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Enzymes activities, hydroxymethylfurfural content and pollen spectrum of some Algerian honey

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Forty honey samples from *Apis mellifera* were collected in different parts of Algeria during 2007 to 2010 and were analysed for parameters including hydroxymethylfurfural (HMF), invertase and diastase activities. The spectra of pollen of the honeys collected in those areas were also studied. The results show that the amylase activity ranged from 4.40 to 30.15 DN, with only one sample having a low diastase number than 8 DN. The invertase activity ranged from 0 to 138.38 UE. Invertase is generally present in small amounts and is inactivated by heating. The HMF values in our study were low (<40 mg/kg). 85% of samples (34 samples) had a HMF content less than 15 mg/kg HMF. The results of mellissopalynology show that the NPG/10 g for all samples of honey ranged from 8×10^3 to 2.01×10^6 ; all samples studied are original flower honey. We also note that the dominant pollen in the majority of samples was: pollen of *Eucalyptus*, *Trifolium* sp, *Echium* sp, *Hedysarium corornarium* and to a lesser extent, *Asteraceae* (mostly *Carduus*), *Apiaceae*, *Ericaceae*, *Rosaceae* and *Rutaceae*. In general, the samples were found to meet the requirements of the international honey standards.

Key words: Honey, hydroxymethylfurfural (HMF), diastase activity, invertase activity, mellissopalynology.

INTRODUCTION

Honey, produced by the honeybee, is a natural supersaturated sugar solution, which is mainly composed of a complex mixture of carbohydrates and contains certain minor constituents like proteins, enzymes (invertase, glucose oxidase, catalase, phosphatases), amino and organic acids (gluconic acid, acetic acid, etc.), lipids, vitamins (ascorbic acid, niacin, pyridoxine etc.), volatile chemicals, phenolic acids, flavonoids, and carotenoid-like substances and minerals (Blasa et al., 2006). The composition of honey depends on the plant species visited by the honeybees and the environmental, processing and storage conditions (Bertoncelj et al., 2007,

Guler et al., 2007).

Enzymes are the most important and also the most interesting honey components. They are accountable for the conversion of nectar and honeydew to honey, and serve as a sensitive indicator of the honey treatment. In some countries, the specification of enzymes is a binding legal indicator (Bogdanov et al., 1987; Oddo et al., 1999). Honey naturally contains small amounts of enzymes. The predominant enzymes in honey are diastase (amylase), invertase (α -glucosidase) and glucose oxidase. Others, including catalase and acid phosphatase, can also be present. Honey's enzyme content can vary widely by

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floral source and region. The levels of some enzymes such as diastase are relatively easy to measure and have been used for many years to estimate the extent of heating to which a honey has been exposed. Such information has been required by some countries where heating of honey is believed to reduce or destroy potentially health-promoting properties. In fact, because the enzyme content of fresh honeys can vary widely, enzyme levels in packed honey are a poor indicator of processing and storage conditions (White, 1968).

The HMF content depends on the temperature, the duration of storage or heating, the content of various sugars, pH, the mineral content (especially that of iron) and the water content of honey (Gondarski, 1961; Ivanov, 1978; Pichler et al., 1984). According to some authors (Bogdanov, 1999), fresh honey contains practically no HMF but its concentrations increase during storage. Many studies (Ivanov, 1986; Pichler et al., 1984; Von der Oh, 1992; White, 1964) have shown that HMF levels in natural non-heated honey is almost always under 1 mg%. For 1 to 2 years, the HMF content could increase up to 3 to 4 mg% or more depending on the temperature of storage. The high HMF content in honeys is related to its long-term storage or to its overheating for liquidification of crystallized honey (White et al., 1964).

Pollen analysis has been the traditional method to determine the floral origin of the honey (Hermosin et al., 2003; Von der Ohe, 1994). Usually, honey is considered unifloral if the pollen frequency of that plant is >45%. (Terrab et al., 2003c). The identification and quantification of pollen grains in honey sediment (melissopalynological) is still the most important method for determining the botanical species that make up the pollen spectrum of honey samples. The pollen spectrum shows whether the honey samples are from different geographical and floral regions, and also the flowering time of the bee plants and their value as nectar and pollen suppliers. Through quantitative analysis of pollen grains, it is possible to establish the proportion which each plant, as a nectar supplier contributes to the constitution of the honey. However, this proportion depends on the pollen, and on the structure and biology of the different plant species (Anklam, 1998).

The objective of this study was to determine the enzymes activity of α -glucosidase (invertase), β -amylase (diastase), the contents of hydroxymethylfurfural (HMF = 5-hydroxymethylfuran-2-carbaldehyde) and the contents of pollen spectrum for forty honey samples sorted by their botanical origins.

MATERIALS AND METHODS

Honey samples

The forty fresh honey samples (Table 1) were collected directly from professional beekeepers in various regions in Algeria. The vegetation in these areas can be described as being composed of a mixture of various natural plants. Honey samples were filled into

glass jars after harvesting and stored at 4°C until analysis. Duplicate analyses were performed for each parameters.

Physico-chemicals analysis

Method of determination of enzymes activities

The diastase enzymes (α - and β -amylase) were analysed with the Phadebas procedure. The determination of the honey diastase activity is a photometric method in which a blue tint insoluble cross-linked type of starch is used as a substrate. This is hydrolyzed by the enzyme, giving soluble blue fragments in water, determined photometrically at 620 nm with a Varian spectrophotometer type UV visible Cary 50. The absorbance of the solution is directly proportional to the diastatic activity of the sample.

Measurement of the invertase enzyme (α -glucosidase) was done following the method of Siegenthaler as harmonised by the International Honey Commission (Bogdanov, 1997). The activity can also be expressed as number invertase. *p*-nitrophenyl - α -D-glucopyranoside (pNPG) as a substrate is used for the analytical determination of the number of sucrose in honey. pNPG is divided into *p*-nitrophenol and glucose by α -glucosidase (invertase). By adjusting the pH value to 9.5 the enzyme reaction is stopped, and at the same time is turned into nitrophenol nitrophenolate anion, which corresponds to the amount of substrate converted and which is determined photometrically at 400 nm with a Varian spectrophotometer type UV visible Cary 50.

Determination of hydroxymethylfurfural (HMF)

The HPLC method, HMF is separated on a reversed-phase column, with water and methanol as the mobile phase, and then detected by UV absorbance. HMF was analysed by HPLC method as harmonised by the International Honey Commission (Bogdanov, 1997). One gram of sample was mixed with 9 ml of distilled water. The mixture was then centrifuged at 15,000 rounds for 10 min to remove any fine debris present in the sample. The supernatant was filtered through a 0.45 μ m membrane and 20 ml of filtrate was injected to HPLC with autosampler of HPLC Agilent 1200 series, a photo diode array detector (DAD) and Nucleosil 5 C18 column. Mobile phase was a mixture of methanol/ water. The detector was set to 280 nm and duplicate analyses were performed.

Pollen analysis

Pollen analysis was carried out using the methods established by the International Commission of Bee Botany described by Louveaux et al. (1978) and Von der Ohe et al. (2004).

Quantitative melissopalynological

Analysis was performed according to Maurizio's method (Louveaux et al., 1978) by counting microscopically the number of pollen present in the honey sediment after centrifuging a honey solution. Pollen in 400 fields of view was counted in four fold. The results were based on the average number in the 400 fields of view and expressed as the number of pollen grains in 10 g honey (PG/10 g) in thousands (10^3) and rounded to the nearest thousand. The honeys were placed into one of the five pollen representativity classes as distinguished by Maurizio (Louveaux et al., 1978; Von der Ohe et al., 2004). Class I includes honeys poor in pollen (PG/10 g < 20×10^3), class II includes honeys with normal pollen representativity (20×10^3 to 100×10^3), class III honeys with over representativity pollen (100×10^3 to 500×10^3), class IV with

Table 1. The geographical origin of the honey samples.

Sample	Geographic origin	Year collected
E1	Soukahras	2010
E2	Oum el bouaghi	2009
E3	Soukahras	2009
E4	Taref	2010
E5	Taref	2010
E6	Guelma	2010
E7	Guelma	2009
E8	Guelma	2010
E9	Guelma	2009
E10	Annaba	2008
E11	Guelma	2008
E12	Skikda	2010
E13	Guelma	2009
E14	Oum el bouaghi	2009
E15	Taref	2007
E16	Taref	2010
E17	Taref	2010
E18	Taref	2008
E19	Soukahras	2009
E20	Annaba	2007
E21	Oum el Bouaghi	2010
E22	Djelfa	2010
E23	Skikda	2010
E24	Guelma	2009
E25	Khenchla	2010
E26	Taref	2009
E27	Taref	2010
E28	Annaba	2010
E29	Taref	2009
E30	Aghwat	2009
E31	Taref	2009
E32	Guelma	2010
E33	Guelma	2010
E34	Oum el bouaghi	2007
E35	Guelma	2010
E36	Skikda	2010
E37	Skikda	2010
E38	Khenchla	2009
E39	Taref	2009
E40	Khenchela	2010

strongly over-represented pollen ($500 \times 10^3 - 10^6$) and class V includes pressed honeys ($PG/10\text{ g} > 10^6$ pollen).

Qualitative melissopalynological analysis

The types of pollen grains with their percentages in the pollen sediment was carried out by dissolving the honey in dilute sulphuric acid, centrifuging the solution and mounting the sediment in Kaiser's Glycerol Gelatine (glycerine jelly). For each sample, pollen were counted and their relative frequency classes were determined, using the terms predominant (> 45%) secondary pollen (16 to 45%),

pollen occurring rarely and sporadically are called important minor pollen (3 to 15%) and minor pollen (< 3%) (Louveaux et al., 1978). For all pollen species in the 40 samples the individual occurrence was calculated and expressed as percentage of the total studied samples in which the determined pollen type was found.

RESULTS AND DISCUSSION

Tables 2, 3 and 4 shows the mean values of the different parameters analysis and the result of pollen spectrum.

Table 2. The results of diastase number (DN) and invertase activity (IS) of honey samples.

Sample	DN(UE) m±SD	IS(UE) m±SD
E1	15.56±0.11	42.26±0.57
E2	11.80±0.39	11.24±0.39
E3	4.40±0.26	42.83±0.68
E4	30.15±0.17	37.43±0.06
E5	18.13±0.26	72.01±0.95
E6	19.13±0.39	45.58±0.07
E7	8.66±0.13	0.00±0.00
E8	12.41±0.50	12.44±0.10
E9	8.20±0.31	0.00±0.00
E10	20.82±0.57	27.16±0.05
E11	9.31±0.25	0.00±0.00
E12	17.52±0.16	103.57±0.02
E13	17.72±0.13	41.93±0.11
E14	8.17±0.45	0.00±0.00
E15	14.12±0.24	12.49±0.00
E16	25.49±0.38	35.82±0.51
E17	16.13±0.04	10.25±0.06
E18	21.70±0.44	23.17±0.52
E19	16.29±0.26	37.81±1.01
E20	20.90±0.08	24.19±0.16
E21	11.47±0.10	0.00±0.00
E22	28.94±0.11	107.66±0.51
E23	18.78±0.39	105.02±1.00
E24	16.29±0.26	29.43±0.12
E25	17.10±0.17	90.50±0.15
E26	18.53±0.26	78.75±0.15
E27	15.62±0.39	72.71±1.00±
E28	20.70±0.13	31.65±0.10
E29	17.40±0.50	16.23±0.08
E30	23.14±0.31	138.38±0.60
E31	18.84±0.57	138.25±0.29
E32	14.74±0.25	3.48±0.40
E33	14.41±0.16	5.81±0.13
E34	10.34±0.13	10.47±0.35
E35	18.57±0.45	12.65±0.27
E36	20.72±0.24	47.03±0.06
E37	17.10±0.11	105.53±0.39
E38	17.00±0.12	110.14±0.07
E39	24.11±0.12	111.52±0.19
E40	17.56±0.28	30.67±0.34

Diastase

Diastase is the common name for the enzyme α -amylase. It is found in nectar and is also added by the honeybee during the collection and ripening of nectar. Diastase digests starch to simpler compounds. The amylase activity ranged from 4.40 to 30.15 DN (Table 2); only one sample had a low diastase number than 8 DN.

The floral origin of honey also influences its diastase content. For example, citrus and clover honeys tend to contain less diastase. Other factors may affect diastase values: the natural difference in pH among honeys, nectar flow and foraging patterns of the bees. Long storage at moderate temperatures and exposure to high temperatures will inactivate diastase in honey. Diastase levels do not correlate with honey quality. Therefore,

specifying the diastase level will not guarantee quality (Babacan et al., 2002). Numerous studies also indicate that honey shows significant variations in amylase content based on composition, pH value and floral source. In fact, research reveals that heating is not the only factor influencing amylase content in honey (White, 1992; Crane, 1980; Low, 1988; Babacan and Rand, 2007).

Invertase

The invertase activity ranged from 0 to 138.38 UE (Table 2). Invertase is the enzyme that hydrolyzes sucrose to fructose and glucose. It is added to the nectar by the bee. The resulting chemical reaction is a key step in the ripening of nectar to honey. Invertase has been considered responsible for most of the chemical changes that take place during the conversion of nectar to honey. Invertase is generally present in small amounts and is inactivated by heating (Babacan et al., 2005).

Hydroxymethylfurfural (HMF)

Since HMF can be formed either by Maillard reaction (heating of reducing sugars in the presence of proteins), or by dehydration under acidic conditions. The HMF values in our study were low (<40 mg/kg) (Table 3), which is fixed at 40 mg/kg in the honey standards (Codex Alimentarius Commission, 2001; European Commission, 2002). An exception has been made for honeys from tropical regions for which this limit is 80 mg/kg. 85% of samples (34 samples) had a HMF content less than 15 mg/kg. HMF content are widely recognized as parameters indicating the freshness of honey (Mendes et al., 1998; Terrab et al., 2002). The presence of HMF is an indicator for spoiled, adulterated or products that were exposed to heat stress or bad storage conditions. On the other hand, HMF is considered an irritant and is irritating to eyes, upper respiratory tract, skin and mucous membrane. No positive or negative definite reports associating HMF with a cancer risk in humans were identified in available literature (FPA, 2006). However the National Institute of Environmental Health Sciences nominated HMF for testing based on the extensive human exposure, lack of adequate data characterizing its toxicity and carcinogenicity. Miller (1994) in studies based on mice and rats, proposed that sulphonation of HMF may lead to multigenicity and carcinogenicity. Janzowski et al. (2002) studied the DNA damaging potential and reactivity of HMF towards cellular glutathione as an assessment of multigenicity of HMF. It is also reported that HMF damages striated muscles and viscera by combining with protein and thus causing the accumulation of poisons in the body (Pamplona et al., 1995; Chi et al., 1998).

Pollen analysis

The results from quantitative melissopalynological analysis, summarised in Table 3, show that the NPG/10 g for all samples ranged from 8×10^3 to 2.01×10^6 . 22 of the 40 samples were poor in pollen ($< 20 \times 10^3$ in 10 g) and belonged consequently to class I; 14 samples belonged to class II, 3 samples were in class III and one sample in class V; they contained more than 10^6 pollen in 10 g. The observed that pollen distribution is quite normal for Mediterranean honey (Persano Oddo and Piro, 2004) and also corresponded with the results of published study of Ouchemoukh et al. (2007), who found in their study of 11 Algerian samples, lower PG/10 g values ranging from 20×10^3 till 40×10^3 and a study of Makhloufi et al. (2007). However many factors influence the number of pollen in honey. It is known that the pollen richness depends upon the pollen production of the plant, the weather conditions, the distance of the beehive to the flower field, the filtering by the bee's proventriculus and consequently the pollen's diameter, and the mode of honey extraction (Von der Ohe, 1994). The results from qualitative melissopalynological analysis, summarised in Table 4, show that predominant pollen is found in 9 samples. It is generally accepted that a minimum content of 70% *Eucalyptus* pollen is necessary to classify *Eucalyptus* honey as unifloral. *Eucalyptus* honeys are considered among the best honeys and are very valuable from a consumer's point of view (Terrab et al., 2003a). The results show that all honey samples studied are original flower honey. We also note that the dominant pollen in the majority of samples are: pollen of *Eucalyptus*, *Trifolium* sp, *Echium* sp and *Hedysarium coronarium*, Brassicaceae, Asteraceae (mostly *Carduus*) and to a lesser extent, *Rubus* and *Citrus*. Our results of pollen analysis are consistent with Louveaux and Abed (1984), Chefrouf et al. (2007), Ouchemoukh et al. (2007) and Makhloufi et al. (2007, 2010). The distribution of observed pollen is quite normal for honey of the Mediterranean (Persano Oddo and Piro, 2004). Also, Louveaux and Abed (1984) found that eucalyptus is one of the most important honey plants in Algeria and Terrab et al. (2003c) concluded that the single-flower honeys from *Eucalyptus camaldulensis* Dehnh are very common in northern Morocco. They mentioned *Plantago* sp. *Thymelaceae* as accompanying pollens while Ricciardelli and Vorwohl (1980) found the *Acacia* and *Gleditsia* species *H. coronarium* as pollens companions for Libya. According to the same authors, Tunisian *eucalyptus* honeys were characterized by *Citrus* species, *Acacia* and *Erica*, *Olea europaea*, and *H. coronarium* as pollens carers (Seijo et al., 2003). We also found a pollen of *Citrus* in our honey in the important minor pollen case. Pollen *Citrus* is generally under-represented in honey. Unifloral honey citrus were also collected in northwestern Morocco (Terrab et al., 2003b), Spain, *Cyprus* (Ricciardelli and Vorwohl, 1980) and Tunisia (Louveaux and Abed, 1984). Analysis of pollen honey (melissopalynology)

Table 3. The results of HMF and pollen number (NPG) of honey samples.

Sample	HMF (mg/kg) m±SD	Number of pollen in 10 g m±SD	Pollen class
E1	10.99±0.67	42600±23	II
E2	21.89±0.35	800±321	I
E3	12.99±0.84	205000±220	III
E4	12.56±0.25	17700±111	I
E5	8.77±0.29	15400±3	I
E6	1.62±0.01	5810±321	I
E7	11.67±0.90	200000±24	III
E8	7.04±0.74	8600±219	I
E9	12.08±0.12	14000±211	I
E10	33.96±0.23	10000±36	I
E11	12.56±0.43	17800±24	I
E12	1.72±0.25	83600±90	II
E13	1.99±0.92	57900±86	II
E14	36.31±0.44	30100±287	II
E15	8.49±0.51	50000±221	II
E16	14.41±0.33	40600±32	II
E17	10.09±0.72	16200±21	I
E18	14.26±0.22	32400±230	II
E19	12.60±0.78	28200±100	II
E20	38.84±0.39	14800±36	I
E21	37.36±0.45	29600±287	II
E22	1.64±0.59	16000±310	I
E23	2.67±0.29	22000±400	II
E24	16.99±0.01	10400±18	I
E25	5.72±0.23	18400±190	I
E26	7.34±0.59	18800±98	I
E27	6.50±0.31	17600±148	I
E28	32.85±0.90	12800±226	I
E29	7.83±1.14	8000±118	I
E30	3.33±0.05	33700±216	II
E31	4.42±0.04	115400±221	III
E32	6.64±0.70	18100±614	I
E33	10.52±0.02	2010000±513	V
E34	14.09±0.55	18600±107	I
E35	8.61±0.29	3400±105	I
E36	0.86±0.77	4000±125	I
E37	0.90±0.01	67800±22	II
E38	2.70±0.56	36000±203	II
E39	4.32±0.18	17900±201	I
E40	3.66±1.01	30100±115	II

is very important to the quality control of honey. However, the wealth of pollen depends on the production of plant pollen, weather conditions, distance from the hive to flower field, filtering by glandular of the bee and the method of extraction of honey (Von der Ohe, 1994).

Conclusion

On the basis of HMF and enzymes activities of forty

Algerian honey samples we can note that the results obtained agreed with requirements of international and European Community Directives. Although the enzymes activities and the HMF content of honey usually bear no direct relationship to the botanical origin of the product of some honey types; like *Citrus* honey, they are characterized by naturally low enzyme content. In general, these tree parameters are used as quality criteria. The strong heating and too long storage damage the enzyme activity

Table 4. Pollen types present in the honey samples (in percentages).

Sample	Predominant pollen (>45%)	Secondary pollen (16–45%)	Minor pollen (3–15%)	Important minor pollen (<3%)
E1		<i>Echium</i> (29%), <i>Eucalyptus</i> (18%)	<i>Hedysarium coronarium</i> (10%), <i>Trifolium sp</i> (7%)	<i>Carduus</i> , <i>Apiaceae</i> , <i>Asteraceae</i> , <i>Mentha spp</i> , <i>Arbutus unedo</i> , <i>Erica arborea</i> , <i>Lavandula stoechas</i>
E2		<i>Trifolium sp</i> (25%), <i>Eucalyptus</i> (19%)	<i>Hedysarium coronarium</i> (4%)	<i>Raphanus</i> , <i>Asphodelus</i> , <i>Papilionaceae</i> , <i>Lotus</i> , <i>Cistus</i> , <i>Citrus</i> , <i>Rubus</i> , <i>Erica</i> , <i>Borrago</i> , <i>Apiaceae</i>
E3	<i>Echium</i> (49%)	<i>Eucalyptus</i> (18%), <i>Trifolium sp</i> (17%)	<i>Hedysarium coronarium</i> (3%), <i>Lavandula stoechas</i> (3%)	<i>Cistus</i> , <i>Mentha</i> , <i>Erica arborea</i> , <i>Carduus</i> , <i>Rhamnaceae</i> , <i>Citrus</i> , <i>Apiaceae</i> , <i>Acacia spp</i> , <i>Fabaceae</i>
E4	<i>Eucalyptus</i> (50%)	<i>Trifolium sp</i> (23%)	<i>Hedysarium coronarium</i> (7%), <i>Echium</i> (4%)	<i>Carduus</i> , <i>Apiaceae</i> , <i>Asteraceae</i> , <i>Mentha spp</i> , <i>Arbutus unedo</i> , <i>Erica arborea</i>
E5		<i>Hedysarium coronarium</i> (21%), <i>Echium</i> (16%)	<i>Brassicaceae</i> (7%), <i>Trifolium sp</i> (6%), <i>Eucalyptus</i> (6%), <i>Fabaceae</i> (5%), <i>Carduus</i> (5%), <i>Asteraceae</i> (3%)	<i>Rhamnaceae</i> , <i>Asteraceae</i> , <i>Erica arborea</i> , <i>Prunus/Pyrus</i> , <i>Mimosa pudica</i> , <i>Lamiaceae</i> , <i>Helianthus</i> , <i>Apiaceae</i>
E6		<i>Eucalyptus</i> (16%), <i>Echium</i> (19%)	<i>Hedysarium coronarium</i> (10%), <i>Fabaceae</i> (6%),	<i>Carduus</i> , <i>Apiaceae</i> , <i>Asteraceae</i> , <i>Mentha spp</i> , <i>Arbutus unedo</i> , <i>Erica arborea</i>
E7		<i>Trifolium sp</i> (33%), <i>Eucalyptus</i> (16%)	<i>Echium</i> (7%), <i>Hedysarium coronarium</i> (4%)	<i>Cistus</i> , <i>Mentha</i> , <i>Erica arborea</i> , <i>Carduus</i> , <i>Rhamnaceae</i> , <i>Citrus</i> , <i>Apiaceae</i> , <i>Acacia spp</i> , <i>Fabaceae</i>
E8	<i>Echium</i> (55%)	<i>Eucalyptus</i> (20%), <i>Trifolium sp</i> (16%)	<i>Hedysarium coronarium</i> (3%), <i>Fabaceae</i> (3%)	<i>Cistus</i> , <i>Mentha</i> , <i>Erica arborea</i> , <i>Carduus</i> , <i>Lavandula stoechas</i> , <i>Myrtus</i> , <i>Rubus</i> , <i>Citrus</i> , <i>Apiaceae</i>
E9		<i>Eucalyptus</i> (20%), <i>Echium</i> (16%)	<i>Trifolium sp</i> (11%), <i>Hedysarium coronarium</i> (10%), <i>Fabaceae</i> (7%), <i>Carduus</i> (3%), <i>Erica arborea</i> (3%)	<i>Apiaceae</i> , <i>Lavandula asphodelus</i> , <i>Lavandula stoechas</i> , <i>Lamiaceae</i> , <i>Brassicaceae</i> , <i>Taraxacum</i> , <i>Euphorbiaceae</i>
E10		<i>Eucalyptus</i> (21%), <i>Trifolium sp</i> (17%)	<i>Echium</i> (12%), <i>Hedysarium coronarium</i> (11%), <i>Prunus/Pyrus</i> (9%), <i>Fabaceae</i> (6%), <i>Carduus</i> (5%), <i>Erica arborea</i> (5%)	<i>Allium spp</i> , <i>Apiaceae</i> , <i>Lavandula asphodelus</i> , <i>Lavandula stoechas</i> , <i>Brassicaceae</i> , <i>Myrtus</i> , <i>Erodium sp</i> , <i>Euphorbiaceae</i> , <i>Daucus carota</i>
E11		<i>Eucalyptus</i> (40%)	<i>Trifolium sp</i> (10%), <i>Erica arborea</i> (9%), <i>Erica sp</i> (3%), <i>Hedysarium coronarium</i> (3%)	<i>Cistus</i> , <i>Mentha</i> , <i>Erica arborea</i> , <i>Carduus</i> , <i>Lavandula stoechas</i> , <i>Rhamnaceae</i> , <i>Citrus</i> , <i>Apiaceae</i> , <i>Acacia spp</i> , <i>Lavandula asphodelus</i> , <i>Lamiaceae</i> , <i>Brassicaceae</i>
E12	<i>Eucalyptus</i> (63%)	<i>Hedysarium coronarium</i> (17%)	<i>Echium</i> (6%), <i>Carduus</i> (5%)	<i>Acacia spp</i> , <i>Trifolium sp</i> , <i>Asteraceae</i> , <i>Lavandula stoechas</i> , <i>Olea</i> , <i>Cistus</i> , <i>Helianthemum</i>
E13		<i>Trifolium sp</i> (21%), <i>Eucalyptus</i> (17%), <i>Hedysarium coronarium</i> (19%)	<i>Erica arborea</i> (9%), <i>Erica sp</i> (3%)	<i>Adonis sp</i> , <i>Cistus</i> , <i>Mentha</i> , <i>Carduus</i> , <i>Lavandula stoechas</i> , <i>Rhamnaceae</i> , <i>Citrus</i> , <i>Apiaceae</i> , <i>Acacia spp</i> , <i>Lavandula asphodelus</i>
E14		<i>Hedysarium coronarium</i> (25%), <i>Trifolium sp</i> (18%)	<i>Eucalyptus</i> (10%)	<i>Rubus</i> , <i>Asphodelus</i> , <i>Lotus</i> , <i>Lyptus</i> , <i>Citrus</i> , <i>Cistus</i> , <i>Rosaceae</i> , <i>Mentha</i> , <i>Erica</i> , <i>Borrago</i>
E15		<i>Eucalyptus</i> (40%), <i>Echium</i> (17%)	<i>Hedysarium coronarium</i> (6%), <i>Rubus</i> (5%)	<i>Citrus sp</i> , <i>Apiaceae</i> , <i>Lavandula asphodelus</i> , <i>Lamiaceae</i> , <i>Acacia sp</i> , <i>Carduus</i> , <i>Erica arborea</i> , <i>Lavandula stoechas</i>
E16	<i>Eucalyptus</i> (66%)	<i>Echium</i> (17%)	<i>Trifolium sp</i> (14%), <i>Borrago officinalis</i> (3%)	<i>Acacia spp</i> , <i>Asteraceae</i> , <i>Lavandula stoechas</i> , <i>Molva</i>
E17		<i>Eucalyptus</i> (32%), <i>Echium</i> (20%)	<i>Trifolium sp</i> (12%), <i>Rubus</i> (10%), <i>Erica arborea</i> (8%)	<i>Cistus</i> , <i>Mentha</i> , <i>Carduus</i> , <i>Lavandula stoechas</i> , <i>Pyrus/Molus</i> , <i>Citrus</i> , <i>Apiaceae</i>
E18		<i>Eucalyptus</i> (29%), <i>Echium</i> (25%)	<i>Trifolium sp</i> (15%), <i>Liliaceae</i> (7%), <i>Citrus</i> (3%)	<i>Asteraceae</i> , <i>Lavandula stoechas</i> , <i>Cistus</i> , <i>Mentha</i> , <i>Erica arborea</i>
E19		<i>Eucalyptus</i> (35%), <i>Hedysarium coronarium</i> (20%)	<i>Trifolium sp</i> (15%), <i>Lavandula stoechas</i> (3%)	<i>Cistus</i> , <i>Mentha</i> , <i>Erica arborea</i> , <i>Carduus</i> , <i>Rhamnaceae</i> , <i>Citrus</i> , <i>Apiaceae</i> , <i>Acacia spp</i> , <i>Fabaceae</i>

Table 4. Contd.

E20	<i>Eucalyptus</i> (69%)	<i>Hedysarium coronarium</i> (20%)	<i>Trifolium sp</i> (16%), <i>Hedysarium coronarium</i> (14%), <i>Rubus</i> (5%)	<i>Lamiaceae</i> , <i>Mentha</i> , <i>Erica arborea</i> , <i>Carduus</i> , <i>Rhamnaceae</i> , <i>Malva</i> , <i>Salix</i> , <i>Allium</i> , <i>Citrus</i> , <i>Cistus</i> , <i>Apiaceae</i> , <i>Acacia spp</i> , <i>Fabaceae</i>
E21		<i>Hedysarium coronarium</i> (22%), <i>Eucalyptus</i> (18%)	<i>Echium</i> (6%)	<i>Trifolium sp</i> , <i>Fabaceae</i> , <i>Carduus</i> , <i>Rosaceae</i> , <i>Apiaceae</i> , <i>Asteraceae</i> , <i>Erica arborea</i>
E22		<i>Eucalyptus</i> (42%)	<i>Rubus</i> (15%)	<i>Trifolium</i> , <i>Tamarix</i> , <i>Carduus</i> , <i>Rosaceae</i> , <i>Lamiaceae</i> , <i>sp</i> , <i>Mentha sp</i> .
E23	<i>Eucalyptus</i> (59%)	<i>Trifolium sp</i> (18%), <i>Echium</i> (17%)	<i>Hedysarium coronarium</i> (4%), <i>Lavandula stoechas</i> (4%)	<i>Lamiaceae</i> , <i>Mentha</i> , <i>Erica arborea</i> , <i>Carduus</i> , <i>Rhamnaceae</i> , <i>Citrus</i> , <i>Apiaceae</i> , <i>Acacia spp</i> , <i>Fabaceae</i>
E24		<i>Eucalyptus</i> (20%), <i>Trifolium sp</i> (16%), <i>Echium</i> (16%)	<i>Hedysarium coronarium</i> (6%), <i>Fabaceae</i> (3%)	<i>Cistus</i> , <i>Mentha</i> , <i>typeGenista</i> , <i>Lotus sp</i> , <i>Carduus</i> , <i>Lavandula stoechas</i> , <i>Rosaceae</i> , <i>Rhamnaceae</i> , <i>Citrus</i> , <i>Apiaceae</i> , <i>Acacia spp</i>
E25		<i>Eucalyptus</i> (29%)	<i>Echium</i> (14%), <i>Trifolium sp</i> (11%), <i>Hedysarium coronarium</i> (4%)	<i>Asteraceae</i> , <i>Borago officinalis</i> , <i>Lavandula stoechas</i> , <i>Rubus sp</i> , <i>Rosaceae</i> , <i>Menth</i>
E26		<i>Eucalyptus</i> (32%), <i>Trifolium sp</i> (18%)	<i>Echium</i> (12%)	<i>Citrus</i> , <i>Cistus</i> , <i>Mentha</i> , <i>typeGenista</i> , <i>Erica</i> , <i>Borago officinalis</i> , <i>Arborea</i> , <i>Carduus</i> , <i>Lavandula stoechas</i> , <i>Rhamnaceae</i> , <i>Lotus</i> , <i>Rosaceae</i> , <i>Apiaceae</i> , <i>Acacia spp</i>
E27		<i>Eucalyptus</i> (38%), <i>Echium</i> (20%)	<i>Hedysarium coronarium</i> (13%), <i>Carduus</i> (7%)	<i>Citrus sp</i> , <i>Apiacea</i> , <i>Lavandula asphodelus</i> , <i>Lamiaceae</i> , <i>Erica sp</i> , <i>Acacia sp</i> , <i>Arborea</i> , <i>Apiaceae</i> , <i>Myrtus communis</i> , <i>Lavandula stoechas</i>
E28	<i>Eucalyptus</i> (69%)	<i>Trifolium sp</i> (19%)	<i>Hedysarium coronarium</i> (11%), <i>Salix</i> (4%), <i>Sinapiss sp</i> (3%)	<i>Carduus</i> , <i>Apiaceae</i> , <i>Malva</i> , <i>Pyrus</i> , <i>Citrus</i> , <i>Rosaceae</i> , <i>Medicago</i> , <i>Asteraceae</i> , <i>Mentha spp</i> , <i>Arbutus unedo</i> , <i>Erica arborea</i> , <i>Lotus</i> , <i>Lavandula stoechas</i>
E29		<i>Hedysarium coronarium</i> (21%), <i>Echium</i> (16%), <i>Eucalyptus</i> (16%)	<i>Trifolium sp</i> (6%), <i>Fabaceae</i> (5%), <i>Carduus</i> (5%)	<i>Brassicaceae</i> , <i>Mentha sp</i> , <i>Borago officinalis</i> , <i>Lotus</i> , <i>Rosaceae</i> , <i>Rhamnaceae</i> , <i>Asteraceae</i> (échinulé), <i>Erica arborea</i>
E30		<i>Eucalyptus</i> (37%)	<i>Hedysarium coronarium</i> (14%)	<i>Rubus</i> , <i>Rosaceae</i> , <i>Carduus</i> , <i>Rosaceae</i> , <i>Lamiaceae sp</i> , <i>Acacia sp</i>
E31		<i>Echium</i> (19%), <i>Trifolium sp</i> (18%), <i>Eucalyptus</i> (16%)	<i>Hedysarium coronarium</i> (11%), <i>Fabaceae</i> (5%), <i>Rosaceae</i> (3%)	<i>Erica arborea</i> , <i>Carduu</i> , <i>Citrus</i> , <i>Rosaceae</i> , <i>Asteraceae</i> , <i>Prunus/Pyrus</i> , <i>Mimosa pudica</i> , <i>Lamiaceae</i> , <i>Helianthus</i> , <i>Apiaceae</i>
E32		<i>Eucalyptus</i> (16%), <i>Echium</i> (19%)	<i>Hedysarium coronarium</i> (10%), <i>Fabaceae</i> (6%)	<i>Carduus</i> , <i>Apiaceae</i> , <i>Asteraceae</i> , <i>Mentha spp</i> , <i>Arbutus unedo</i> , <i>Erica arborea</i>
E33		<i>Eucalyptus</i> (20%)	<i>Echium</i> (14%), <i>Trifolium sp</i> (13%), <i>Hedysarium coronarium</i> (4%)	<i>Cistus</i> , <i>Mentha</i> , <i>typeGenista</i> , <i>Ericaarborea</i> , <i>Cistus</i> , <i>Rosaceae</i> , <i>Boragoofficinalis</i> , <i>Carduus</i> , <i>Rhamnaceae</i> , <i>Citrus</i> , <i>Apiaceae</i> , <i>Acacia spp</i> , <i>Fabaceae</i>
E34	<i>Neant</i>	<i>Hedysarium coronarium</i> (22%),	<i>Eucalyptus</i> (11%), <i>Echium</i> (7%),	<i>Trifoliumsp</i> , <i>Fabaceae</i> , <i>Carduus</i> , <i>Rosaceae</i> , <i>Apiaceae</i> , <i>Asteraceae</i> , <i>Menth aspp</i> , <i>Arbutusunedo</i> , <i>Ericaarborea</i> ,
E35		<i>Eucalyptus</i> (45%)	<i>Trifolium sp</i> (15%), <i>Erica arborea</i> (4%), <i>Hedysarium coronarium</i> (3%)	<i>Erica sp</i> , <i>Echium</i> , <i>Cistus</i> , <i>Mentha</i> , <i>typeGenista</i> , <i>Carduus</i> , <i>Asteraceae</i> , <i>Lavandula stoechas</i> , <i>Rhamnaceae</i> , <i>Citrus</i> , <i>Apiaceae</i> , <i>Acacia spp</i> , <i>Lavandula asphodelus</i> , <i>Lamiaceae</i> , <i>Brassicaceae</i>
E36		<i>Eucalyptus</i> (33%), <i>Hedysarium coronarium</i> (18%)	<i>Echium</i> (14%), <i>Carduus</i> (5%)	<i>Acacia spp</i> , <i>Trifolium sp</i> , <i>Asteraceae</i> , <i>Mentha sp</i> , <i>Rosaceae</i> , <i>Citrus</i> , <i>Lavandula stoechas</i>
E37	<i>Trifolium sp</i> (51%)	<i>Eucalyptus</i> (23%)	<i>Hedysarum coronarium</i> (8%)	<i>Erica sp</i> , <i>Citrus</i> , <i>Cistus</i> , <i>Boraginoceae</i> , <i>Arborea</i> , <i>Carduus</i> , <i>Rhamnaceae</i> , <i>Apiaceae</i> , <i>Acacia spp</i> , <i>Fabaceae</i>
E38		<i>Eucalyptus</i> (32%)	<i>Trifolium sp</i> (13%), <i>Hedysarium coronarium</i> (10%)	<i>Erica sp</i> , <i>Echium</i> , <i>Erica arborea</i> , <i>Cistus</i> , <i>Citrus</i> , <i>Apiaceae</i> , <i>Acacia spp</i> , <i>Lamiaceae</i> , <i>Brassicaceae</i>

Table 4. Contd.

E39	<i>Eucalyptus</i> (35%), <i>Trifolium sp</i> (18%)	<i>Hedysarium coronarium</i> (15%)	<i>Citrus sp</i> , <i>Apiacea</i> , <i>Mentha</i> , <i>Cistus</i> , <i>Carduus</i> , <i>Lavandula asphodelus</i> , <i>Rosaceae</i> , <i>Lamiacea sp</i> , <i>Acacia sp</i> , <i>Erica arborea</i> , <i>Apiaceae</i> , <i>Myrtus communis</i> , <i>Lavandula stoechas</i>
E40	<i>Hedysarium coronarium</i> (35%), <i>Eucalyptus</i> (22%)	<i>Trifolium sp</i> (15%), <i>Erica arborea</i> (9%)	<i>Cistus</i> , <i>Mentha</i> , <i>typeGenista</i> , <i>Erica sp</i> , <i>Carduus</i> , <i>Rhamnaceae</i> , <i>Citrus</i> , <i>Lavandula asphodelus</i> , <i>Brassicaceae</i>

and increase the HMF content. However, honey samples differ in quality on account of various factors such as season, origin of honey, activity of the bee, food of the bee, period and technique of extraction of honey, conditions of storage and the freshness of honey. The analysis of palynological results therefore revealed that the dominant pollen in the majority of samples are: pollen of *Eucalyptus*, *Trifolium sp*, *Echium sp* and *H. coronarium*, Brassicaceae, Asteraceae (mostly *Carduus*) and to a lesser extent, *Rubus* and *Citrus*, *Apiaceae* and *Ericaceae* are most frequently found in the pollen spectrum of Algerian honeys.

Conflict of Interest

The authors have not declared any conflict of interests.

REFERENCES

- Anklam E (1998). A review of the analytical methods to determine the geographical and botanical origin of honey. *Food Chem.* 63:549–562.
- Babacan S, Pivarnik LF, Rand AG (2002). Honey amylase activity and food starch degradation. *J. Food Sci.* 67(5):1625-1630.
- Babacan S, Rand A (2005). Purification of amylase from honey. *J. Food Sci.* 70(6):1625-1630.
- Bertoncelj J, Dobersek U, Jamnik M, Golob T (2007). Evaluation of the phenolic content, antioxidant activity and colour of Slovenian honey. *Food Chem.* 105:822-828.
- Blasa M, Candiracci M, Accorsi A, Piacentini MP, Albertini MC, Piatti E (2006). Raw Millefiori honey is packed full of antioxidants. *Food Chem.* 97:217-222.
- Bogdanov S (1997). Nature and origin of the antibacterial substances in honey. *Lebensmittel Wissenchar und Technol.* 30:748-753.
- Bogdanov S (1999). Honey Quality, methods of analysis and international regulatory standards: Review of the work of the international honey commission. *Mitt. Gebiete Lebensm. Hyg.* 90:108-125.
- Bogdanov S, Rieder K, Rüegg M (1987). New quality criteria for honey studies. *Apidologie* 18(3): 267-278.
- Babacan S, Rand AG (2007). Characterization of honey amylase. *J. Food Sci.* 72:50-55.
- Chefrour A, Battesti MI, Ait K, Tahar A (2007). Melissopalynologic and physicochemical analysis of some north-east algerian honeys. *Eur. J. Sci. Res.* 18:389-401.
- Chi W CB, Zhang YH, Cao LY, Guo (1998). Investigation of the restriction on the formation of 5- HMF Spec. *J. Pharm. People's Mil. Surgar* 14:101-104.
- CODEX ALIMANTAIRE (1993). Standard for honey. Ref. Nr. CL 1993/14-SH, FAO. Rome.
- Codex Alimentarius Commission (2001). Codex standard 12, Revised Codex Standard.
- Crane E (1980). *A Book of Honey.* Charles Scribner's Sons, New York.
- Food Products Association (2006). Emerging Chemical Contaminants Update. 1(2) www.fpa-food.org/Upload/library/07112006200748.pdf.
- Gondarski H (1961). Über den Einfluß von Wassergehalt und pH des Honigs auf den Grad der Wärmeschädigung der Invertase. *Zeitschrift f. Bienenforschung* 5:191-198.
- Guler A, Bakan A, Nisbet C, Yavuz O (2007). Determination of important biochemical properties of honey to discriminate pure and adulterated honey with sucrose (*Saccharum officinarum* L.) syrup. *Food Chem.* 105(111):9-1125.
- Hermosin I, Chicon RM, Dolores Cabezudo M (2003). Free amino acid composition and botanical origin of honey. *Food Chem.* 83:263–268.
- Ivanov TZ (1978). Studies on the composition and characteristics on the Bulgarian Bee Honey. *Agricultural Science. Agricultural Academy. Sofia* pp. 23-25.
- Ivanov TZ (1986). Quality, standardisation and qualification of honey products. *Survey. Agric. Academy. Sofia* pp. 7-31.
- Janzowski CV, Glaab E, Sammimi RJ, Schlatte G, Eisenbrand (2002). 5-Hydroxymethyl furfural: Assessment of mutagenicity, DNA-damaging potential and reactivity towards cellular glutathione. *Food Chem. Toxicol.* (38):801-809.
- Louveaux J, Abed L (1984). Les miels d'Afrique du nord et leur spectre pollinique. *Apidologie* 15:145–170.
- Louveaux J, Maurizio A, Vorwohl G (1978). Methods of Melissopalynology. *Bee World* 59:139-153.
- Low NH (1988). Carbohydrate analysis of Western Canadian honeys and their nectar sources to determine the origin of honey oligosaccharides. *J. Apic. Res.* 27(4):244.
- Makhloufi C, Jacob DK, Giancarlo RD, Ali C, Riad S (2010). Characterization of algerian honeys by palynological and physico-chemical methods. *Apidologie* 41:509–521.
- Makhloufi C, Schweitzer P, Azouzi B, Persano OL, Choukri A, Laaredij H (2007). Some properties of algerian honeys. *Apiacta* 42:73–80.
- Mendes E, Brojo PE, Ferreira IMPLVO, Ferreira MA (1998). Quality evaluation of Portuguese honey. *Carbohydrate. Polymer* 37(3):219-223.
- Miller JA (1994). Recent studies on the metabolic activation of chemical carcinogens 1. *Cancer Res.* 54:1879-1881.
- Ouchemoukh S, Louaileche H, Schweitzer P (2007). Physicochemical characteristics and pollen spectrum of some algerian honeys. *Food Control* 18:52–58.
- Oddo LP, Piazza MG, Pulcini P (1999). Invertase activity in honey. *Apidologie* 30:57-65.
- Pamplona R, Bellmunt MJ, Portero M, Riba D, Prat J (1995). Chromatographic evidence for amadori product formation in rat liver aminophospholipids. *Life Sci.* 57:873-879.
- Oddo PL, Piro R (2004). Main European unifloral honeys: descriptive sheets. *Apidologie* 35(1):S38–S81.
- Pichler FJ, Vorwohl G, Gierschner K (1984). Faktoren die die Bildung von Hydroxymethylfurfural im Honig beeinflussen. *Apidologie.* 15:171-188.

- Ricciardelli D'AG and Vorwohl G (1980). Sortenhonige im Mittelmeergebiet, *Rivista Agricoltura Subtropicale e Tropicale* 74:89–118.
- Seijo MC, Aira MJ, Mendez J (2003). Palynological difference in pollen content of *Eucalyptus* honey from Australia. Portugal and Spain. *Grana* 42:183–190.
- Terrab A, Diez MJ, Heredia FJ (2003b). Palynological, physico-chemical and colour characterization of Moroccan honeys. II. Orange (*Citrus* sp.) honey. *Int. J. Food Sci. Technol.* 38:387–394.
- Terrab A, Diez MJ, Heredia FJ (2003a). Palynological, physico-chemical and colour characterization of Moroccan honeys. I. River and gum (*Eucalyptus camaldulensis Dehnh*) honey. *Int. J. Food Sci. Technol.* 38:379–386.
- Terrab A, Diez MJ, Heredia FJ (2003c). Palynological, physico-chemical and colour characterization of Moroccan honeys. III. Other unifloral honey types. *Int. J. Food Sci. Technol.* 38:395–402.
- Terrab A, Diez MJ, Heredia FJ (2002). Characterization of Moroccan unifloral honeys by their physicochemical characteristics. *Food Chem.* 79:373–379.
- The Council of the European Union (2002). Council Directive 2001/110/ec of 20 december relating to honey. *Off. J. Eur. Commun.* 10:47–52.
- Von Der Ohe W, Persano Oddo L, Piana M.L, Morlot, M, Martin P (2004) harmonized methods of melissopalynology. *Apidologie* 35:18–25.
- Von der Ohe W, von der Ohe K (1992) Honigqualität. Der Einfluß der Temperatur, *Deutsches Imker-J.* 3:78–82 .
- Von der Ohe W (1994). Unifloral honeys: chemical conversion and pollen reduction. *Grana* 33:292–294.
- White JW (1992). Quality Evaluation of Honey: Role of HMF and Diastase Assays. *Am. Bee J.* 132: 737-743, 792-794.
- White JW Jr, Kushnir I, Subers MH (1964). Effect of storage and processing temperatures on honey quality. *Food Technol.* 18:153–156.
- White JW, Subers MH, Schepartz AI (1968). The identification of inhibine, the antibacterial factor in honey, as hydrogen peroxide and its origin in a honey glucose-oxidase system. *Biochim. Biophys. Acta* 73:57–70.