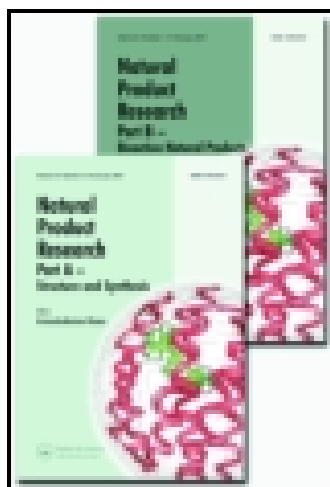


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### Volatiles, color characteristics and other physico-chemical parameters of commercial Moroccan honeys

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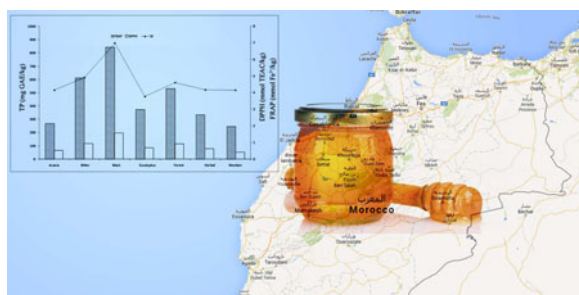
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## Volatiles, color characteristics and other physico-chemical parameters of commercial Moroccan honeys

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Seven commercial Moroccan honeys were considered for chemical characterisation. Volatile fraction, total polyphenols content, antioxidant and antiradical activities were evaluated by employing different analytical methodologies. Several physical parameters such as refractive index, pH, water content, solids content and colour were measured. Volatile fraction revealed an abundant presence of *cis*- and *trans*-linalool oxide in the seven studied samples. The presence of high levels of compounds related to the Maillard reaction, like furfural and hydroxymethylfurfural, could be the result of thermal treatments used to liquefy commercial honeys or of long storage times. The CIE  $L^*a^*b^*C_{ab}^*h_{ab}^\circ$  chromatic coordinates confirmed the advanced stage of the Maillard reaction, showing  $L^*$  values lower than the common values found for honey of similar typologies.

**Keywords:** furfural; hydroxymethylfurfural; chromatic coordinates; HS-SPME

### 1. Introduction

Honey is a common, natural sweetener present worldwide. It is obtained from honeybees, which process the nectar of flowers or the secretions of living parts of plants. The floral source extracted by bees is the main variable that determines the composition of the honey (Anklam 1998). Taking into account that the floral secondary metabolites vary with respect to the geographic location of the plant, the chemical composition of the honey is also often related to the geographical area of production.

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The major constituents of honey are sugars, mainly glucose and fructose, and water, followed by minor components such as proteins, minerals, polyphenols, vitamins, free amino acids and several volatiles.

The presence of organic acids and considerable water activity, combined with the high concentration of sugars, gives rise to favourable conditions for the formation of furanic aldehydes. The production of this class of compounds is mainly due to two different pathways: the Maillard reaction (Martins & Jongen 2000) and the dehydration of hexoses catalysed by acid (Beliz & Grosh 1999). The most important furanic aldehyde in foodstuffs is 5-hydroxymethyl-2-furfuraldehyde (HMF), whose concentration is related to the quality and freshness of food. Indeed, it is well known that the concentration of HMF approaches zero in fresh and untreated food, whereas its concentration increases as a result of thermal treatment and storage time. Other parameters such as colour or diastase index have been related to the freshness or quality of honey (Mendes et al. 1998; Martins & Jongen 2000). Therefore, understanding the chemical and physical characteristics of honey is very important in order to evaluate the quality of the sweetener or to detect the presence of adulterants (Cordella et al. 2002).

Among the Arab countries, Egypt comes first in producing honey, generating 16,000 tons of honey yearly. Second to Egypt is Morocco, which produces up to 3500 tons of honey from both the industrial sector and local beekeepers. There are about 35,000 apiarists who keep up to 380,000 hives (Ministere de l'agriculture 2006). Although Morocco has an ancient tradition of honey production, the number of papers published about the characteristics of local honey is scarce and there is little available data on the chemical composition of honey. Previous research has focused mainly on the physicochemical characterisation of several Moroccan honeys, including colour determination, mineral composition and sugar fingerprint (Terrab, Diez, et al. 2002; Terrab, Vega-Perez, et al. 2002; Terrab, Diez, et al. 2003; Terrab, Gonzalez, et al. 2003; Bettar et al. 2015; Chakir et al. 2015). Recently, Aazza et al. (2014) reported a study of several Moroccan honeys in which they screened some physicochemical properties and antioxidant activity. They found high values of HMF, as well as a high melanoidin content. They related these results to inadequate processing and storage conditions of the honeys. Within the recent techniques for the analysis of honey components solid-phase microextraction (SPME) followed by gas chromatography mass spectrometry (GC-MS) has been used to obtain representative chemical composition of aroma components and to give objective data linked with honey processing (Jerković et al. 2011). The aroma and sweet taste are the most important sensory properties of honey that relate to consumer preference. In addition, as reported by several authors, volatile composition could be a source of chemical marker used for the authenticity assessment of honeys (Manyi-Loh et al. 2011). With regard of polar fraction, high performance liquid chromatography (HPLC) technique is the main tool used to investigate the chemical composition of polar compounds such as polyphenols.

There appear to be no prior studies that report any screening of the volatile composition of Moroccan honeys. In this study, we report the volatile characterisation determined by the headspace (HS)-SPME/GC-MS of seven commercial Moroccan honeys. HPLC was also used for a screening of selected marker compounds of the polar fraction. In addition antioxidant and antiradical activities were evaluated by ferric ion reducing antioxidant power (FRAP) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) antioxidant assay, and the total polyphenol (TP) content was evaluated by Folin–Ciocalteu reagent. Several physical–chemical parameters were measured, including the colour chromatic attributes obtained by transmittance data.

## 2. Results and discussion

The physicochemical parameters were very homogeneous among the samples studied. The refractive index ranged from 1.49028 (eucalyptus) to 1.49300 (herbal). The water content varied

between 17.4 g/100 g of herbal honey and 18.2 g/100 g of bitter honey. The solids content ranged from 79.7 to 70.4°Bx. The pH showed an average value of 4.43. The measured physicochemical parameters (see the Supplemental material) were in accordance with the international requirements as expected for commercial honeys (Council Directive 2001).

The volatiles determined by the HS-SPME/GC-MS analysis of Moroccan honeys are reported in Table 1. The volatile organic compound (VOC) chemical composition of the honey samples showed some similarities. Linalool oxide, in its two isomer forms, was present as the main compound in all honeys, and its sensory term equivalent is representative by flower aroma as reported by Acree and Arnn (2004). The contribution of linalool derivatives to the aroma, particularly in samples where they are more abundant such as herbal honey, could produce very impactful result on sensory characteristic of the honey.

Although it occurred in a lower percentage than the linalool derivatives, furfural was always present in all analysed honeys, ranging from 3.89% in eucalyptus honey to 11.08% in black honey. The presence of furfural is very likely related to Maillard reactions (Sancho et al. 1992). As well as honey thermal treatment or excessive storage time could also be linked to the presence of linalool derivatives in the volatile fraction; Castro-Vázquez et al. (2008) reported that the concentration of linalool derivatives in the volatiles of Orange honey strongly increased during the storage time, especially when the honeys were stored at 40°C.

Unlike the linalool derivatives and furfural, some volatiles were found in specific samples; nonanol was found in only Mountain honey at 0.2%, whereas cuminaldehyde, matching 0.8%, was present only in bitter honey. The existence of p-isopropylbenzaldehyde in the chemical composition is perhaps due to the presence of *Cuminum cyminum* in the area of honey production.

Although Radovic et al. (2001) reported the absence of phenylacetaldehyde in the volatile fraction as a typical feature of Acacia honeys, we detected it in a high value (about 29%) in our sample, supporting the recent results of a similar study by Plutowska et al. (2011). Phenylacetaldehyde is also a typical product of Strecker degradation and therefore its presence in our samples could be related to heating/long storage time, while its absence in the Acacia samples studied by Radovic et al. (2001), could indicate freshness of the samples.

The analysis of the volatile fraction of our Acacia honey sample showed the presence of typical markers, such as *cis*-linalool oxide, nonanol and decanal, all of which have been indicated representative for Acacia honeys (Radovic et al. 2001; Plutowska et al. 2011).

The chromatographic profile of our Eucalyptus' sample shows few peaks mainly represented by *cis*- and *trans*-linalool oxide (83%) and no botanical markers were found.

The concentration of typical VOCs representative of honey decreases considerably according to the time and temperature of storage conversely linalool derivatives concentration increase in stored honey (Castro-Vázquez et al. 2008). Taking into account the high values of HMF (24 mg/kg), the presence of furfural and the lightness value ( $L^* = 41$ ), the absence of typical markers in the volatiles of Eucalyptus honey, could be related to long storage period and/or heating times.

The HPLC with photodiode array detection fingerprinting of the seven analysed Moroccan honeys showed very similar profiles that differed mainly with regard to signal intensity, with the black honey sample being the richest one (data not shown). Selected polar markers found in different unifloral honeys (4-hydroxybenzoic acid, kynurenic acid, 4-hydroxyphenylacetic acid, riboflavin, phenyl lactic acid, *p*-coumaric acid, benzoic acid, lumichrome, methyl syringate, (*E/E*)-abscisic acid, (*Z/E*)-abscisic acid, salicylic acid and methyl benzaldehyde) were not detected. The HPLC data, as well as GC result, showed a general similarity among the seven honeys, suggesting common nectariferous sources.

The HMF analysis (data reported in supplementary material) showed that it is highly represented in all honey samples. It had an average value of  $27.49 \pm 5.91$  mg/kg, and ranged

Table 1. Main compounds in the volatile fraction for different honey samples isolated by HS-SPME.

	Acacia (%)	Bitter (%)	Black (%)	Eucalyptus (%)	Forest (%)	Herbal (%)	Mountain (%)	RI	ID	Sensory terms equivalents <sup>a</sup>
Dimethyl sulphide	ND	ND	3.6	3.6	2.3	1.1	ND	522	RI/MS	
Furfural	9.7	4.9	11.1	3.9	8.3	4.6	4.2	830	RI/MS	Bread, almond, sweet
Benzaldehyde	5.7	6.7	ND	ND	3.7	1.3	5.2	958	RI/MS/STD	Almond, burnt sugar
Trisulphide, dimethyl	ND	0.6	ND	ND	ND	ND	ND	967	RI/MS	Sulphur, fish, cabbage
Octanal	ND	1.1	1.0	ND	ND	ND	ND	1002	RI/MS	Fat, soap, lemon, green
Benzeneacetaldehyde	28.7	13.5	4.9	ND	8.4	4.7	43.3	1043	RI/MS	Honey, sweet
<i>cis</i> -Linalool oxide	36.0	26.8	46.0	68.4	35.4	63.3	34.2	1073	RI/MS	Flower
<i>trans</i> -Linalool oxide	8.2	6.9	10.0	14.3	7.7	14.9	6.7	1088	RI/MS/STD	Flower
Linalool	0.9	1.4	3.2	ND	2.8	ND	ND	1100	RI/MS/STD	Flower/lavender
Nonanal	5.6	ND	ND	ND	ND	ND	2.8	1101	RI/MS	Fat, citrus, green
Hotrienol	ND	8.8	3.6	6.9	10.3	6.4	ND	1104	RI/MS	Hyacinth
2-Phenylethanol	ND	9.3	2.4	ND	2.0	ND	ND	1114	RI/MS	
Lilac aldehyde <sup>b</sup>	ND	1.2	1.4	ND	2.6	ND	ND	1143	RI/MS	
Lilac aldehyde <sup>b</sup>	ND	1.5	2.2	ND	3.4	0.4	ND	1151	RI/MS	
Nerol oxide	ND	1.0	ND	ND	ND	0.8	ND	1154	RI/MS/STD	Oil/flower
Lilac aldehyde <sup>b</sup>	ND	0.7	1.1	ND	1.7	ND	ND	1166	RI/MS	
Nonanol	ND	ND	ND	ND	ND	ND	0.2	1169	RI/MS	Fat, green
Ethyl octanoate	ND	ND	ND	ND	0.9	ND	ND	1197	RI/MS	
Safranal	ND	ND	2.9	2.8	ND	ND	0.3	1201	RI/MS	
Decanal	2.5	ND	ND	ND	1.3	ND	1.5	1203	RI/MS	
Cuminaldehyde	ND	0.8	ND	ND	ND	ND	ND	1240	RI/MS/STD	
Benzeneacetic acid, ethyl ester	ND	1.6	ND	ND	ND	ND	ND	1244	RI/MS	
Benzeneacetaldehyde, Alpha ethylidene-	ND	5.2	ND	ND	3.1	ND	0.8	1272	RI/MS	Soap, orange peel, tallow
Total identified	97.3	92	93.4	99.90	93.9	97.5	99.2			

Note: ND, not detected.

<sup>a</sup>Acree and Arm (2004).<sup>b</sup>Undetermined isomers.

from 18.87 mg/kg in mountain honey to 34.98 mg/kg in black honey. A previous study conducted by Aazza et al (2014) confirmed a high concentration of HMF in Moroccan honeys; some of their samples fell beyond the upper limit of several quality normatives (Codex Alimentarius 2001), with an average value of about 45 mg/kg.

The colour of honey varies with respect to several parameters, such as mineral content (González-Miret et al. 2005), botanical origin, Maillard reaction and fructose caramelisation. In addition, some authors have reported a positive correlation between colour and polyphenol content, as well as between colour and antioxidant activity (Bertoncelj et al. 2007). The colour CIE coordinates were obtained by transmittance data (see Supplementary material) in Cartesian form ( $L^* a^* b^*$ ) as well as in polar form ( $L^* C_{ab}^* h_{ab}^\circ$ ). The lightness of Moroccan honeys ranged from 9.24 of black honey to 56.74 of mountain honey; a comparison with literature data show that these values are closer to those obtained for the darker honeys like honeydew, buckwheat, or heater honey. If this can be acceptable for some multifloral honeys such as black, forest and bitter honeys, some concerns arise for the eucalyptus and acacia samples. This is especially true for acacia honey, which shows chromatic coordinates atypical for a unifloral honey (Tuberoso et al. 2014). Taking into account the high HMF values found in all honeys, as well as the presence of furfural in all samples, it is possible to conclude that the dark colour of honey is mainly due to the melanoidins content, which is linked to long storage time and heating treatment. In a previous study of several Moroccan honeys by Terrab, Diez, et al. (2002), they found that honeys with lighter colours (average  $L^*$  values, in absorbance measurement, of about 22) shown HMF values (average amount about 20 mg/kg) lower than our commercial honeys. This supports our conclusion on colour of the studied commercial honeys and Maillards products.

The antioxidant activity of the samples was evaluated as ‘radical scavenging ability’ towards the DPPH and towards the capability of those antioxidants or reductants, present in the samples, to reduce the  $Fe^{3+}/Fe^{2+}$  couple. Results of antioxidant (FRAP test expressed as mmol of  $Fe^{2+}$ ) and antiradical [DPPH test expressed as mmol of trolox equivalents antioxidant capacity (TEAC)] assays are reported in Table 2; black honey showed the best result in both assays, reaching 6.74 mmol of  $Fe^{2+}$  and 1.58 mmol TEAC. As reported by Thaipong et al. (2006), DPPH and FRAP show a good reproducibility. Indeed, a strong determination factor of 0.96 has been found between the data obtained from the two assays. Furthermore, plotting our results of colour lightness against antioxidant activity, a good correlation  $R^2 = 0.90$  ( $L^*$  vs FRAP) and  $R^2 = 0.88$  ( $L^*$  vs DPPH) was found, which supports the result of several researchers (e. g. Bertoncelj et al. 2007).

Table 2. TP amount, antiradical and antioxidant activity.

Honey sample	TP <sup>a</sup> (mg GAE/kg)		FRAP <sup>b</sup> (mmol $Fe^{2+}$ /kg)		DPPH <sup>c</sup> (mmol TEAC/kg)	
	Mean	± SD	Mean	± SD	Mean	± SD
Acacia	520.65	29.93	2.15	0.21	0.52	0.01
Bitter	616.20	20.23	4.88	0.19	0.94	0.05
Black	874.04	16.95	6.74	0.83	1.58	0.03
Eucalyptus	472.06	54.71	2.99	0.09	0.68	0.01
Forest	577.23	19.74	4.25	0.41	0.91	0.01
Herbal	523.06	17.41	2.67	0.07	0.62	0.05
Mountain	520.32	34.10	1.96	0.05	0.41	0.01

<sup>a</sup> Expressed as mg GAE/kg.

<sup>b</sup> FRAP: expressed as mmol of  $Fe^{2+}$ /kg of honey.

<sup>c</sup> DPPH: expressed as mmol of TEAC/kg of honey.

As reported in literature, the main compounds responsible for the antioxidant activity in honey are polyphenols (e.g. Petretto et al. 2015); The TP amount was screened by the spectrophotometric Folin–Ciocalteu method and the amount range from 874 mg of gallic acid equivalents (GAE)/kg in black honey to 472 mg GAE/kg in eucalyptus honey. The result of the Folin–Ciocalteu method showed a high content of polyphenol compounds in all studied honeys. Bertoncelj et al. reported a TP value in Slovenian acacia honey approximately 10 times lower than our acacia samples. Plotting the TP content against antioxidant activity showed significant correlations:  $R^2 = 0.82$  (TP vs FRAP) and  $R^2 = 0.88$  (TP vs DPPH). Similar values of total phenols, especially for the multifloral honeys, were detected in other commercial Moroccan honeys (Aazza et al. 2014).

### 3. Conclusions

Seven commercial Moroccan honey samples were characterised in order to shed some light on the quality of common honeys available in the local markets of Casablanca. Of the physical and chemical parameters studied, the most significant were the volatile composition, colour, antioxidant activity and polyphenolic content.

The analysis of the volatile fraction revealed an abundance of linalool derivatives in all of the samples. In the case of acacia honey, the HS analysis showed the presence of the typical markers that support the declared botanical origin. The percentages of furfural observed could be related to the Maillard process, which is caused by thermal treatments commonly carried out on commercial honeys or by long storage times. The advanced stage of the Maillard reaction in the honeys was also highlighted by the presence of HMF, which was more abundant in the samples with a darker colour.

Despite the relatively high values of HMF (sometimes close to the legal limit of 40 mg/kg), the quality of the studied commercial Moroccan honeys could be considered good. The results showed relatively high amounts of TP (average value 586 mg GAE/kg) as well as relatively high values of antioxidant activity (average DPPH value 0.81 mmol TEAC/kg; average FRAP value 3.66 mmol Fe<sup>2+</sup>/kg).

### Supplementary material

Experimental details relating to this paper are available online at <http://dx.doi.org/10.1080/14786419.2015.1056186>.

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### Disclosure statement

No potential conflict of interest was reported by the authors.

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