

Physicochemical Analysis For Standardizing Quality Criteria of Libyan Eucalyptus (*Eucalyptus* sp.) Honey

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Abstract

The physicochemical characteristics of Libyan *Eucalyptus* honey were measured including: moisture with 18.00%, sugars (fructose; 40.00, glucose; 32.00%, sucrose; 1.50% & maltose; 4.70%), total proteins; 0.08%, total amino acids; 0.11%, total acidity; 25.00meq./gk, HMF; 4.60mg/kg, color; light amber, ash; 0.17%, total soluble solids; 82.00% and insoluble solids; 0.07%, and electrical conductivity; 0.44 mS/cm. Maturity, purity and genuineness criteria, compared to those recommended by Codex Alimentarius and European Standard Drafts, indicated that the quality of the tested honey was very good. Eucalyptus (*Eucalyptus* sp.) was the most abundant pollen in the tested honey which is considered to be unifloral. Potassium was the dominant mineral in the honey tested followed by sodium, nitrogen, phosphorous, iron and magnesium. Detectable amounts of nickel, zinc, cadmium and lead were also found.

Keywords: *Eucalyptus* honey – Physicochemical analysis – Minerals - Melissopalynology - Quality criteria - Libya.

Introduction

Beekeeping in Libya has been practiced, traditionally, since early times in the east region (Jabal Akhdar), while modern beekeeping has developed only in the recent decades. The successful introduction of modern hives is described by Brittan (1956) who described nectar sources and types of honey produced. The number of hives increases by 20% every year, and a surplus of honey is obtained. Migratory beekeeping is also practiced and honey prices increase. Nine types of honey are

obtained including *Eucalyptus* and *Citrus* honeys (El-Mabrook, 1996). Two main honey flows occur in north Libya (December and June). The main honey plants in Libya including; *Acacia* spp., *Pinus* spp., *Cupressus* spp., *Thymus vulgaris*, *Lantana camara*, *Hibiscus rosa-sinensis*, *Eucalyptus cawaldulensis*, *Medicago sativa* and many wild plants (Hussein, 2000). Of those types, *Eucalyptus* honey (*Eucalyptus* sp.) is one of the main honeys produced and consumed in Libya especially in the north where its extensive trees flowering in November and December.

Since, there is a few or rare scientific information published worldwide about Libyan honey, this work aimed to determine the main quality criteria of Libyan *Eucalyptus* honey via estimating the typical physicochemical indicators of maturity, purity and also determine its origin by examining pollen spectrum.

Material and Methods

A. Sampling:

Eucalyptus honey was obtained during December, 2003 from 13 honey bee colonies headed by local hybrid Italian, *Apis mellifera ligustica*, queens, reared in Dadant's hives situated in the apiary of the High Institute for Agricultural Technology at Ghiran, Tripoli, Libya. After centrifuging of sealed honey combs (by an electrical extractor), honey was strained and kept in a stainless-steel ripener for nearly one week. The ripened honey then was packed in glass bottles and kept at room temperature away from direct light.

B. Physicochemical analyses

The physicochemical estimations were carried out in the laboratories of the Faculty of Agriculture at Fayoum, Cairo University

and Department of Honeybee Research, Agricultural Research Center, Ministry of Agriculture, Egypt as the following:

1-Moisture content, total soluble, insoluble solids and density: An Abbe refractometer was used to record total soluble solids (T.S.S.) and refractive index value. After correction for temperature, the reading was converted to moisture content (%) using the Table given by White *et al.* (1962). For specific gravity (density) and total insoluble solids, the methods given by the A.O.A.C. (1990) was used.

2-Optical density (O.D.) and viscosity: 10g honey diluted to 100ml with distilled water was centrifuged for 10min at 3000rpm. The absorbance and transmission of the filtrate supernatant was measured at 530nm against distilled water as a blank using a Spectronic 20 (Bausch & Lomb, USA) spectrophotometer. For viscosity, the method described by A.O.A.C. (1990) was applied using a viscosimeter.

3-Electrical conductivity (E.C.): Honey solution 20 %, based on the dry substance, was tested using a conductometer and ascertained by the equation: $C = 0.14 + 1.74A$ given by Piazza *et al.* (1991) where; C is the electrical conductivity in milli Siemens per cm (mS/cm) and A is the ash content in g/100g honey.

4-Ash: According to the A.O.A.C. (1990),10g honey were ashed by high temperature (550 °C) adjusted in a burning muffle for 5 hrs. The obtained ash was weighed after cooling to room temperature. Preheating of honey on a gas flame up to darkness is necessary to avoid foaming.

5-Minerals and heavy metals: Atomic absorption spectrometry was used to determine mineral content (Mg, Fe, Ca, Mn, Ni, Zn, Pb, Cd &Cu). The calcined ash of honey (obtained in step 4) was dissolved in 100ml 2N HCL. It was then measured in a Zeiss (model AA 55, Germany) atomic absorption spectrometer with the appropriate lamp for

each ion measured. The K & Na contents were determined using a Jenway (model PFP7, UK) flame photometer.

6-Total nitrogen and total protein contents: The N content was determined by the Kjeldahl method outlined by the A.O.A.C. (1990). The obtained content was multiplied in 6.25, as a factor, to calculate total protein content.

7-Phosphorous content: Was colorimetrically determined using the method given by Chen *et al.* (1956).

8-Acidity: Was determined according to White *et al.*(1962) and FAO (1986).

9-Total free amino acids: Was estimated according to the method described by Rosein (1957).

10-Hydroxymethylfurfural (H.M.F.): Was determined following the method of White (1979).

11-Sugar determination by HPLC: The concentration of main honey sugars (fructose, glucose, sucrose and maltose) were determined by high performance liquid chromatography (HPLC) according the method outlined by Bogdanov and Bauman (1988); cited from Nafea (2004).

C. Pollen examination (melissopalynology)

Based on the method of Louveaux *et al.* (1978). Samples of 20g honey were dissolved in 20ml warm distilled water (about 40 °C). The solution was centrifuged for 15 minutes at 3000 rpm. The supernatant was carefully discarded and the sediment was weighed (g). The sediment was then dispersed in 10ml distilled water to remove sugars, then centrifuged again for 10 min. The supernatant was decanted off and the sediment spread over 2x2 cm of a glass slide. After drying by slight heating, not above 40 °C, the sediment was mounted with glycerine-gelatine liquid medium and spread under cover glass. Microscopical examination (40x) for pollen grains was made and divided into categories

explained as very frequent (VF); grains were more than 45% of the total, frequent (F); 16-45%, rare (R); 3-15% and sporadic (S); less than 3%.

Results and Discussion

Tables (1,2&3) show the mean values of basic characteristics obtained from the physicochemical analyses and the percentage of pollen types found in the tested Libyan *Eucalyptus* honey. The obtained data could be discussed as the following:

I. Physicochemical analyses

The moisture content is the only composition criteria, which as a part of the honey standard has to be fulfilled in world honey trade. A maximum value of 21g/100g honey is suggested in the draft for a new standard (Lüllmann, 1997).

It is worth noting that the obtained water (18%) and sucrose (1.5%) contents are in keeping with values obtained for this same type of honey by different authors (Mateo-Castro& Bosch-Reig, 1984 and Serra-Bonvehi, 1989). Martinez-Gomez *et al.* (1993) reported 16.19% and 2.02% for the same parameters, respectively for Spanish *Eucalyptus* honey. The obtained values also were less than the limits set by recent international regulations recommended by European Standard Draft (ESD) and Codex Alimentarius Draft (CAD) being $\leq 21\%$ and $\leq 5\%$ for water and sucrose contents. They reported also $\leq 10\%$ sucrose for *Eucalyptus* honey (Bogdanov, 1999).

Table (1). Physicochemical characteristics of tested Libyan *Eucalyptus* honey.

No.	Parameter	Value	No.	Parameter	Value
1	Specific gravity	1.42	12	pH	3.20
2	Optical density (530 nm)	0.13	13	Free acidity (meq./kg)	24.00
3	Color grade	Light amber	14	Lactone (meq./kg)	1.00
4	Viscosity (poise)	48.10	15	Total acidity (meq./kg)	25.00
5	Total soluble solids (%)	82.00	16	Fructose (%)	40.00
6	Total insoluble solids (%)	0.07	17	Glucose (%)	32.00
7	Sediment content (%)	0.25	18	Sucrose (%)	1.50
8	Granulation	1.77	19	Maltose (%)	4.70
9	Electric conductivity (mS/cm)	0.44	20	HMF (mg/kg)	4.61
10	Ash content (%)	0.17	21	Total amino acids (%)	0.11
11	Moisture content (%)	18.00	22	Total protein (%)	0.08

Table (2). Minerals and heavy metals of tested honey (mg/kg honey).

Element	Value
N	131.60
P	40.00
K	414.00
Mg	32.90
Ca	3.44
Na	381.00
Fe	7.42
Mn	3.07
Zn	1.60
Cu	0.10
Ni	2.60
Pb	1.10
Cd	0.45

Table (3). Approximate pollen grains (%)
in tested honey.

Type of pollen	Value
<i>Eucalyptus</i> sp.	>60
<i>Acacia</i> sp.	<30
Herbs (different)	<7
Others (unidentified)	<3

The obtained insoluble solids (0.07%) and ash (0.17%) values indicate the cleanness of the product, however, since these values are less than the limits allowed by ESD and CAD for floral honeys ($\leq 0.1\%$ and $\leq 0.6\%$, resp.). On the other hand, coincides with the results of Chandler *et al.* (1974) for Australian *Eucalyptus* honey and also Martinez-Gomez *et al.* (1993) gave 0.077% and 0.206%, resp. for Spanish *Eucalyptus* honey. It seems that the permitted maximum in the Codex and European Standards of 0.1g/100g honey is too high. Mostly lower values, in the range of 0.005g to 0.05g/100g are found. Wax, which is not determined by the Codex method, is a major source of water-insoluble contamination (Bogdanov, 1999).

The obtained ash (0.17%) and electrical conductivity (0.44 mS/cm) corresponds with all of the values being homogeneously distributed around this finding. This result is in keeping with that found by Huidobro & Simal (1984) and Martinez-Gomez *et al.* (1993); 448.6 $\mu\text{S}/\text{cm}$ though it is lower than that reported by Serra-Bonvehi (1989) and Accorti *et al.* (1986) for this type of honey and also Bogdanov (1999) proposed ≤ 0.8 mS/cm E.C. for honey in general. In this regard, the EU (1996) mentioned that ash content is a quality criterion for honey botanical

origin. However, the blossom honeys having a lower ash content than honeydew honeys. At present, this measurement is generally replaced by the measurement of electrical conductivity. The ash content could be kept as a quality factor during a transition period, until conductivity is accepted as a worldwide standard.

The obtained pH (3.2) corresponded to that of a floral honey. For total acidity and HMF, which were used to determine the degree of deterioration of the honey, were all found to be within acceptable limits. A mean value of 4.61mg/kg for HMF and 25meq./kg for total acidity point to the high degree of freshness of the tested honey. Meanwhile, the obtained values are very lower than those suggested by ESD (40mg/kg & ≤ 40 meq./kg) and CAD (60mg/kg & ≤ 50 meq./kg) for HMF and total acidity, resp. But, the present findings agree with those of Martinez-Gomez *et al.* (1993) who had 3.630 mg/kg HMF and 28.28 meq./kg total acidity in Spanish *Eucalyptus* honey.

Sugar analysis gave results that were similar in glucose and fructose contents to those obtained by Serra-Bonvehi (1989) and Mateo-Castro and Bosch-Reig (1984) for Spanish *Eucalyptus* honeys, but slightly lower for fructose than Australian *Eucalyptus* honeys (Chandler *et al.*,1974). The difference in the latter case is probably due to the procedure used (apparent sucrose). Also, Martinez-Gomez *et al.* (1993) reported 31.63% & 38.75% for glucose and fructose, respectively in Spanish *Eucalyptus* honey.

The amount of total nitrogen found was 131.60 mg/kg. This value is lower than those obtained by Chandler *et al.* (1974), Wootton *et al.* (1976) for the same type of honey. Also Martinez-Gomez *et al.* (1993) reported higher nitrogen value (0.055%) for Spanish *Eucalyptus* honey.

Concerning minerals (Table 2), potassium appeared in the greatest content (414.00 mg/kg) followed by sodium (381.00 mg/kg).

Phosphorous and magnesium came after with 40.00 & 32.90 mg/kg, respectively. Iron, calcium and manganese were represented in lower concentrations; 7.42, 3.44 & 3.07 mg/kg, respectively. Noticeable contents of tested heavy metals (as environmental pollutants) were detected (Ni; 2.60 mg/kg, Zn; 1.60 mg/kg, Pb; 1.10 mg/kg, Cd; 0.45 mg/kg), while Cu had the lowest content (0.10 mg/kg).

In comparison with other authors, the obtained potassium, magnesium, iron and manganese values are keeping with the trend found by Serra-Bomvehi (1989). Matinez-Gomez *et al.* (1993) reported 285.60, 39.05, 8.73 & 3.93 ppm for the same minerals, respectively, but they reported higher calcium content; 155.30 ppm and low content of sodium; 77.7 ppm. Other variations in honey mineral contents were recorded by White (1978) and Feller-Delmasy *et al.* (1989)

II. Pollen analysis:

Honey pollen is important for the determination of the botanical and the geographical origin of the honey. Pollen examination (Table 3) showed that *Eucalyptus* (*Eucalyptus* sp.) pollen was dominant (60% of the total) means that tested honey is nearly unifloral. Pollen of *Acacia* sp. was about 20% of the total, while other pollen type of various wild herbs (7%) and unidentified pollens (3%) were less represented.

In this respect, although the *Eucalyptus* pollen was dominant in the tested honey, Serra-Bonvehi (1989) considered that a honey is unifloral only when it contains over 70% of one type of pollen.

Conclusion

Comparing the physicochemical parameters of the tested honey with those of international regulations, the obtained results can assert that the honey analyzed was of good quality, had acceptable values for maturity, purity, and cleanness and these values could be used to set quality controls for this type of honey.

References

- Accorti, M.; Persano-Oddo, L.; Piazza, M.G. and Sabatini, A. (1986). Schede di caratterizzazione delle principali qualità di miele italiano. *Apicultora* No.2; appendix; 36 pp. *Api. Abst.*, 1325/1987.
- A.O.A.C. (1990). Association of Official Analytical Chemists, official methods of analysis 15th Ed., Arlington, index of method number 920. 181.
- Bogdanov, S. (1999). Honey quality and international regulatory standards: review by the International Honey Commission. *Bee World*, 80 (2): 61-69.
- Bogdanov, S. and Bauman E. (1988). Bestimmung von Honigzucker mit HPLC. *Mitt. Gebiere Lebensm. Hyg.*, 79: 198-206.
- Brittan, O. (1956). Introduction of modern beekeeping to Cyrenacia (Libya). *Bee Craft*, 37 (12): 145-146; 38 (1): 4-5.
- Chandler, B.V.; Fenwick, D.; Orlova, T. and Reynolds, T. (1974). Composition of Australian honeys. CSIRO, Australia; Technical paper, Division of food Research No.38; 39 pp. *Apic. Abst.*, 845/1975.
- Chen, J. R.; Toribara, T. Y. and Warner, H. (1956). Assay of inorganic phosphate, total phosphate and phosphatases. *Anal. Chem.*, 28, 1756.
- El-Mabrook, A.S. (1996). Beekeeping in Libya. 1st International Arab Apicultural Congress, Beirut, August; pp. 29-31.
- EU (1996). European Union, Proposal for a directive of the European council relating to honey. Document 96/0114; Bruxelles, Belgium.

- FAO (1986). Food and Agriculture Organization. Manuals of food quality control; food analysis: quality, adulteration and tests of identity, 14/8, Rome, Italy.
- Feller-Delmasy, M. J.; Vincent, B. and Beaulieu, F. (1989). Teneur en minéraux et origine géographique des miels du Canada. *Apidologie* 20(1): 77-91. *Apic. Abst.*, 1280/1989.
- Huidobro, J.F. and Simal, J. (1984). Determinación del color y la turbidez en las mieles. *Anales de Bromatologica* 36 (2): 225-245. *Apicultural Abstracts*, 648/1988.
- Hussein, M.H. (2000). A review of beekeeping in Arab countries. *Bee World*, 81 (2): 56-71.
- Louveaux, J, Maurizio, A. and Vorwohl, G. (1978). Methods of melissopalinalogy. *Bee World*; 59 (4): 139 – 157.
- Lüllmann (1997). Annual reports of the Institute for Honey Analysis. IHA; Bremen, Germany.
- Martinez-Gomez, M.E.; Guerra-Hernandez, E.; Montilla-Gomez, J.Y. and Molins-Marin, J.L. (1993). Physicochemical analysis of Spanish commercial *Eucalyptus* honeys. *J. Apic. Res.*, 32 (3/4): 121-126.
- Mateo-Castro, R. and Bosch-Reig, F. (1984). Estudio sobre la composición glucídica de algunos tipos de mieles monofrales comerciales españolas. *Procede del 2o Congreso Nacional Apícola, Fundación Principado de Asturias, Gijón, Oviedo, Spain: pp. 358- 369.*
- Nafea, E. A. A. (2004). The biological effect of honeybee products as environmentally safe substances against some pathological microorganisms. Ph.D. Thesis. Department of Agricultural Science, Institute of Environmental Studies & Research, Ain Shams University, Egypt, pp. 36-38.

- Piazza, M. G.; Accorti, M. and Persano-Oddo, L. (1991). Electrical conductivity, ash, colour and specific rotatory power in Italian unifloral honeys. *Apicultora*, 7: 51-63.
- Rosein, H. (1957). A modified ninhydrin colorimetric analysis for amino acids. *Arch. Biochem. Biophys.*, 67:10-51.
- Serra-Bonvehi, J. (1989). Características físico-químicas y composición de la miel de eucalipto (*Eucalyptus* sp.) producción en España. *Anales de Bromatología*, 41 (1): 41-56. *Apic. Abst.*, 242/1993.
- White, J. W. Jr. (1978). Honey. *Advances in Food Research* 24: 287-374. *Apic. Abst.*, 999/1980.
- White, J.W.Jr.(1979). Determination of Hydroxymethylfufural (HMF). *J. Assoc. Anal. Chem.*, 62 (3): 509-511.
- White, J.W.Jr.; Riethof, M. L.; Subers, M.H. and Kushnir, L. (1962). Composition of American honey. *USDA. Tech. Bull.*, 1261: 1-124.
- Wootton, M.; Edwards, R. A. and Faraji-Haremi, R. (1976). Effect of accelerated storage conditions on the chemical composition and properties of Australian honeys. *J. Apic. Res.*, 15 (1): 29-34.

التحليل الطبيعي والكيميائي لقياس صفات الجودة لعسل اليوكالبتوس (السرول) الليبي

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الملخص العربي

أجريت التحاليل الطبيعية والكيميائية لدراسة خواص الجودة فى مكونات عسل اليوكالبتوس (السرول) الناتج من طوائف نحل العسل (هجين إيطالى) مربية فى خلايا مقاس دادنت فى منحل المعهد العالى للتقنية الزراعية بمنطقة الغيران-طرابلس-ليبيا. وقد شملت المحتوى المائى بنسبة 18.00%، والسكريات (40.00% فركتوز، و32.00% جلوكوز، و1.50% سكروز، و4.70% مالتوز)، و0.08% للبروتين الكلى، و0.11% للأحماض الأمينية الكلية، و25.00 ملليمكافى للحموضة الكلية و 4.60 مجم/كجم للهيدروكسيميثيل فورفورال، ودرجة اللون عنبرى فاتح، ونسبة الرماد 0.17%، والمواد الصلبة الكلية الذائبة 82.00% والغير ذائبة 0.07% أما درجة التوصيل الكهربى فكانت 0.44 مللى سيمنز/سم. وقد أوضحت النتائج أن قيم صفات الجودة المختبرة لتقدير نقاء وسلامة ونضج العسل محل الدراسة كانت جيدة جدا مقارنة بتلك الموصى بها عالميا من خلال دستور الأغذية والمواصفات القياسية الأوروبية. كما احتوى هذا العسل على أعلى نسبة من حبوب نبات اليوكالبتوس مقارنة بالأنواع الأخرى التى وجدت فيه مما يمكن من تسميته بهذه التسمية. ودل تحليل المعادن وبعض العناصر على أن عنصر البوتاسيوم هو السائد يليه الصوديوم ثم النيتروجين ثم الفوسفور والمغنيسيوم والحديد. كما تم تسجيل كميات قليلة من بعض العناصر الثقيلة كالنيكل والزنك والرصاص والكاديوم.

الكلمات الدالة: عسل اليوكالبتوس - التحليل الطبيعي والكيميائي - المعادن - تحليل حبوب اللقاح - صفات الجودة - ليبيا.