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Microbiological and Physico-Chemical Properties of Moroccan Honey

NAMAN MALIKA, FAID MOHAMED† AND EL ADLOUNI CHAKIB
Chouaib Doukkali University, College of Science, Department of Biology, PO BOX 10 Eljadida, Morocco.
†Hassan II Institute of Agronomy and Veterinary Medicine Department of Food engineering and Technology PO Box 6202 Rabat-Institute, Morocco.
[1]Corresponding author's e-mail: faidmohamed@yahoo.fr.

ABSTRACT

A total of 10 samples of Moroccan honey from different botanical origins including unifloral (*Eucalyptus*, *Thymus*, *Euphorbia*) and multifloral types were evaluated for their physico-chemical including pH, water activity and moisture, ashes, free lactone, electrical conductivity and total acidity and microbiological properties including the standard plate count (SPC), total colifoms, spore forming bacteria, fungi (yeasts and moulds). Results showed an aw of 0.55, the free acidity approach 22 meq/kg, the lactone acidity was around 7.5 meq/kg and total acidity was 29.5 meq/kg. An average pH of 4.2, while moisture was about 19,658 %, and the ash content was 0.32 g/kg, and the conductivity was 0.5183 ms/cm. Results of the microbiological characteristics showed that the microbial profile were very low for all the microorganisms studied, the SPC varied from 0 to 200cfu/g, whereas total coliforms were not detected in any sample, and fungi (yeasts and moulds) were also present at low levels and counts in many honey samples were below 100 cfu/g.

Key Words: Honey; Microbiology; Physico-chemical properties; Flora; Morocco

INTRODUCTION

Honey had been used in earlier times for its medicinal properties in many cultures throughout the world (Ransome, 1992). The use of honey as a therapeutic compound has been explored by the medical profession in more recent times. Scientific reports showed that honey exhibits important biochemical therapeutic activities (Molan, 1992) as it cures. Several studies showed the higher activity of honey over many of well known antibiotics (Al-Waili & Saloom, 1999). Honey showed powerful antibacterial effect against pathogenic and non pathogenic microorganisms, yeasts and fungi even against those that may develop resistance to many antibiotics. It had been demonstrated in earlier studies that honey can accelerate many diseases healing and also had bactericidal properties (Molan, 1996). Honey is thus able to destroy all of the bacteria that cause surgical infections and it had also shown to be able to of control post operative wound infections caused by *S. aureus*, *E. coli*, *Pseudomonas spp* (Efem *et al.,* 1992). Ceyhan and Ugur (2001) reported that honey may inhibit the growth of a wide range of microorganisms.

Honey has several important properties in addition to its composition and taste, also it is a sugar solution of high osmolarity which inhibits bacteria. The high sugar concentration tie-up water molecules, so that bacteria would have insufficient water to support their growth (Efem, 1988). The water content usually is only 15-21% (w/w) (White, 1975). Thus, the natural acidity of honey will inhibit many pathogens. Minimum pH values for some species range between 4.0-4.5. The predominant acid found in honey is gluconic acid. Its presence in all types of honey originates from the activity of glucose oxidase. This enzyme is of a considerable interest since it causes the production of hydrogen peroxide which not only stabilizes the ripening of nectar against spoilage but it has a microbicidal action.

Therefore, the factors mentioned above are considered in the concept of the microbiological stability to preserve honey during the ripening process. The aim of this study was to assess the physico-chemical and microbiological properties of some types of natural honey from different botanical origins including unifloral and multifloral.

MATERIALS AND METHODS

Sample collection. Eight samples of honey were selected from different botanical origins and locations in Morocco. The samples were stored at -20°C, in the black until analysis.

Physico-Chemical Determinations

pH was measured by pH-meter (Consort C832) in a solution containing 10 g of honey in 75 mL of distilled water (AOAC, 1990).

The free, lactonic and total acidity were determined as follows, by the titrimetric method: the addition of 0.05 N NaOH, is stopped at pH 8.50 (free acidity), immediately a volume of 10 mL 0.05 N NaOH is added and, without delay, back-titrated with 0.05 M HCl from 10 mL to pH 8.30 (Lactonic acidity). Total acidity was obtained by adding free plus lactone acidities. Results were expressed as

meq/kg (AOAC, 1990).

The moisture content was determined by drying a weighed amount of the product at 105°C until a constant weight was obtained.

Ash content was determined by ignition at 550°C in a furnace to constant mass (AOAC, 1990).

The activity of water (aw) was measured using an Aqualab Cx2 instrument (Deacagon Devices, Pullman, Washington, USA). 5 g of each sample were used to determine the aw.

Electrical conductivity of a solution of 20 g dry matter of honey in 100 mL distilled water is measured using an electrical conductivity cell at 20°C (HANNA instruments, HI 933100). The result is expressed in milliSiemens per centimeter (mS. cm^{-1}) (AOAC, 1995).

Microbiological determinations. Ten grams of each sample was mixed with 90 mL of saline water (8.5 g L^{-1}) to prepare the initial dilution. This was used as the mother dilution for further serial dilutions.

Standard plate count (SPC). Appropriate serial dilutions (10^{-1} to 10^{-3}) of the samples in saline water were plated on standard plate count agar (PCA) (Biokar, France). The plates were incubated at 30°C for 48 h.

Bacillus. The initial dilution was heat activated at 80°C for 10 minutes and cooled immediately in iced water. Aerobic spore forming bacteria were plated on plate count agar (PCA). The plates were incubated at 30°C for 48 h.

Coliform counts. Coliform counts were enumerated on Deoxycholate Citrate Lactose Agar (DCLA) (Difco, USA). Plates were incubated at 37°C for 24 h.

Yeasts. Count of yeasts was accomplished by surface plating dilutions on Potato Dextrose Agar (PDA) (Difco, USA) and incubating at 25°C for 72h.

Moulds. Moulds were enumerated on Sabouroud agar (biokar, France). The plates were incubated at 25°C for 7 days.

RESULTS AND DISCUSSION

Physico-Chemical characteristics. The physico-chemical properties of the different samples of honey are reported in Table II. pH values were in the same range for most of the samples and may lay between 4 and 4.5 except for one sample in which the pH was 3.8. Our results are in the range reported by White (1975) who has mentioned that honey is characteristically quite acidic, its pH being between 3.2 and 4.5. Acidification has been shown to promote healing by causing oxygen release from haemaglobin (Leveen *et al.,* 1973). In fact, the presence of gluconic acid in all honey originates largely from the activity of glucose oxidase which the bees add during ripening (Ruiz-Argueso *et al.,* 1973). The pH of honey is low enough to slow down or prevent the growth of many species of bacteria, but this acidity may be neutralized in the body by the buffering liquid fluids. The values of ash content varied in the range of 0.17 -0.56 with an average of 0.32 g kg^{-1}. The value was observed in multifloral samples (8 & 9) followed by the sample from *Thymus broussunetti, Eucalyptus globulus* and finally *Euphorbia resinefera* which has the highest value of ash (0.57 g kg^{-1}). Ash represents the direct measure of the inorganic residues after honey carbonization. This variability in the ash content can be explained by the floral origin (Vit *et al.,* 1998).

The moisture content of honey varied from 17.8 % in multifloral (9), to 21.8% in *Thymus broussunetti*. The average was 19.65%. This moisture variation can be explained by the composition and floral origin of honey samples. The strong interaction of sugar with water molecules may decrease the water available for microorganisms. Honey is hygroscopic and will remove moisture from the air. The low moisture content of honey also forms an important part of the system which protects honey from attack by microorganisms. The hyperosmotic nature of honey would prevent the growth of bacteria and yeasts as it draws water out of the organism, killing them by dessication.

The moisture content of honey is widely related to the harvest season in Morocco and the level of maturity released in the hive. This parameter is highly important for the shelf-life of the honey during storage.

The values of the water activity varied from 0.53 to 0.058 with an average of 0.55. The aw of honey varies slightly, it is obviously related to the floral source of nectar. Honey is a supersaturated sugar solution with a low water activity, which means that there is insufficient water available to support the growth of bacteria and yeasts. Although some yeasts can survive in high water content, causing spoilage of the honey. Mean values for the honey have been reported as 0.56 and 0.58 (Ruegg & Blanc, 1981). Many species of bacteria have their growth completely inhibited by the aw being in the range 0.94-0.99 (Scott, 1957) and the aw of ripened honey is between 0.56-0.62 (Tysset *et al.,* 1980) does not support the growth of yeasts. Fungi are generally much more tolerant to low aw than bacteria (Leistner & Roedel, 1975).

The results of the different unifloral and multifloral honeys for the free and total acidity are summarized in Table III. Values for the acidity vary from 26 to 39.5 meq kg^{1} honey. The highest value was found in multifloral honey (8) and the lowest value in *Eucalyptus globulus* honey (4). These results showed that the total acidity was not significantly different of honey samples from the same plant (*Thymus*, *Eucalyptus* & *Euphorbia*), but there can be a large variation in the total acidity of the multifloral honey. These results were in agreement with previous works in Spain reported by Garcia (Garcia *et al.,* 2001). The authors found that the total acidity value was in the normal range, except for four samples that showed acidity values over 40 meq/kg honey. So, the acidity of honey contributes to its stability against microorganisms and to flavor. The total acidity was below maximum limits set internationally (40 meq/kg). It has been suggested by previous works (Vit *et al.,* 1998) which reported mean values for acidity vary from 30.40 to

Table I. Microbial profiles (in cfu/g) of the honey samples

Honey samples	Microorganisms count (cfu/g)				
	SPC	*Bacillus sp*	TC	Yeasts	Moulds
Thymus broussonetti (1)	<10	<10	<10	<10	<10
Thymus broussonetti (2)	100	<10	<10	<10	<10
Eucalyptus globulus (1)	<10	<10	<10	<10	<10
Eucalyptus globulus (2)	<10	10	<10	<10	10
Euphorbia resinifera (1)	200	200	<10	10	<10
Euphorbia resinifera (2)	200	200	<10	30	<10
Euphorbia resinifera (3)	20	10	<10	<10	10
Multifloral (1)	10	30	<10	20	<10
Multifloral (2)	100	10	<10	<10	10
Multifloral (3)	10	<10	<10	<10	<10

SPC: Standard Plate Count; TC: Total coliforms.

Table II. Physico-chemical characteristics of honey samples from different botanical origins.

Samples	pH	Ash (g/kg)	Moisture (%)	aw	E.C.
Thymus broussonetti (1)	4.5	0.1968	19.98	0.556	0.271
Thymus broussonetti (2)	4.42	0.2367	21.80	0.570	0.215
Eucalyptus globulus (1)	4.10	0.3727	20.00	0.543	0.429
Eucalyptus globulus (2)	4.00	0.3575	18.4	0.537	0.498
Euphorbia resinifera (1)	4.35	0.49	21.2	0.556	0.608
Euphorbia resinifera (2)	4.20	0.50	20.00	0.554	0.761
Euphorbia resinifera (3)	4.28	0.5669	18.8	0.558	0.78
Multifloral (1)	3.80	0.1776	18.6	0.587	0.532
Multifloral (2)	4.25	0.189	17.8	0.554	0.541
Multifloral (3)	4.15	0.204	20	0.547	0.590
Averages	4.205	0.32	19.658	0.5562	0.5183

E.C.: Electrical conductivity.

Table III. Free, lactone acidity and total acidity of the different honey samples

Samples	Free acidity (meq/kg)	Lactone acidity (meq/kg)	Total acidity (meq/kg)
Thymus broussonetti (1)	25	7.5	32.5
Thymus broussonetti (2)	23	12	35
Eucalyptus globulus (1)	20	10	30
Eucalyptus globulus (2)	22.5	3.5	26
Euphorbia resinifera (1)	31.5	6	37.5
Euphorbia resinifera (2)	33	4.1	37.1
Euphorbia resinifera (3)	29	9	38
Multifloral (1)	34	5.5	39.5
Multifloral (2)	21	12	33
Multifloral (3)	22	7.5	29.5
Averages	26.1	7.71	33.81

48.27 meq/kg of honey. The acidity is determined fundamentally by the content of gluconic acid and glucolactone.

Electrical conductivity values varied from 0.215 to 0.761 with an average of 0.518 mS/cm. The electrical conductivity was lowest in honey sample of *Thymus broussonetti* (1) 0.215 mS/cm and of *Thymus broussonetti (1)* 0.271 mS/cm, the highest value was found in honey samples of *Euphorbia resinefera* (2) 0.761 mS/cm. The values of electrical conductivity were not significantly different between the four groups of honey samples. The values of electriocal conductivity of honey samples of *Thymus broussonetti* were similar to previous works (Soria *et al.,* 2004), who reported that artisanal honeys may have a minimum value of electrical conducvity of 0.119 mS/cm and a maximum of 1.515 mS/cm.

Values of the electrical conductivity should be ≤ 0.7 mS/cm. The conductivity is a good criterion related to botanical origin of honey and thus is very often used in routine honey control instead of the ash content. Ash and electrical conductivity are two parameters bound to honey minerals content. Ash represents a direct measure of the inorganic residue after honey carbonization, while the electrical conductivity measures all ionisable organic and inorganic substances. The relationship between the two parameters has been shown by several authors (Accorti *et al.*, 1983; Piazza *et al.,* 1991; Sancho *et al.,* 1991).

The conductivity value obtained is similar to the mean value published by Terrab *et al.* (2003), who have reported that mean value of conductivity of honey is 0.240 ms/cm.

Electrical conductivity depends of the mineral content of the honey. As the differences in the electrical conductivity of the various honeys are attributable to their differing geographical and botanical origins, it serves to characterize varietal honeys.

Microbiological characteristics. Microbial counts in the different samples of honey are reported in table I. This shows low levels for all of the samples. The standard plate counts (SPC) were found in low numbers in most samples of honey with a minimum count of 10 cfu/g and a maximum count of 2 10^2 cfu/g. Spores of *Bacillus* are found in most of the samples. They were detected chiefly in honey from *Eucalyptus globulus* (2), *Euphorbia resinefera* (1, 2 & 3) and multifloral (1 & 2), but they were not detected in honey from *Thymus broussonetti* (1 & 2), Eucalyptus (1) and multifloral (3).

Total coliforms were not detected in any sample. This may be explained by the evidence that honey is well preserved against bacteria so that theses microorganisms would not survive unfavorable conditions.

Yeasts were detected in low counts and only in some samples. Counts vary from 10 to 30 cfu/g. Moulds were present only in honey from *Eucalytus globulus* (2), *Euphorbia resinefera* (3) and multifloral (2).

The standard plate counts (SPC) and *Bacillus* counts were high in most of honey samples, and their numbers varied from one sample to another. The standard plate count and *Bacillus* spores were naturally found in honey. Our results are in agreement with the data reported by Gilliam (1997). However, total coliforms were absent in all sample including unifloral honey (*Thymus*, *Euphorbia* and *Eucalyptus*) and multifloral. Coliforms are an indicator of sanitary quality of honey. Few samples of honey contain detectable levels of yeasts. Although yeast counts in many honey samples were below 100 cfu/g. This range may approach data reported by Snowdon and Cliver (1996). The low number of moulds would be most probably related to the environmental conditions but it is not significant (10cfu/g). Therefore, honey has inherent antimicrobial properties that can delay the growth of many microorganisms. The microorganisms that may be found in

honey are mostly yeasts and spore forming bacteria, but no disease causing bacterial species have been found in honey (Molan, 2001). The high counts of bacteria detected in honey are due to contamination from exogenous sources. It was demonstrated by Nakano *et al.* (1994) that multiplication of *Clostridium botulinum* in honey bees explains the heavy contamination of honey. Also Nakano and Sakagauchi (1991) have shown that the high count of *C. botulinum* in the honey may have been due to the possible stimulation of growth by *Bacillus alvei* or some other microorganisms at some stages of honey ripening. Another investigation claimed that spore of *Bacillus* larvae were detected in Argentinian honey (Alippi, 1995). Generally, honey may contain organisms from bees, soil, air and dust that are introduced during post-harvest handling (Jay, 1992).

Results have shown that there is a large variation in the count of microorganisms of different samples from the same plant source. All Samples of honey are characterized by a low count of microorganisms. These factors added to the low aw and pH help well in the preservation and the stability of the product. Factors mentioned above would cause hostile conditions for the growth and the surviving of microorganisms. The low water activity and pH would cause the destruction of microorganisms initially present in honey and told about an acceptable bacteriological quality of Moroccan honey.

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