.0	0.0	0.0	0.0	0.2	0.0	0.0
Anacardiaceae	*Rhus*	*N*	0.0	0.1	0.0	0.2	0.5	0.0	0.8	0.0	0.0	0.0	0.0	0.0
Apiaceae	*Apium*	*N*	0.4	0.5	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Apiaceae	*Bupleurum*	*N*	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.2	0.0	0.0	0.0
Apiaceae	*Coriandrum*	*N*	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	1.4	0.0	0.0	0.0
Apiaceae	*Eryngium campestre* t	*N*	0.4	0.9	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.4	1.8	0.0
Apiaceae	*Foeniculum vulgare* t	*N*	0.0	0.5	0.2	0.0	0.0	33.9	0.0	0.0	0.0	0.7	0.0	0.0
Apiaceae	Other Apiaceae	*N*	1.6	0.0	0.4	0.2	0.0	0.0	0.6	0.0	0.0	0.4	0.0	0.0
Apiaceae	*Pimpinella anisum* t	*N*	0.0	0.6	12.6	11.0	7.2	0.0	12.4	0.4	1.3	16.5	32.2	0.0
Arecaceae	*Chamaerops*	*P*	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.2	0.0	0.0	0.0
Arecaceae	*Phoenix dactylifera*	*P*	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	*Anthemis t*	*N*	0.0	0.8	0.0	0.2	0.2	0.2	0.0	0.0	0.2	0.0	0.0	0.0
Asteraceae	*Aster t*	*N*	0.0	0.0	0.0	0.0	0.2	0.0	0.4	0.9	0.2	0.0	0.0	0.0
Asteraceae	*Atractylis* serratuloides	*N*	0.8	0.5	0.0	0.0	0.0	0.0	0.6	0.2	0.2	0.0	0.0	0.0
Asteraceae	*Bellis t*	*N*	0.0	0.0	0.6	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	*Carthamus*	*N*	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	Centaurea t	N	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.7	2.1	0.0
Asteraceae	*Chrysanthemum* t	N	0.0	0.5	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	1.1	0.3
Asteraceae	*Echinops*	*N*	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	*Galactites t*	*N*	0.0	0.1	0.6	0.2	0.0	0.2	0.4	1.1	0.3	0.0	0.0	0.3
Asteraceae	*Launaea*	*N*	0.4	0.0	0.4	0.0	0.2	0.0	0.0	0.0	0.0	0.0	2.1	0.7
Asteraceae	*Scorzonera t*	*N*	0.2	0.6	0.0	0.0	0.0	0.0	0.6	0.0	0.5	0.0	0.0	0.0
Asteraceae	*Otros* Asteraceae	*N*	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Boraginaceae	*Borago* officinalis	*N*	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Boraginaceae	*Echium*	*N*	0.0	0.0	0.0	0.0	0.0	0.9	1.0	0.0	0.3	0.0	0.5	1.7
Boraginaceae	*Phacelia*	*N*	0.0	0.0	0.2	0.0	0.0	0.0	1.4	0.0	0.0	0.0	0.0	0.0
Brassicaceae	*Brassica napus* t	*N*	0.2	0.3	1.2	0.9	1.1	0.2	0.4	0.2	0.0	0.0	4.2	0.0
Brassicaceae	Other Brassicaceae	N	0.0	0.0	0.0	1.4	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
Brassicaceae	*Eruca sativa* t	*N*	0.0	0.0	0.6	0.2	7.2	0.0	0.0	0.0	1.1	0.0	0.0	0.0
Brassicaceae	*Sinapis alba t*	*N*	0.4	0.4	4.3	1.6	2.8	0.0	0.0	0.0	0.6	0.0	0.0	0.0
Buxaceae	*Buxus* sempervirens	*P*	0.0	0.0	0.2	0.0	0.7	0.0	0.0	0.2	1.1	0.0	0.0	0.0
Capparaceae	*Capparis* spinosa	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.1	0.0
Caryophyllaceae	*Other*	*N*	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Caryophyllaceae	*Paronychia*	*P*	51.7	37.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chenopodiaceae	*Chenopodium* t	N	0.0	0.3	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cistaceae	*Cistus*	*P*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.2	0.0
Cistaceae	*Helianthemum*	*P*	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Convolvulaceae	*Convolvulus*	*N*	0.0	0.1	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0
Crassulaceae	*Sedum*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
Cucurbitaceae	*Citrullus*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0
Cucurbitaceae	Cucurbita	N	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cyperaceae	*Carex*	*N*	0.0	0.1	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Ericaceace	*Erica*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.4	1.5	0.0	0.0	0.0	9.8
Ericaceace	*Arbutus*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	11.2
Euphorbiaceae	*Crozophora*	*N*	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.5	0.0
Euphorbiaceae	*Euphorbia t*	*N*	0.6	2.1	0.0	0.0	0.5	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Acacia*	*P*	0.0	0.0	0.2	0.2	0.0	1.6	0.0	0.0	0.0	0.0	22.5	0.0
Fabaceae	*Arachis* hypogea	*N*	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Astragalus*	*N*	0.0	0.0	0.0	4.6	2.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Ceratonia* siliqua	*P*	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Genista t*	*N*	12.8	25.0	0.8	0.0	0.3	18.4	2.7	2.5	5.1	0.7	0.0	0.0
Fabaceae	*Hedysarum* coronarium	*N*	0.6	0.5	0.0	0.0	1.6	0.2	45.1	76.6	24.7	0.0	0.0	0.0
Fabaceae	*Lotus t*	*N*	0.0	0.1	0.2	0.0	0.8	4.6	0.0	0.0	0.0	0.0	0.0	1.4
Fabaceae	*Onobrychis*	*N*	0.0	0.1	0.8	0.0	1.6	0.0	0.0	7.7	2.2	0.0	21.2	0.7
Fabaceae	*Ononis* natrix	*N*	0.0	0.0	4.9	0.2	2.1	0.4	6.2	3.0	1.0	0.2	0.0	0.0
Fabaceae	Other Fabaceae	N	0.0	0.0	0.0	0.2	0.0	8.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Retama*	*N*	0.0	0.0	0.0	3.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Trifolium pratense t*	*N*	0.0	0.0	0.2	0.0	0.3	0.0	3.3	1.5	0.2	1.2	0.0	0.0
Fabaceae	*Trifolium repens t*	*N*	0.0	0.0	2.4	1.6	0.2	0.0	0.4	0.0	1.3	2.7	0.0	10.1
Fabaceae	*Vicia*	*N*	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fagaceae	*Quercus*	*P*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	9.8
Lamiaceae	*Phlomis*	*N*	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lamiaceae	*Rosmarinus officinalis* t	*N*	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lamiaceae	*Teucrium scorodonia* t	*N*	0.0	0.0	0.0	0.0	0.2	0.0	0.6	0.0	0.0	0.0	0.0	0.0
Lamiaceae	*Thymus t*	*N*	0.0	0.1	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lythraceae	*Lythrum*	*N*	0.0	0.0	0.0	0.0	0.0	1.5	0.2	0.0	0.0	0.2	0.0	0.0
Lythraceae	*Punica* granatum	*N*	0.0	0.0	0.0	0.7	0.0	0.5	0.0	0.2	1.1	0.0	0.0	0.0
Myrtaceae	*Eucalyptus*	*N*	1.4	0.5	32.4	58.9	27.3	12.8	16.1	2.3	50.6	72.8	0.7	1.7
Myrtaceae	*Myrtus*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.9	0.0	43.0
Nitrariaceae	*Peganum* harmala	*N*	0.0	4.9	0.0	0.0	0.2	0.0	0.4	0.0	0.5	0.0	0.0	0.0
Oleaceae	*Fraxinus t*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0
Oleaceae	*Olea* europaea	*P*	2.0	1.8	1.4	0.0	8.0	0.0	0.2	0.0	0.0	0.2	1.6	3.8

Oxalicaceae	*Oxalis*	*N*	0.0	0.0	0.2	1.1	0.2	0.2	0.0	0.0	0.2	0.0	0.0	0.0
Papaveraceae	*Papaver rhoeas t*	*P*	0.6	2.8	2.6	2.3	0.2	0.9	0.0	0.0	0.6	2.0	5.7	2.8
Plantaginaceae	Plantago	P	0.2	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.2	0.0
Poaceae	*Poaceae*	*P*	0.2	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.7
Rhamnaceae	Rhamnus t	N	0.0	0.0	0.0	0.0	0.0	0.0	4.4	0.2	0.0	0.0	0.0	0.0
Rhamnaceae	*Ziziphus lotus*	*N*	19.6	12.9	0.6	0.0	0.7	9.8	0.0	0.0	3.2	0.0	0.0	0.0
Rosaceae	*CrataegusT*	*N*	0.0	0.1	1.6	1.4	3.3	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Rosaceae	Other Rosaceae	N	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rosaceae	*Prunus t*	*N*	0.6	0.0	0.4	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.7
Rutaceae	Citrus	N	4.0	0.1	0.4	0.0	0.3	0.2	0.2	0.0	0.0	0.0	0.2	0.0
Salicaceae	*Salix*	*N*	0.0	0.0	0.2	0.9	0.2	0.0	0.0	0.2	0.0	0.0	0.0	0.0
Tamaricaceae	*Tamarix pq?*	*P*	0.8	0.1	25.3	6.2	1.6	0.2	0.0	0.0	0.0	0.0	0.0	0.0
Tamaricaceae	Tamarix gr?	P	0.0	0.0	0.0	0.0	24.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Crassulaceae	Opuntia ficus-indica	N	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0
Globulariaceae	Globularia	N	0.0	0.0	0.0	0.0	0.0	1.6	0.0	0.0	0.0	0.0	0.0	0.0
Smilacaeae	Smilax	N	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0
Others	Others	N	0.0	0.1	3.6	0.9	0.0	0.7	0.4	0.4	1.0	0.4	0.9	0.0
	sum		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

1.3. Samples marked as (R) and (H)

Family	Pollen type	apicultural value	R1	R2	R3	R4	R5	R6	R7	R8	R9	R10	R-	H1	H2	H3	H4	H5	X4
Anacardiaceae	*Rhus*	*N*	0.0	0.0	0.3	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.9	0.0	0.0	0.0	0.0	0.0
Apiaceae	*Apium*	*N*	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Apiaceae	*Bupleurum*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	51.6	0.0	0.0	0.0	0.0	0.0	0.5
Apiaceae	*Coriandrum*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.3	8.7	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.3
Apiaceae	*Eryngium* campestre t	*N*	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Apiaceae	*Ferula* communis t	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.3	0.0	0.5	0.6	0.0	0.0	0.0	0.0	0.0	3.0
Apiaceae	*Foeniculum* vulgare t	*N*	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
Apiaceae	Other Apiaceae	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Apiaceae	*Pimpinella* anisum t	*N*	0.0	0.0	3.5	0.6	0.0	0.0	5.9	1.1	0.0	0.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Arecaceae	*Chamaerops*	*P*	0.0	0.0	0.0	0.0	0.2	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Arecaceae	*Phoenix dactylifera*	*P*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	1.2
Asparagaceae	*Muscari*	*N*	0.3	0.0	0.0	0.0	0.6	0.0	0.5	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
	Asphodelus	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	*Anthemis t*	*N*	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.1	1.3	0.0	0.0	2.6	0.0	0.0	0.8
Asteraceae	*Aster t*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	*Atractylis serratuloides*	*N*	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	3.2	0.6	0.0	0.0	0.0	1.8	0.7	3.1
Asteraceae	*Bellis t*	*N*	0.0	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.3	0.0	0.0	0.0
Asteraceae	*Artemisia*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	Centaurea t	N	0.3	0.5	0.2	0.4	0.2	0.2	0.0	0.2	0.0	3.3	0.4	0.0	0.0	0.0	0.0	0.0	3.5
Asteraceae	*Chrysanthemum* t	N	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	*Echinops*	*N*	0.0	0.0	0.0	0.0	0.0	2.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	*Galactites t*	*N*	0.0	0.0	0.2	0.0	0.0	0.0	0.3	0.6	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	*Launaea*	*N*	0.3	0.0	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	*Scorzonera t*	*N*	0.0	0.0	0.0	0.6	0.0	0.6	0.2	1.6	0.0	0.4	0.0	0.0	0.0	0.2	0.0	0.0	0.0
Asteraceae	*Otros* Asteraceae	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.3	1.1	0.2
Boraginaceae	*Borago* officinalis	*N*	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Boraginaceae	*Echium*	*N*	0.3	0.3	0.3	0.0	0.4	0.2	0.0	0.0	0.0	0.0	1.1	0.0	0.0	0.2	0.0	0.2	0.0
Boraginaceae	*Phacelia*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.2	1.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Brassicaceae	*Brassica napus* t	*N*	0.0	0.0	0.0	0.2	0.0	2.7	0.0	0.2	0.2	0.8	3.9	0.2	0.0	0.2	0.0	0.0	0.2
Brassicaceae	Other Brassicaceae	N	0.0	0.0	0.0	0.0	0.2	0.2	0.0	0.2	1.1	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0
Brassicaceae	*Eruca sativa* t	*N*	6.2	6.9	6.1	18.4	10.6	1.3	0.0	0.0	0.0	0.0	6.6	43.5	48.3	85.8	90.6	93.8	0.0
Brassicaceae	*Sinapis alba t*	*N*	0.0	0.0	0.0	0.0	0.0	2.5	0.0	0.3	0.2	0.0	0.1	0.0	0.0	0.2	0.0	0.0	0.0

Brassicaceae	*Raphanus*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Buxaceae	*Buxus* sempervirens	*P*	0.0	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Capparaceae	*Capparis* spinosa	*N*	2.3	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.4	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Caryophyllaceae	*Other*	*N*	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
Caryophyllaceae	*Paronychia*	*P*	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Chenopodiaceae	*Chenopodium* t	N	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.5	1.8	1.3	0.0
Cistaceae	*Cistus*	*P*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cistaceae	*Helianthemum*	*P*	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	9.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Convolvulaceae	*Convolvulus*	*N*	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Crassulaceae	*Sedum*	*N*	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2
Cucurbitaceae	Cucurbita	N	0.0	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cyperaceae	*Carex*	*N*	0.0	0.0	0.0	0.0	0.0	0.2	2.6	3.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ericaceace	*Erica*	*N*	0.0	0.3	0.0	0.0	0.2	2.3	0.0	0.0	0.0	0.3	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Euphorbiaceae	*Crozophora*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Euphorbiaceae	*Euphorbia t*	*N*	0.0	0.0	0.0	0.0	0.0	0.2	0.3	0.3	3.2	0.0	1.0	0.0	0.0	0.8	0.5	1.1	0.3
Fabaceae	*Acacia*	*P*	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Arachis* hypogea	*N*	0.0	0.5	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Astragalus*	*N*	0.0	0.0	0.0	0.8	0.0	2.8	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Genista t*	*N*	0.0	0.0	6.5	2.0	0.0	0.6	0.0	0.3	0.0	29.0	3.2	0.0	0.0	2.4	0.0	0.0	40.6
Fabaceae	*Hedysarum coronarium*	*N*	0.0	0.5	0.0	0.0	0.0	0.0	74.3	0.0	0.0	0.3	0.7	0.0	0.2	0.0	0.0	0.0	0.0
Fabaceae	*Lotus t*	*N*	0.0	0.0	0.0	0.0	0.2	0.9	0.0	0.5	0.0	6.7	0.0	13.8	14.6	0.0	0.0	0.0	0.0
Fabaceae	*Onobrychis*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Ononis* natrix	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.9	0.6	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	Other Fabaceae	N	0.0	0.0	0.9	1.4	0.2	3.8	0.0	9.0	0.4	0.0	0.3	0.2	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Retama*	*N*	87.6	84.1	22.2	60.0	83.1	63.4	10.3	50.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Psoralea*	*N*	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.5	0.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Spartium junceum* t	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	90.6	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Trifolium pratense* t	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	10.9	0.0	0.0	0.0	0.0	1.3	0.2	0.0
Fabaceae	*Trifolium repens t*	*N*	0.0	0.0	0.0	0.0	0.0	0.9	0.2	0.3	0.0	0.0	0.0	0.0	0.0	3.7	0.0	0.0	0.0
Fabaceae	*Vicia*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.6	0.0	0.0	0.0	0.0
Fagaceae	*Quercus*	*P*	0.0	0.0	0.0	0.2	0.0	0.4	0.0	0.0	0.0	0.0	0.1	1.2	0.0	0.0	0.0	0.0	0.0
Lamiaceae	*Other* Lamiaceae	*N*	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lamiaceae	*Teucrium scorodonia* t	*N*	0.0	0.3	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.0	0.0
Lamiaceae	*Thymus t*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	8.9	0.0	4.3	3.2	0.0	0.0	0.0	0.0
Lythraceae	*Punica* granatum	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.3	2.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Myrtaceae	*Eucalyptus*	*N*	0.7	0.0	42.7	0.4	0.0	0.0	0.5	8.7	0.0	0.3	0.0	0.0	0.4	0.8	0.0	0.0	0.5
Myrtaceae	*Myrtus*	*N*	0.0	0.0	6.3	5.2	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Nitrariaceae	*Peganum harmala*	*N*	0.0	1.8	8.9	6.0	0.0	0.0	1.0	0.0	0.0	0.0	0.6	31.1	25.3	0.0	0.0	0.0	7.4
Oleaceae	*Fraxinus t*	*N*	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Oleaceae	*Olea europaea*	*P*	0.3	0.0	0.0	0.0	0.7	4.9	0.7	1.0	0.0	0.0	0.1	0.0	0.0	0.2	0.0	0.0	0.2
Oxalicaceae	*Oxalis*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Papaveraceae	*Papaver rhoeas t*	*P*	0.3	0.8	0.0	0.0	0.6	0.8	0.0	1.3	0.2	0.0	0.6	0.2	0.0	0.3	0.0	0.0	0.0
Plantaginaceae	*Plantago*	P	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Poaceae	*Poaceae*	*P*	1.3	1.0	0.0	0.0	0.7	1.5	0.7	0.6	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0
Rhamnaceae	*Ziziphus lotus*	*N*	0.0	0.0	0.0	0.2	0.2	0.9	0.0	0.0	0.0	0.5	0.0	0.2	0.0	1.1	0.0	0.0	37.3
Rosaceae	*Prunus t*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rutaceae	Citrus	N	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
Salicaceae	*Salix*	*N*	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Tamaricaceae	*Tamarix pq?*	*P*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	1.1	0.0
Tamaricaceae	*Tamarix gr?*	P	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	23.5	0.0	0.0	0.0	0.0	0.0	0.0
Thymelaeaceae	*Thymelaea*	N	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Globulariaceae	*Globularia*	N	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	16.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Others	Others	N	0.0	1.0	0.7	1.8	1.1	1.5	0.8	2.1	1.3	0.0	1.0	0.8	3.9	0.5	0.3	0.4	0.7
	Populus	P	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Scabiosa	N	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	sum		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

1.4. Samplesmarked as (M)

Family	Pollen type	apicultural value	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10	M11	M12	M13
Apiaceae	*Coriandrum*	*N*	0.0	0.0	0.1	0.1	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Apiaceae	*Pimpinella anisum* t	*N*	0.0	0.3	0.1	0.0	1.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.3
Araliaceae	*Hedera helix* t.	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0
Arecaceae	*Chamaerops*	*P*	0.0	0.0	3.2	4.2	0.8	1.0	0.6	1.2	6.5	0.0	3.9	0.0	0.0
Arecaceae	*Phoenix dactylifera*	*P*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.4	0.0	3.7	0.0
Asparagaceae	*Urginea*	*N*	0.0	0.0	0.0	0.0	0.3	0.2	0.3	0.0	0.6	0.4	0.8	0.0	0.0
Asparagaceae	*Muscari*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.2	0.0	0.0
Asteraceae	*Anthemis t*	*N*	0.3	0.0	0.0	0.0	0.1	0.5	0.0	0.0	0.0	0.2	0.0	0.3	0.3
Asteraceae	*Aster t*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.4	0.0	0.2
Asteraceae	*Atractylis* serratuloides	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
Asteraceae	*Artemisia*	*N*	0.0	0.0	2.8	3.1	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	Centaurea t	N	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Asteraceae	*Chrysanthemum* t	N	0.0	0.0	0.0	0.0	0.0	0.2	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	*Echinops*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	*Galactites t*	*N*	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Asteraceae	*Launaea*	*N*	0.7	0.2	0.0	0.0	0.0	0.2	0.0	0.7	0.0	0.0	0.2	0.0	0.3
Asteraceae	*Scorzonera t*	*N*	0.0	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	1.1	0.0
Asteraceae	*Cichorium*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.0	0.2	0.0	0.0
Betulaceae	*Carpinus*	*P*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Boraginaceae	*Echium*	*N*	0.0	0.0	0.0	0.0	0.3	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Brassicaceae	*Brassica napus* t	*N*	0.0	0.0	0.9	0.7	0.0	0.0	0.3	0.0	0.2	0.6	0.4	0.9	0.3
Brassicaceae	*Diplotaxis*	*N*	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Brassicaceae	Other Brassicaceae	N	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.4	0.4	0.2	0.0	0.0
Brassicaceae	*Eruca sativa* t	*N*	3.7	3.2	3.4	4.4	17.3	9.1	14.4	11.0	2.6	0.6	1.2	3.2	1.0
Brassicaceae	*Sinapis alba t*	*N*	0.2	0.0	0.0	0.0	5.7	4.7	0.0	0.0	0.6	0.2	0.2	0.0	0.2
Brassicaceae	*Capsella*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.2
Capparaceae	*Capparis spinosa*	*N*	2.8	6.1	69.4	61.4	51.5	56.6	21.7	70.1	10.8	10.8	10.6	0.0	5.8
Caryophyllaceae	*Other*	*N*	0.2	0.5	0.0	0.0	0.0	0.0	0.0	0.5	0.0	0.4	0.2	0.0	0.6
Chenopodiaceae	*Chenopodium* t	N	0.0	0.0	0.1	0.0	0.0	0.9	0.1	0.0	0.0	0.0	0.0	0.0	0.0
Cistaceae	*Cistus*	*P*	0.0	0.0	0.0	0.0	0.5	0.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cucurbitaceae	*Citrullus*	*N*	0.5	0.0	0.1	0.1	0.0	0.5	0.3	0.0	0.0	0.6	0.0	0.0	0.6
Cucurbitaceae	Cucurbita	N	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cupressaceae	*Cupressus*	*P*	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cyperaceae	*Carex*	*N*	0.0	0.2	0.0	0.0	0.1	0.0	0.0	0.0	0.4	0.4	0.6	0.3	0.6
Ephedra	*Ephedra*	*N*	0.0	0.0	0.4	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Ericaceace	*Erica*	*N*	0.0	0.0	0.1	0.0	0.1	0.3	0.0	0.0	0.0	0.2	0.6	0.0	0.0
Euphorbiaceae	*Euphorbia t*	*N*	1.3	1.4	0.8	1.2	1.3	1.4	1.0	2.3	3.2	0.8	0.4	0.9	1.8
Fabaceae	*Astragalus*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	4.5	0.0	0.0	0.0
Fabaceae	*Genista t*	*N*	46.9	38.3	13.0	16.0	8.3	12.9	49.2	3.9	0.2	1.0	73.4	83.0	0.0
Fabaceae	*Hedysarum* coronarium	*N*	31.8	35.1	0.0	0.0	0.0	3.8	9.1	0.0	0.0	0.0	0.0	0.0	0.0
	Hedysa/Zygophyllum	*N*	0.0	0.0	5.1	5.7	5.9	0.0	0.0	1.0	0.9	1.0	1.0	0.3	39.5
Fabaceae	*Onobrychis*	*N*	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Ononis* natrix	*N*	0.7	0.0	0.0	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	Other Fabaceae	N	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Retama*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	37.4
Fabaceae	*Pisum* sativum	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	5.4	0.0	0.0	0.0	0.0	0.0
Fabaceae	*Spartium junceum* t	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	65.4	70.6	0.0	0.0	0.0
Fabaceae	*Trifolium pratense t*	*N*	0.0	0.0	0.0	0.0	0.0	0.7	0.0	0.0	0.6	1.8	1.8	0.0	0.0
Fagaceae	*Quercus*	*P*	0.0	0.0	0.0	0.0	0.1	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Lamiaceae	*Lavandula t*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Lamiaceae	*Vitex*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0	0.0	0.0

Lythraceae	*Punica* granatum	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.6	0.4	3.7	0.0
Myrtaceae	*Eucalyptus*	*N*	0.0	0.0	0.0	0.0	0.6	1.2	0.3	0.0	0.0	0.0	0.0	0.3	0.0
Nitrariaceae	*Peganum* harmala	*N*	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	1.1	0.2	0.6	0.0	0.0
Oleaceae	*Fraxinus t*	*N*	0.0	0.0	0.0	0.1	0.3	0.2	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Oleaceae	*Olea* europaea	*P*	0.2	0.0	0.1	0.9	3.2	3.4	0.4	0.8	2.2	1.4	1.0	0.3	0.0
Oxalicaceae	*Oxalis*	*N*	0.0	0.0	0.1	0.0	0.0	0.0	0.4	0.0	0.0	0.0	0.0	0.0	0.0
Papaveraceae	*Papaver rhoeas t*	*P*	10.1	13.3	0.0	0.0	0.3	0.5	0.1	0.0	0.2	0.2	0.0	0.0	8.3
Plantaginaceae	Plantago	P	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Plumbaginaceae	*Limonium*	*N*	0.0	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Poaceae	*Poaceae*	*P*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
Rhamnaceae	Rhamnus t	N	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.3	0.0
Rhamnaceae	*Ziziphus lotus*	*N*	0.0	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Rosaceae	*CrataegusT*	*N*	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Salicaceae	*Salix*	*N*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.2	0.0	0.0	0.0
Tamaricaceae	*Tamarix pq?*	*P*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.0
Tamaricaceae	Tamarix gr?	P	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	1.7	0.8	0.0	0.3	0.0
Crassulaceae	Opuntia ficus-indica	N	0.0	0.0	0.0	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Thymelaeaceae	Thymelaea	N	0.0	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Others	Others	N	0.7	0.3	0.0	0.3	0.4	0.0	0.0	3.0	1.1	0.0	0.6	0.6	0.6
	sum		100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0

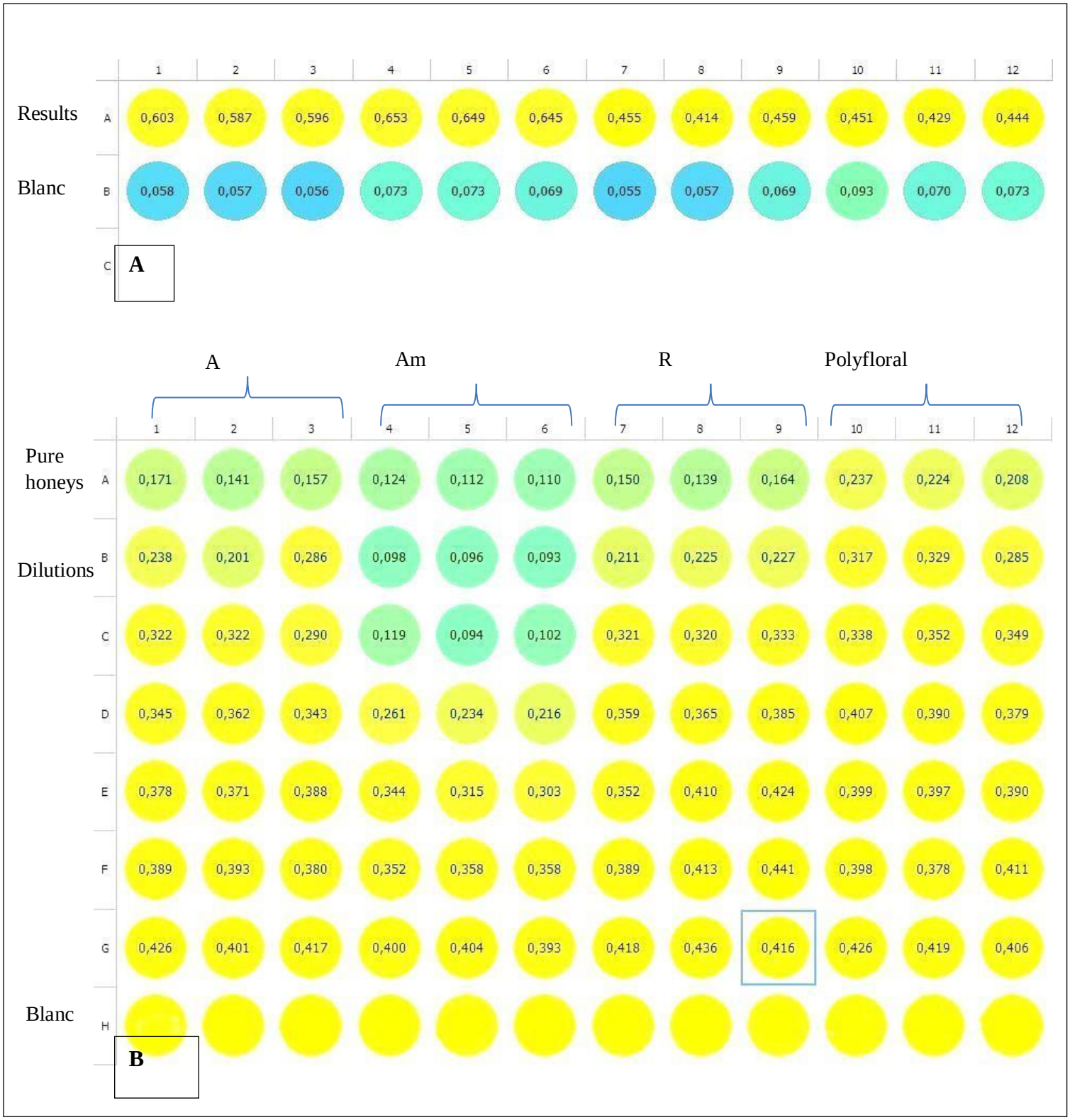
Annexe 2 : Biological part

1. Plate of the results of the antioxidant activity of four representative samples (A. Am. R6 and R6)

A. Polyphenol results

B. DPPH results

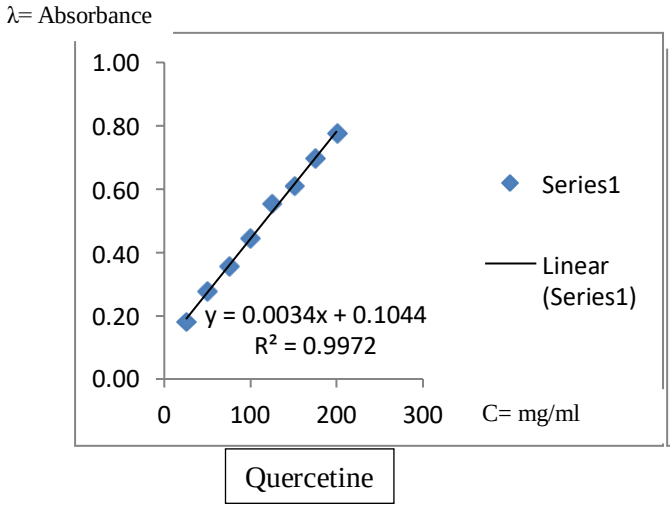
C. ABTS results



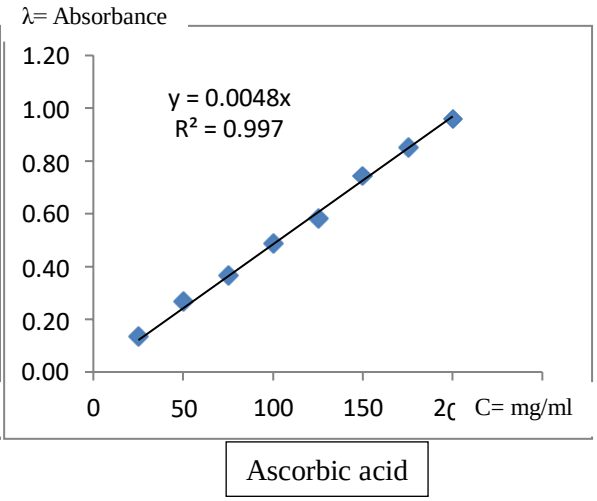
	1	2	3	4	5	6	7	8	9	10	11	12
A	0,082	0,066	0,072	0,066	0,066	0,071	0,068	0,070	0,078	0,080	0,077	0,081
B	0,061	0,056	0,056	0,069	0,054	0,055	0,063	0,066	0,073	0,109	0,139	0,145
C	0,197	0,196	0,163	0,076	0,050	0,059	0,214	0,187	0,225	0,292	0,252	0,272
D	0,329	0,338	0,300	0,165	0,207	0,157	0,355	0,359	0,372	0,416	0,386	0,386
E	0,433	0,412	0,409	0,325	0,311	0,327	0,431	0,427	0,437	0,426	0,378	0,446
F	0,480	0,464	0,476	0,419	0,420	0,380	0,492	0,494	0,487	0,503	0,500	0,516
G	0,541	0,509	0,484	0,493	0,497	0,493	0,507	0,512	0,528	0,514	0,523	0,545
H												

C

2. Calibration curves of Quecetin and ascorbic acid (TPC) and (TFC)



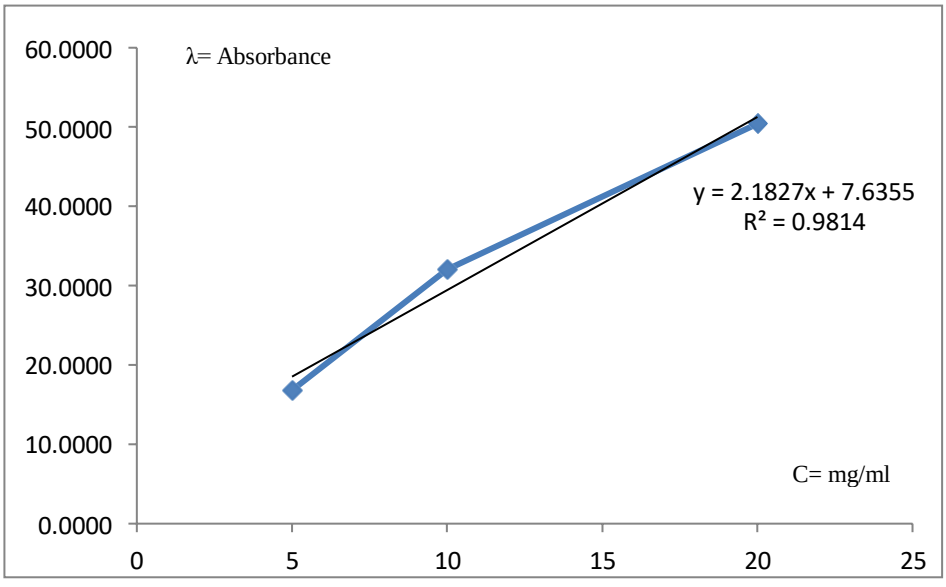
Quercetine



Ascorbic acid

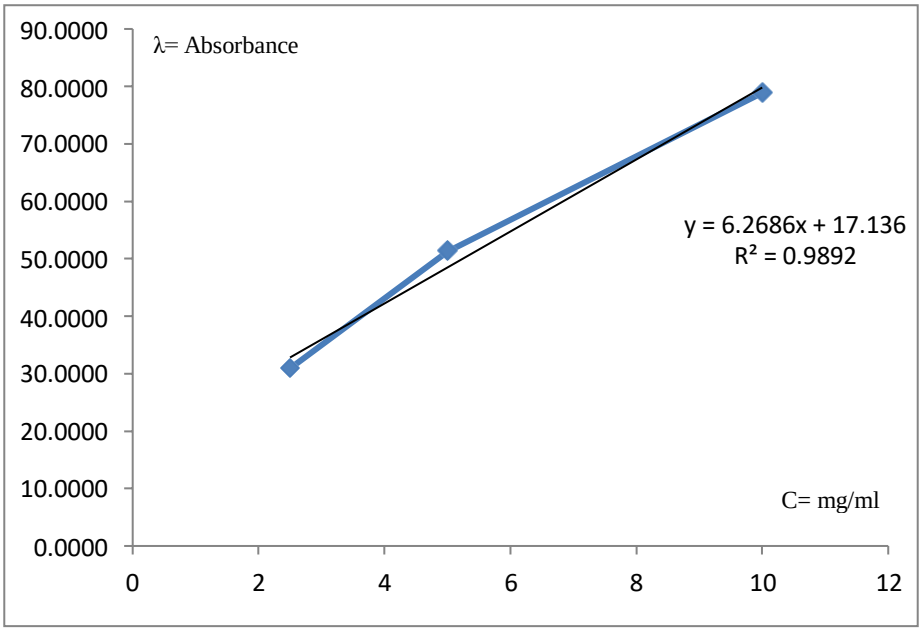
3. IC $_{50}$ of DPPH and ABTS results of main samples

	Y	Bx	+	A	IC_{50}	M	S.D.
A1	50	2.3673	+	17.017	13.93	13.43	0.65
A1	50	2.6696	+	16.097	12.70		
A1	50	2.3038	+	18.536	13.66		
A1	50	2.4469	+	17.21667	-1687.95		
Am1	50	11.607	+	14.718	3.04	2.94	0.09
Am1	50	13.27	+	11.191	2.92		
Am1	50	12.5042	+	14.19	2.86		
Am1	50	12.4604	+	13.36633	2.94		
R5	50	2.5368		16.216	13.32	13.54	0.45
R5	50	2.7582		13.475	13.24		
R5	50	2.7307		11.618	14.06		
R5	50	2.675233	+	13.76967			
R6	50				#DIV/0!	#DIV/0!	#DIV/0!
R6	50				#DIV/0!		
R6	50	2.1827		7.6355	19.41		
R6	50	2.1827		7.6355	#DIV/0!		



Annexe 4: IC $_{50}$of ABTS .+ results of main samples

	Y	Bx	+	A	IC50	M	S.D.
A1	50	11.004	+	10.691	3.57		
A1	50	10.289	+	13.554	3.54		
A1	50	11.436	+	11.036	3.41		
A1	50	10.90967	+	11.76033	-1687.95	3.51	0.09
Am1	50	26.68	+	2.4534	1.78		
Am1	50	26.243	+	3.0861	1.79		
Am1	50	25.328	+	6.3739	1.72		
Am1	50	26.08367	+	3.971133	1.76	1.76	0.04
R5	50	10.319		10.243	3.85		
R5	50	13.06		1.6845	3.70		
R5	50	13.658		2.0734	3.51		
R5	50	12.34567	+	4.666967		3.69	0.17
R6	50	7.0687		11.735	5.41		
R6	50	6.0393		19.371	5.07		
R6	50	6.2686		17.136	5.24		
R6	50	6.458867		16.08067	5.24	5.24	0.17



4. Descriptive diagram of the alpha amylase inhibition test plate

	1	2	3	4	5	6	7	8	9	10	11	12
A	As_1	As_1	As_1	A_{b1}	As_2	As_2	As_2	A_{b2}	As	As	As	A_b
B	As_1	As_1	As_1	A_{b1}	As_2	As_2	As_2	A_{b2}	As	As	As	A_b
C	As_1	As_1	As_1	A_{b1}	As_2	As_2	As_2	A_{b2}	As	As	As	A_b
D	As_1	As_1	As_1	A_{b1}	As_2	As_2	As_2	A_{b2}	As	As	As	A_b
E	As_1	As_1	As_1	A_{b1}	As_2	As_2	As_2	A_{b2}	As	As	As	A_b
F	As_1	As_1	As_1	A_{b1}	As_2	As_2	As_2	A_{b2}	As	As	As	A_b
G	As_1	As_1	As_1	A_{b1}	As_2	As_2	As_2	A_{b2}	As	As	As	A_b
H	Ae	Ae	Ae	Ae	Ae	Ae	Ac	Ac	Ac	Ac	Ac	Ac

Annexe 3: Volatile compounds part: Calcul method of Linear retention index (LRI)

Non-isothermal Kovats retention indices (from temperature-programming. using definition of Van den Dool and Kratz)

LRI c = 100n + 100(tx-tn) / (tn+1 − tn) = =100*(((RT of present compound-RT of previous alcan)/(RT of equivalent alcan- RT of previous alcan))+ N of carbon of previous alcan)

Using Alcanes as references and their carbon numbers with their RT.

Annexe 4 : **Sensorial analysesquestionnaire part**

Rifka NAKIB, PhD Student, Food Science. U. mmTizi Ouzou (Algeria)/ U. Vigo (Spain)

SENSORIAL HONEY DESCRIPTION

Name: .....

Date: .....

Sample code: .....

Observe and taste the samples then indicate your appreciation of the characters listed below by ticking the appropriate box:

Organoleptic characteristics

1. **Texture :**

Liquid ☐ Creamy ☐ Crystallized ☐

2. **Colour:**

Liquid	White		Light amber		Amber		Dark amber		Dark	
Crystallized	White		Straw (paja)		Gold		Orange		Brown	

3. **Smell/Odor:**

Fruity	**Candy**	**Floral**	**Vegetal**	**Chemical**	**Animal**	**Degraded**
	Caramel	Orange blossom	Fresh grass	Lactic		Soap
	Vanilla	Lavender	Mint			Smoked
		Violet	Leaves			Burned
	Other	Rose	Resin	**Other:**		Caramelized
		Other:	Wood			**Other:**
			Pepper			
			Cinnamon			
			Other:			

Intensity Smell 0 5 10

Persistence Smell 0 5 10

4. Flavor :

Sweetness	Sourness	Saltiness	Bitterness
0 5 10	0 5 10	0 5 10	0 5 10
0 5 10	0 5 10	0 5 10	0 5 10
Comments:			

5. Aroma

Fruity	Candy	Floral		Vegetal		Chemical		Animal		Degraded	
	Vanilla	Orange blossom		fresh grass		Lactic		Dog		Soap	
		Rose		Mint		Camphorated		Urine		Burned	
	Caramel	Lavender		Wood		Yeast		Leather		Smoked	
		Violet		Leaves		Other:		Wax			
	Other	Other:		Resin				Other:		Caramelized	
				Pepper						Other:	
				Cinnamon							
				Other:							

Intensity of main aroma:

ASTRINGENCY

NO		YES	

SPICY

NO		YES	

Comments:.....
.....
.....

SENSORIAL HONEY DESCRIPTION

Questions

Answer the questions in the appropriate space:

1. Can you classify the samples on groups according to some similarities?
   If so, what are you basing it on?

Groups	Groupe 1	Groupe 2	Groupe 3	Groupe 4
Sample code				
Common characteristics				

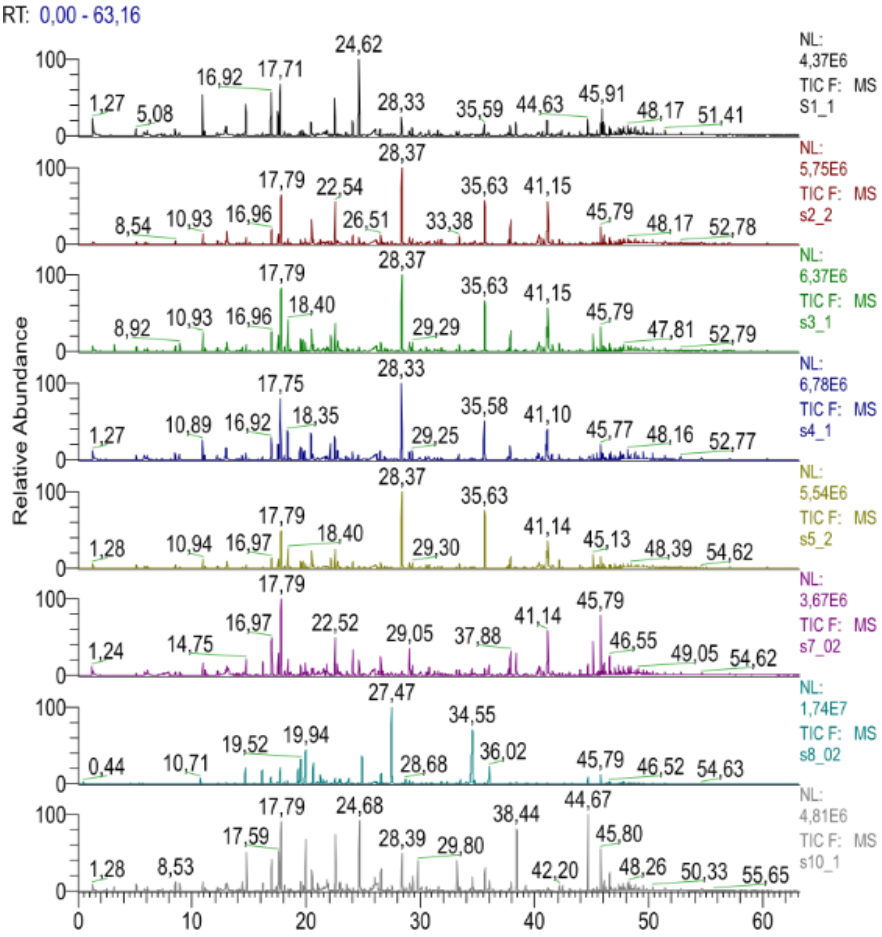
2. The presented honeys are from an Algerian origin, rarely studied.
   Can you approach each group to a type of (Plant, food or honey type) that you already know? (Quoting the character based on (flavor, aroma, smell...)
   _Groupe 1: .....
   _Groupe 2:.....
   _Groupe 3:.....
   _Groupe 4:.....

3. Which honey group do you prefer? Why ?
   .....
   What is your impression of a crystallized honey? Justify
   .....
   .....

Thank you for your collaboration

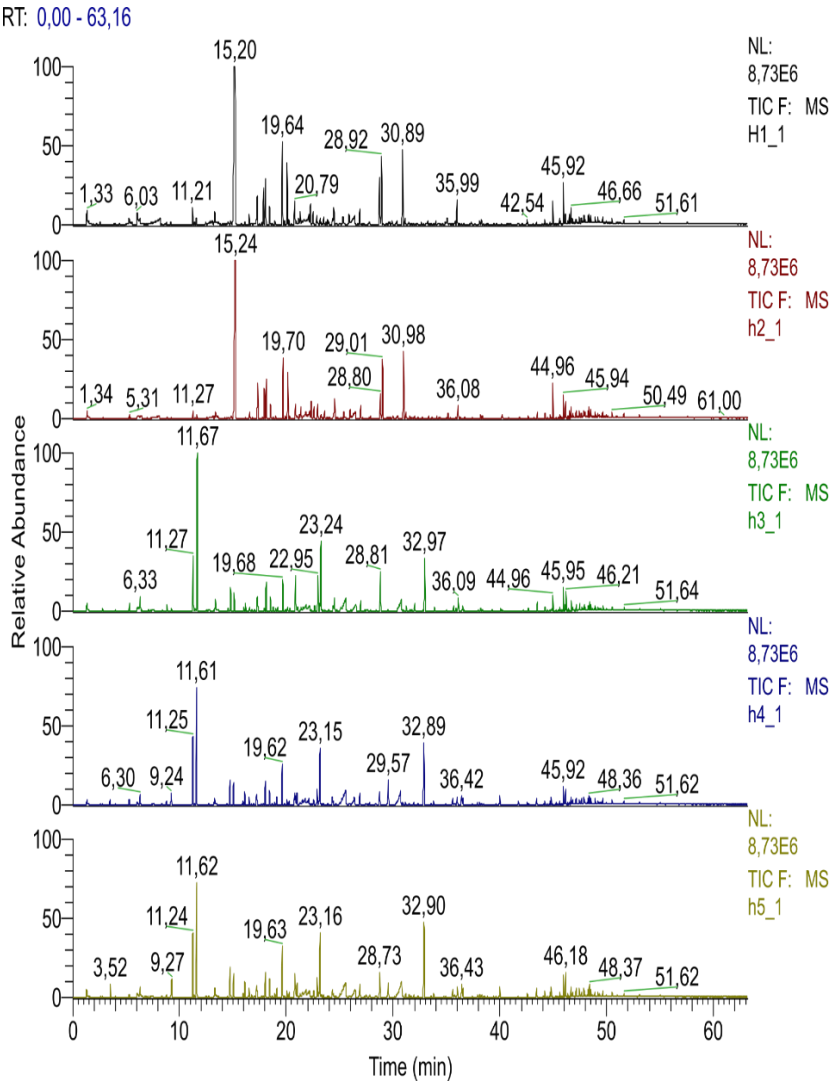
Annexe 5: **Chromatograms of volatile compounds**

1. Volatile profile of *Atractylis serratuloides* honey samples.



2. Volatile profile of *Eruca sativa* honey samples.

3.



Volatile profile of *Retama spherocarpa* honey samples