

MICROBIOLOGICAL QUALITY OF MOROCCAN LABELED *EUPHORBIA RESINIFERA* HONEY

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ABSTRACT

In the present work, microbiological profile of thirty-seven samples of labeled honey were collected in a Protected Geographical Indication “PGI” area of Tadla-Azilal region, which is an endemic zone of *Euphorbia resinifera* plant. A profile was assessed using conventional microbial methods, like enumeration, detection and/or germs identification, in accordance with ISO norms. This is the first study in which a honey with Moroccan “PGI” was tested, in order to assess its compliance with bacteriological recommendations. Coliforms (Total and fecal Coliforms), *Salmonella* spp., *Shigella* spp., *Sporus of Bacillus cereus* and *Clostridium perfringens* were not detected. The numbers of Standard Plate Count “SPC” were less than 10² CFU.g⁻¹ for all samples. The molds and yeasts were found among samples and 32% and 40% of samples were positive, respectively. However, no samples showed a higher value than recommended limit [10² CFU.g⁻¹]. We conclude that samples of labeled euphorbia honey of Tadla-Azilal analyzed present good commercial quality parameters (SPC, molds and yeasts “absence of unwanted fermentations”), a good sanitary quality (absence of coliforms and *S. aureus*) and are safe (*Slam.*, *Shig.*, *Sporus of B. cereus* and *C. perf.*). Standardization (regulation and specifications) and a rationalization of beekeeping techniques throughout *Euphorbia* “PGI” area studied may further sustainably improve the quality of this unique honey, and ensure it over the years.

Keywords: Morocco, labeled *Euphorbia resinifera* honey, Bacteriological Quality

INTRODUCTION

The *Euphorbia resinifera* is one of the specific and endemic plants of Moroccan Atlas Mountains (Picture 1). Generally, the *Euphorbia* plants have high adverse effect level (due to the Latex component, which is a powerful alkaloid), so they has been studied for their antifungal and antibacterial properties (Kamba *et al.*, 2010; Benmehdi *et al.*, 2013). In addition, the honeys produced from these plants confirms the antibacterial and antifungal activity (Malika *et al.*; 2004, Crousilles, 2014; Bouhlali *et al.*, 2016). Likewise, generally the intrinsic properties of honey (osmolality, pH, hydrogen peroxide, phenolic components and flavonoids) affect the growth and survival of microorganisms by bacteriostatic or bactericidal action. (Adock, 1912; White *et al.*, 1962; Iurlina and Fritz, 2005; Kačaniová *et al.*, 2009; Adenekan *et al.*, 2010). Furthermore, the low pH, the low water activity and the high sugar content of undiluted honeys prevent the growth of many species of microorganisms (Snowdon and Cliver, 1996, Snowdon, 1999). In consequence, *Euphorbia* honey can be expected to contain a small number and limited varieties of microorganisms. It can be noted that vegetative forms of human disease-causing bacteria have not been found in honey and, as bacteria do not replicate in honey, a high count of vegetative bacteria is indicative of a recent contamination from a secondary source (Snowdon and Cliver, 1996; McKee *et al.*, 2003; Antúnez *et al.*, 2004). The microorganisms of interest are those that withstand the concentrated sugar, acidity and antimicrobial character of honey. These microorganisms; indicative of sanitary or commercial quality, include yeasts, molds, coliforms, *Salmonella*, *Shigella* and some microorganisms such as sporus-forming bacteria, like *Bacillus cereus* (*B. cereus*) and *Clostridium perfringens* (*C. perf.*), which under certain conditions (e.g. germination and growth in a non-heated-treated product) could cause illnesses in humans (Snowdon and Cliver, 1996; Al-Waili *et al.*, 2012). Otherwise, the Moroccan standards for honey quality (Moroccan Norm 08.05.600, 2012) inspired essentially from Codex Alimentarius Standards (Codex Stan, 2001) and the specifications of the label "GPI" (Moroccan Order,

2012) includes several chemical and physical parameters but do not require microbiological analysis.

However, the use of adequate hygienic practices during the product handling is required (Moroccan Law 28-07, 2010; Moroccan Norm 08.0.000, 2008). In addition, various studies have been carried on the palynological and physicochemical parameters of Moroccan *Euphorbia* honey (Chakir *et al.*, 2011; Aazza *et al.*, 2014, Terrab *et al.*, 2014; Bettar *et al.*, 2015), but a microbiological contamination has not been extensively investigated.



Figure 1 *Euphorbia resinifera* plant of the “PGI” Tadla-Azilal region of Morocco. ©Photo. Moujanni

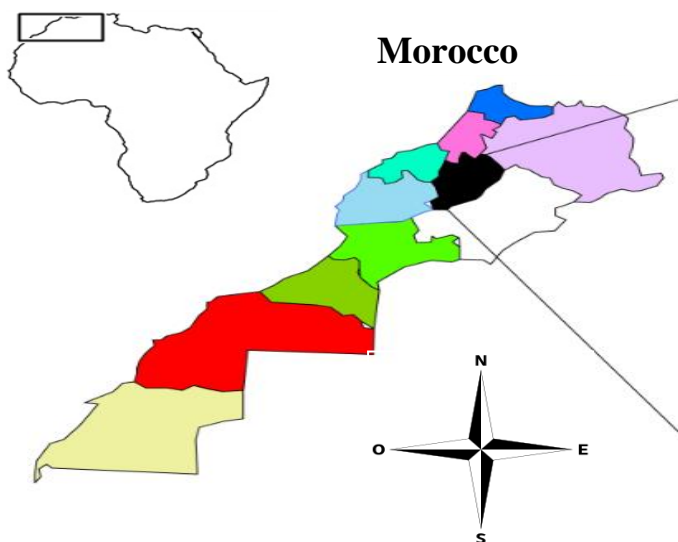
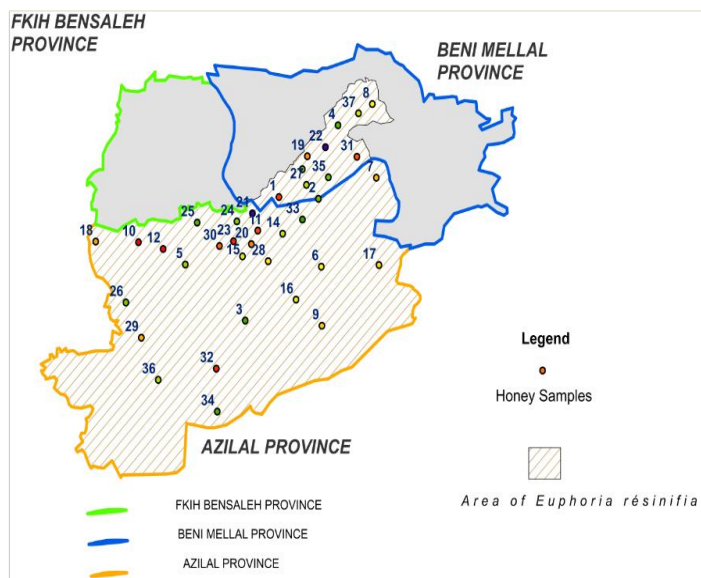


Figure 1 Distribution of samples of labeled monofloral *Euphorbia resinifera* honeys in “PGI” production area of Tadla-Azilal

Euphorbia resinifera honey of Tadla-Azilal region is the first honey labeled “Protected Geographical Indication –PGI–” in Morocco (Moroccan Order, 2012; ADA, 2014). This label was published in the EU Official Journal through the public consultation documents on geographical indications of the Kingdom of Morocco (European Commission, 2013/C).

In this context and in concordance, with the importance and good status of this unique monofloral honey, we decided to investigate about its bacteriological profile targeting the major microbiological contaminants (*SPC*, *Total coliform*, *Fecal coliform*, *Sporus of Bacillus cereus* and *Clostridium perfringens*, *Staphylococcus aureus*, *Salmonella spp.*, *Shigella spp.*) and its fungal profile (*Molds and Yeasts*) which may cause undesirable fermentation. Their counts being indicative of honey’s commercial and sanitary quality and safety.

MATERIAL AND METHODS

Sampling

Thirty-seven (37) samples of honey “GPI” mono-floral *Euphorbia resinifera* were supplied directly by the beekeepers of the “PGI” area affiliated to U.C.A.T.AZ cooperative or working individually (Picture 2). Their distribution is indicated in Figure 1.

The samples had not been heated or pasteurized. The productions years of all samples were 2013 and 2014 (Table 1). Upon collection, 250g or 500g of each sample are put in clean commercial labeled container and stored at room temperature pending analysis (Table 1).



Figure 2 “PGI” Tadla-Azilal production area with aggregate *Euphorbia resinifera* plant

Table1 Information on honey samples studied

Sample	Locality name	Harvest Year
P1	FoumOudi	2014
P2	FoumElaancer	2013
P3	AitMhamed	2013
P4	Tanougha	2013
P5	Tabia	2014
P6	Tabaroucht	2013
P7	Ait Hamza	2013
P8	Elksibah	2013
P9	Tilougguite	2013
P10	Rfala	2013
P11	Afourer	2013
P12	Ben Driss	2012
P13	BeniMellal	2013
P14	Timoulilt	2013
P15	Bin Elouidane-AitOuarda	2013
P16	AitMazigh	2013
P17	Anergui	2013
P18	Bzou	2013
P19	FoumElaancer	2014
P20	AitOuarda	2014
P21	AitAamir	2014
P22	Tagzirt	2014
P23	Anergui	2013
P24	Afourer	2013
P25	BeniAayat	2014
P26	FoumJemaa	2014
P27	BeniMellal	2014
P28	Azilal	2014
P29	Tanant	2014
P30	Ouaouzaght - Damnat	2014
P31	Tagzirt	2014
P32	AitAbbass -AitMassad	2014
P33	Assaksi - Tagleft	2014
P34	AitBououlli	2014
P35	FoumElaancer -Tagzirt	2014
P36	Ouaoula-AitMhamed	2014
P37	Elksibah	2014

PGI: Protected Geographical Indication

UCATAZ: Union of Beekeepers Cooperative of “PGI” Tadla-Azilal Region, Morocco.

Microbiological analysis.

Ten grams of each sample were mixed with 90mL of Buffered Peptone Water (Biokar) to prepare the initial dilution. This was used at the mother dilution for further serial dilution.

Standard Plate Count (SPC) (ISO Norm 4833-1, 2013): Appropriate serial dilutions (between 10 and 100 colonies per plate) of the samples in the Buffered Peptone Water were placed on standard plate count agar (PCA) (Biokar, France). The plates were incubated at 30°C for 72h.

Coliform counts (TC) (ISO Norm 4832, 2006): Were enumerated on Violet Red Bile Lactose Agar (VRBLA). Plates were incubated at 37°C for 24h.

Fecal coliforms (FC) (NF Norm V 08 060, 2009): Were enumerated on Violet Red Bile Lactose Agar (VRBLA). Plates were incubated at 44°C for 24h.

Staphylococcus aureus (*S. aur.*) (ISO Norm 6888-1, 2003): Were enumerated on Baird Parker growth medium (Biolife). Plates were incubated at 37°C for 24h and 48h.

Shigella detection (Shig.) (Lampel KA, 2001): 25g of the sample was homogenized in 225mL of selenite broth (Biokar) and the volume was transferred to an Erlenmeyer flask and incubated at 35°C for 20h. After this period, a loopful of this broth was plated onto Petri dishes containing XLD agar (Biolife) and *Salmonella-Shigella* agar (SS, Biokar). After incubation for 48h at 35°C, five characteristic colonies of *Shigella* were biochemically tested on TSI agar (Biokar) and API-20E Biomerieux). The colonies were also serologically tested.

Clostridium perfringens (*C. perf.*) (ISO Norm 7937, 2004): Petri dishes are seeded with a specific quantity of the initial suspension. Other dishes were seeded in the same conditions, using decimal dilutions obtained from the mother suspension. The tryptone sulfite cycloserine (Biolife) was added and then a layer of the same medium is added from above. The dishes were incubated anaerobically at 37°C for 20h. The characteristic colonies are counted. Finally, the characteristic colonies are confirmed and the number of *C. perfringens* per gram of sample is calculated.

Bacillus cereus (*B. cereus.*) (ISO Norm 7932, 2004): Seeding the surface of a solid selective culture medium poured into Petri dishes (MYP Agar) with a specified quantity of the initial suspension. Other dishes were seeded in the same conditions, using decimal dilutions obtained from the mother suspension. The plates are incubated aerobically at 30°C for 18 to 48h. The characteristic colonies are counted and the characteristic colonies are confirmed by hemolysis test and the number of *B. cereus* per gram of sample is calculated.

Salmonella detection (*Salm.*) (ISO Norm 6579/A1, 2007): For the detection of the presence of *Salmonella*, 25g of honey sample was homogenized in 225mL of peptone buffered water (Biokar), transferred to an Erlenmeyer flask and incubated at 35°C for 24h. After the incubation period, 1mL was added to a tube containing 10mL of tetra-thionate broth (Biolife). The Rappaport broth (Biokar) received 0.1mL from pre-enrichment and the tubes were incubated at 37°C and 41.5°C for 24h, respectively. After this period, a loopful of each selective broth was plated into Petri dishes containing xylose lysine desoxycholate agar (XLD-Biolife) and CHRO-Magar (Rambach). After incubation for 24h at 37°C, five typical colonies from each agar plate were biochemically tested on TSI agar (Biokar) and API-20E (Biomerieux). The colonies were also serologically tested with polyvalent somatic and flagellar antisera (Probac).

→ *Mold and yeast counts* (ISO Norm 21527-1, 2008): Petri dishes prepared using a defined selective culture medium (Glucose Chloramphenicol Agar-Biolife) are seeded. In the number of colonies expected, a specific amount of the initial suspension or decimal dilutions sample / suspension are used. Additional Petri dishes can be seeded in the same conditions; using dilutions decimal obtained from the initial suspension. Plates are incubated aerobically at 25°C for five days. Then, if necessary, the agar plates are allowed to stand in daylight for one to two days. Colonies/propagules are then counted, and if necessary (to distinguish yeast colonies of bacteria colonies), the identity of suspicious colonies is confirmed by examination under the binocular or microscope. The number of yeasts and molds per gram is calculated from the number of colonies/propagules/germs obtained on Petri dishes selected to dilution ratios to obtain colonies that can be counted. Molds and yeasts are counted separately.

Statistical analysis

All determinations were made in triplicate and the data was processed using XLSTAT, 2015 software.

RESULTS AND DISCUSSION

Results of the microbiological analyzes are given in Table 2. The standard plate count (SPC) also referred to as the aerobic plate count or the total viable count, is

one of the most common tests applied to indicate the microbiological quality of food.

The Moroccan legislation (Moroccan Order, 2004) does not set values for SPC in honey but establishes only that you follow good hygiene practices in handling and processing of this product because entire microbial load in honey can indicate the possible presence of pathogens (Moroccan Norm 08.5.600, 2010). The SPC were isolated from all samples of honey. Their number varied between 10 and 340CFU.g⁻¹ with a mean value equal to 76.76±82.93CFU.g⁻¹. This result was inferior to those obtained, for the same type of Moroccan honey, by Malika et al., (2005). Compared to other foreign honeys, our results are below Argentinean and French honeys which had main SPC values 244CFU.g⁻¹ and 227 CFU.g⁻¹ respectively (Iurlina and Fritz, 2005; Tysset et al., 1981), while Portuguese commercial honey had better SPC levels [2.10³CFU.g⁻¹] (Gomes et al., 2010). This variation of SPC values could be related to the type of sample, the age and the honey harvest time. In addition, these vegetative forms can be made by secondary contamination, which would also explain the high counts sometimes found in honey (Snowdon and Cliver, 1996).

Coliforms (TC and FC) are indicators of fecal contamination and poor hygienic processing conditions. In this study, TC and FC were not detected (level of quantification is 10CFU.g⁻¹) and suggest a respect of good practices for extraction and processing of honey were followed. Our results corroborate with data found by Rall et al., (2003), Gomes et al. 2010, Iglesias et al., (2012), Rios et al., (2014) and Kunová et al., (2015). The absence of these microorganisms in analyzed honey was expected since bacteria growth needs water activity more than 0.91 (Ribeiro and Seravalli, 2004). Snowdon and Cliver, (1996) already reported that the population of FC in honey varied from 10 to 10²CFU.g⁻¹. In contrast, in 70 samples of honey analyzed in Nigeria Coliforms and *E. coli* were isolated at rate of 95.7% (Kokubo et al., 1984). Also, Dümen et al.,(2013) and Sherwani et al.,(2013),reported respectively that 16% of 80 honey samples were contaminated by coliforms in Turkey and two Pakistani honey samples over six presents coliforms (0.2×10¹ and 0.4×10¹ CFU.g⁻¹)

S. aureus is the causative agent of the numerous outbreaks of foodborne disease worldwide. Poisonings generally occur after an intake of enterotoxins through the alimentary track. The absence of this bacterium in this study constitutes another sanitary index in favor to the quality of this product. In a similar study done on Turkish honeys, 13.4% of the 67 samples analyzed contained *S. aureus* (Dümen et al., 2013).

B. cereus and *C. perfringens*, as producers of spores, are considered as health indicator including uncontrolled land-based, environmental or human contamination. High levels of *B. cereus* in honey constitute a risk to the consumer, as ingestion of 10⁵ spores can result in food-borne illness (Stenfors et al., 2008). The results of this study demonstrate a negative result regarding detection of sporus of *B. cereus* and *C. perfringens*. However, Pucciarelli, (2014) found the incidence of *Clostridium* and *Bacillus* (42.85 and 39% respectively) in yateí honey from Argentina. In addition, Ragazani et al., (2008) studying honey marketed in several Brazilian states found 11% were *Clostridium* genus and 28% of the genus *Bacillus*. Erkan et al.,(2015) and Sherwani et al.,(2013) reported respectively 5.5×10¹±6.3×10¹ *B. cereus* mean count in Turkish honey and presence of *B. cereus* in all (six) samples of Pakistanis (Karachi) honey tested. In respect to safety, none of the 37 samples contained *Salmonella* and *Shigella*. The absence of these pathogens was expected since in addition to its antibacterial properties, honey has low water activity and a pH, which are not in favor to the development of such bacteria (Snowdon and Cliver, 1996; Alves et al., 2015; Matuella and Torres, 2000).

In the same way, study of the microbiologic quality of honey samples produced in the surroundings of a large garbage dump in Brazil showed the absence of *Salmonella*. These results confirm the conclusion of Anses that the *Salmonella* survival duration in honey does not exceed one month (Anses, 2012).

Table 2 Distribution of microorganisms detected in “GPI” Moroccan *Euphorbia resinifera* honeys

N°	Microorganisms count (CFU.g ⁻¹)					Per 25g		Sporus		
	SPC	TC	FC	<i>S.aur</i>	Yeasts	Molds	<i>Salm.</i>	<i>Shig.</i>	<i>C.perf</i>	<i>B.cereus</i>
P1	50	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P2	340	<10	<10	<10 ²	10	<10	Abs	Abs	<10	<10
P3	320	<10	<10	<10 ²	70	90	Abs	Abs	<10	<10
P4	270	<10	<10	<10 ²	20	<10	Abs	Abs	<10	<10
P5	250	<10	<10	<10 ²	93	60	Abs	Abs	<10	<10
P6	60	<10	<10	<10 ²	10	<10	Abs	Abs	<10	<10
P7	90	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P8	30	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P9	50	<10	<10	<10 ²	30	10	Abs	Abs	<10	<10
P10	70	<10	<10	<10 ²	30	10	Abs	Abs	<10	<10

P11	40	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P12	30	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P13	40	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P14	10	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P15	110	<10	<10	<10 ²	20	20	Abs	Abs	<10	<10
P16	30	<10	<10	<10 ²	10	<10	Abs	Abs	<10	<10
P17	80	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P18	20	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P19	30	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P20	70	<10	<10	<10 ²	50	10	Abs	Abs	<10	<10
P21	60	<10	<10	<10 ²	20	10	Abs	Abs	<10	<10
P22	40	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P23	120	<10	<10	<10 ²	80	20	Abs	Abs	<10	<10
P24	10	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P25	70	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P26	10	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P27	50	<10	<10	<10 ²	50	<10	Abs	Abs	<10	<10
P28	50	<10	<10	<10 ²	20	<10	Abs	Abs	<10	<10
P29	10	<10	<10	<10 ²	<10	10	Abs	Abs	<10	<10
P30	20	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P31	50	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P32	50	<10	<10	<10 ²	<10	10	Abs	Abs	<10	<10
P33	50	<10	<10	<10 ²	<10	10	Abs	Abs	<10	<10
P34	10	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P35	60	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
P36	110	<10	<10	<10 ²	60	20	Abs	Abs	<10	<10
P37	80	<10	<10	<10 ²	<10	<10	Abs	Abs	<10	<10
Mean	76.76	<10	<10	<10 ²	38.20	23.33	n.a.	n.a.	<10	<10
SD	82.93	n.a.	n.a.	n.a.	27.20	25.35	n.a.	n.a.	n.a.	n.a.
Min	10	n.a.	n.a.	n.a.	10	10	n.a.	n.a.	n.a.	n.a.
Max	340	n.a.	n.a.	n.a.	93	90	n.a.	n.a.	n.a.	n.a.

n.a.: not applicable- Abs: Absence
 Level of quantification = 10² CFU.g⁻¹ for *S. aureus*
 Level of quantification = 10 CFU.g⁻¹ for all microorganisms tested
 Level of quantification = 10 sporus for *C.perf.* and *B.cereus*

Table 3 Comparing the results of molds and yeasts counts in honey from different countries

References	Nb. samples	Molds and yeast Count CFU.g ⁻¹			Country	Incidence Positives/Total analyzed	Limit recommended CFU.g ⁻¹ [a] [55]
		Min	Max	Mean			
Iurlina and Fritz (2005)	23	0	4.7×10 ²	1.64×10 ²	Argentina	59%	1.0×10 ²
Finola et al., (2007)	23	<1.0×10 ¹	<1.0×10 ¹	-	Argentina	-	1.0×10 ²
Rios et al., (2014)	58	-	-	-	Argentina	17% (7%)	1.0×10 ²
Pucciarelli et al., (2014)	28	1.2*	4.7*	3.02*	Argentina	-	1.0×10 ²
Rall et al., (2003)	100	<1.0×10 ²	1.5×10 ⁵	-	Brazil	64%	1.0×10 ²
Sereia et al., (2010)[b]	11	1.9×10 ²	1.1×10 ³	5.3×10 ²	Brazil	-	1.0×10 ²
Sereia et al., (2010)[c]	6	1.8×10 ¹	2.5×10 ²	1.0×10 ²	Brazil	-	1.0×10 ²
Pontara et al., (2012)	12	<1.0×10 ¹	<1.0×10 ¹	-	Brazil	-	1.0×10 ²
Ananis et al., (2013)	35	<1.0×10 ¹	5.0×10 ²	-	Brazil	45.71%	1.0×10 ²
Alves et al., (2015)	15	2.2×10 ⁷	3.4×10 ⁷	-	Brazil	20%	1.0×10 ²
Giraldo et al., (2013)	7	0	0	0	Colombia	0	1.0×10 ² [d]
Mahmoudi et al., (2016) [e]	34	-	-	-	Iran	5.8%-32.3%	-
Ayansola, (2012) [f]	108	1.0×10 ¹	2.0×10 ³	-	Nigeria	-	-
Ummulkhair, (2014)	15	1.0×10 ⁴	1.2×10 ⁵	-	Nigeria	26.66%	-
Malika et al., (2005)	10	<1.0×10 ¹	3.0×10 ¹	-	Morocco	30%	-
Present study (yeasts)	37	1.0×10 ¹	9.3×10 ¹	3.82×10 ¹	Morocco	32%	-
Present study (molds)	37	1.0×10 ¹	9.0×10 ¹	2.33×10 ¹	Morocco	40%	-
Sherwani, (2013)	6	0	<1.0×10 ¹	-	Pakistan	20%	-
Róžańska and Osek (2012)	245	<5.0×10 ¹	8.0×10 ⁴	-	Poland	-	1.0×10 ²
Gomes et al. (2010)	5	1.1×10 ¹	2.1×10 ¹	-	Portugal	60%	1.0×10 ²
Feás et al., (2010)	45	1.0×10 ¹	8.0×10 ¹	2.2×10 ¹	Portugal	100%	1.0×10 ²
Duman Aydin et al., (2008)	20	1.0×10 ²	1.0×10 ³	-	Turkey	40%	1.0×10 ²

Dûmen et al., (2013)	500	-	-	-	Turkey	16%-32%	-
Erkan et al., (2015) [g]	50	1.0×10^2	1.2×10^3	3.5×10^2	Turkey	26%	-
Erkan et al., (2015) [h]	50	7.4×10^3	1.4×10^5	5.4×10^4	Turkey	46%	-

*Count in log CFU.g⁻¹
a: The value recommended by MERCOSUR (Agreement on the Southern Common Market) and CNEVA.
b: Organic honey
c: Inorganic honey
d: The value specified by Colombian Resolución N°1057
e: Count of fungi (Aspergillus, Penicillium, candida and other yeasts)
f: Count of total heterotrophic fungi
g: Count for Mold vegetative form
h: Count for Yeast vegetative form

The results obtained for standard counting of molds and yeasts showed that 32% and 40% of samples were positive respectively for molds and yeasts.

However, the detection of yeasts and molds remains at low levels ($[1.0 \times 10^1 - 9.3 \times 10^1 \text{CFU.g}^{-1}]$ for yeasts with mean $= 3.82 \times 10^1 \pm 2.72 \times 10^1 \text{CFU.g}^{-1}$ and $[1.0 \times 10^1 - 9.0 \times 10^1 \text{CFU.g}^{-1}]$ for molds with average $2.33 \times 10^1 \pm 2.53 \times 10^1 \text{CFU.g}^{-1}$). Withal, note that no result exceeds the recommended threshold for yeast (10^2CFU.g^{-1}), nor the fermentation honey line ($5.0 \times 10^2 \text{CFU.g}^{-1}$) (Fléché et al., 1997). Furthermore, total mold and yeast counts can vary greatly, typically between 0 and 10^5CFU.g^{-1} , although high counts are not palatable because of the increased rate of fermentation and the honey is unlikely to pass quality control (changing the taste and the flavor of honey) (White, 1975). From that point a view, a few hundred CFU.g⁻¹ of yeast are more likely to be found in honey samples.

Table 3 below gives a comparison of the values found in several studies, conducted in the world relating to molds and yeasts in honey. It appears from reading the table that our results are close to those of Malika et al., (2005), Gomes et al., (2010) and Feás et al., (2010). Finola et al., (2007) and Giraldo et al., (2013) reported no or low values ($< 1.0 \times 10^1 \text{CFU.g}^{-1}$) of molds and yeasts for respectively Moroccan, Portuguese, Argentinian and Colombian honeys.

However, Róžańska and Osek, (2012) from Poland, Mahmoudi et al., (2016) from Iran, Rall et al., (2003) from Brazil, Erkan et al., (2015) from Turkey, Ummulhair, (2014) from Nigeria and other authors reported a higher counts of molds and yeasts (Table 3).

CONCLUSION

At the end of this study, it was observed that *Euphorbia resinifera* honey has an acceptable microbiological profile. In fact, none of the analyzed sample contained any microorganisms that have an impact on human health. Additionally, the low levels of microbial contamination associated to a very low rate of mold and yeast indicate that this honey does not undergo any significant degradation and, therefore, this product always keeps its commercial quality. However, it is has to be emphasized the importance of continuous monitoring throughout the honey processing, to ensure the marketing of a reliable food. It is also recommended for governments and producers, to ensure a continuous control and to set up specification conditions during storage (moderate temperatures, increased humidity, granulation of the honey and elevated yeast counts).

Finally, standardization, by national and/or "PGI" specifications, of microbial contamination limits is very important to further improve the quality of honey, and ensure its sustainability over the years.

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REFERENCES

- AAZZA, S., LYOUSSI, B., ANTUNES, D., MIGUEL, M.G. (2014). Physicochemical characterization and antioxidant activity of 17 commercial Moroccan honeys, *Int. J. Food Sci. Nutr.* 65 (4), 449-457. <http://dx.doi.org/10.3109/09637486.2013.873888>
- ADENEKAN, M.O., AMUSA, N.A., LAWAL, A.O., OKPEZE, V.E. (2010). Physicochemical and microbiological properties of honey samples obtained from Ibadan. *J. Microbiol. Antimicrob.* 2(8), 100-104.[Online] Available in <http://www.academicjournals.org/JMA>
- ADOCK, D. (2016). The effect of catalase on inhibine and peroxide values of various honeys. *J. Apic.*, (1), 38-40.
- AGENCE DE DEVELOPPEMENT AGRICOLE (ADA), Commercialisation des produits du terroir, Maroc. (2016). [In French]. [Online] Available in: <http://www.irizar.ma/ada/web/produitlabelisedocument> (Accessed in October 14, 2016)

- ALVES, T. T. L., CARVALHO DOS SANTOS T.M., CAVALCANTINETO C.C., BEELEN R.N., MESQUITA DA SILVA S.G. COENTRO MONTALDO Y. (2015). Quality of honey sold in the state of Alagoas, Brazil. *Afr. J. Microbiol. Res.*, 9(27), 1692-1698. <http://dx.doi.org/10.5897/AJMR2015.7494>
- AL-WAILI N., SALOM K., AL-GHAMDI A., ANSARI M.J. (2012). Antibiotic, Pesticide, and Microbial Contaminants of Honey: Human Health Hazards. *Sci. World J.*, (9), 1-9. <http://dx.doi.org/10.1100/2012/930849>
- ANANIAS K.R., MACHADO DE MELO A.A., MOURA C.J. (2013). Analysis of moisture content, acidity and contamination by yeast and molds in *Apis mellifera* L. honey from central Brazil. *Braz. J. Microbiol.*, 44 (3), 679-683. <http://dx.doi.org/10.1590/S1517-83822013000300003>
- ANSES, (Agence Nationale de Sécurité Sanitaire de l'Alimentation, de l'Environnement et du Travail). Avis N° 2011-SA-0170, Etude initiale du guide de bonnes pratiques d'hygiène apiculture « production de miel ». 2012. [In French]. [On line], Available in <https://www.anses.fr/fr/system/files/MIC2011sa0170.pdf>. [Accessed in October 20, 2016].
- ANTÜNEZ, K., D'ALESSANDRO B., PICCINI C., CORBELLA E., ZUNINO P. (2004). *Paenibacillus* larvae larvae spores in honey samples from Uruguay: a nationwide survey. *J. Invertebr. Pathol.*, 86, 56-58. <http://dx.doi.org/10.1016/j.jip.2004.03.011>
- AYANSOLA A.A. 2012. Fungal Isolates from the Honey Samples Collected from Retail Outlets in Southwestern Nigeria, *Journal of Biology and Life Science*, 3 (1), 189-199. <http://dx.doi.org/10.5296/jbls.v3i1.1974>
- BENMEHDI H., BOUNOUA N., AMROUCH A., LAHCENE D., MAAZOUZI A. (2013). Phytochemical study, antioxidant, antimicrobial activities of *Euphorbia resinifera*, *Int.Res.J.Pharma.*, 4(9), 44-50. <http://dx.doi.org/10.7897/2230-8407.04910>.
- BETTAR I., GONZÁLEZ-MIRET M. L., HERNANZ D., MARCONI A., HEREDIA F.J., TERRAB A. (2015). Characterization of Moroccan Spurge (*Euphorbia*) honeys by their physicochemical characteristics, mineral contents and color. *Arab. J. Chem.* <http://dx.doi.org/10.1016/j.arabj.2015.01.003>.
- BOUHLALI E.E.T., BAMMOU M., SELLAM K., RAMCHOUN M., BENLYAS M., ALEM C., FILALI-ZEGZOUTI Y. (2016). Evaluation of antioxidant, antibacterial and antifungal activities of eleven monofloral honey samples collected from Morocco. *J. Chem. Pharm. Res.*, 8(3), 299-306. [Online] Available in <http://www.jocpr.com/articles/evaluation-of-antioxidant-antibacterial-and-antifungal-activities-of-eleven-monofloral-honey-samples-collected-from-moro.pdf>.
- CHAKIR, A., ROMANE, A., MARCAZZAN, G.L., FERRAZI, P. (2010). Physicochemical properties of some honeys produced from different plants in Morocco. *Arab. J. Chem.* <http://doi.org/10.1016/j.arabj.2011.10.013>
- CODEX ALIMENTARIUS COMMISSION. (2001). Revised Codex Standard for Honeys, Codex STAN FAO/WHO, 12-1981, Rev.1 (1987), Rev.2 .2001, 215 p.
- CROUSILLES A. (2014). Usages, propriétés antibactériennes et physicochimie de miels marocains, [In French]. Thèse, Diplôme d'État de Docteur en Pharmacie. [Online]. Available in https://www.academia.edu/5640875/Th%C3%A8se_exercice_pharmacie_Usages_propri%C3%A9t%C3%A9s_antibact%C3%A9riennes_et_physicochimie_de_miels_marocains.?auto=download. [Accessed October 15 2016].
- DUMAN AYDIN, B., SEZER, Ç., BILGE ORAL, N. (2008). Offered Pure Honey of Quality Sales in Kars Investigation of the Quality [In Turkish], *Kafkas. Üniv. Vet. Fak. Derg.*, 14 (1), 89-94.
- DÛMEN, E., AKKAYA, H., ÖZ, G.M., SEZGIN, F.H. (2013). Microbiological and parasitological quality of honey produced in Istanbul. *Turk. J. Vet. Anim. Sci.* 37(5), 602-607. <http://dx.doi.org/10.3906/vet-1301-14>
- ERKAN, M.E., VURAL, A., GURAN, H.S., DURMUSOGLU, H. (2015). Microbiological investigation of honey collected from Şırnak province of Turkey, *J. Hellenic. Vet. Med. Soc.*, 66(1), 3-8. [Online]. Available in <http://www.jhvms.com/sites/default/files/JHVMS-2015-651-IN-PRESS-ERKAN-ET-AL.pdf>
- EUROPEAN COMMISSION ACTS. (2013). Geographical indication from the Kingdom of Morocco C232/C. 13-16
- FEÁS X., PIRES J., IGLESIAS A., ESTEVINHO M.L. (2010). Characterization of artisanal honey produced on the Northwest of Portugal by

- melissopalynological and physico-chemical data. *Food and Chem. Toxicol.*, 48, 3462-3470. <http://dx.doi.org/10.1016/j.fct.2010.09.024>.
- FINOLA, M.S., LASAGNO, M.C., MARIOLI, J.M. (2007). Microbiological and chemical characterization of honeys from central Argentina. *Food Chem.*, 100 (4), 1649-1653. <http://dx.doi.org/10.1016/j.foodchem.2005.12.046>
- FLÉCHÉ, C., CLÉMENT, M.C., ZEGGANE, S., FAUCON, J-P. (1997). Contamination des produits de la ruche et risque pour la santé humaine : Situation en France [In French]., *Rev. sci. tech. Off. int. Epiz.*, 16(2), 609-616.
- GIRALDO, A.M.V.; VÉLEZ ACOSTA, L.M., ZULUAGA GALLEGU, R. (2013). Physicochemical and microbiological characterization of *Apis mellifera* sp. honey from Southwest of Antioquia in Colombia, *Ingeniería y Ciencia*, 9 (18), 61-74.
- GOMES, S., DIAS, G.L., MOREIRA LL, RODRIGUES, P., ESTEVINHO, L. (2010). Physicochemical, microbiological and antimicrobial properties of commercial honeys from Portugal. *Food Chem. Toxicol.*, 48, 544-548. <http://dx.doi.org/10.1016/j.fct.2009.11.029>.
- IGLESIAS, A.; FEÁS, X.; RODRIGUES, S.; SEIJAS, J. A.; PILAR VÁZQUEZ-TATO, M.; DIAS, L.G., ESTEVINHO, L.M. (2012). Comprehensive Study of Honey with Protected Denomination of Origin and Contribution to the Enhancement of Legal Specifications, *Molecules*, 17, 8561-8577. <http://doi.org/10.3390/molecules17078561>
- ISO, 21527-1, 2008. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection and Enumeration of Mold and Yeast. International Standards Organization, Switzerland, 2008.
- ISO, 4832:2006. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection and Enumeration of Coliforms – Most Probable Number Technique. International Standards Organization, Switzerland, 2013.
- ISO, 6579/A1-2007. Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection of *Salmonella* spp. International Standards Organization, Switzerland, 2007.
- ISO, 6888-1. (2003). Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection of *Staphylococcus aureus*. International Standards Organization, Switzerland, 2003.
- ISO, 7932, (2004). Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection of *Bacillus cereus*. International Standards Organization, Switzerland, 2004.
- ISO, 7937, (2004). Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection of *Clostridium perfringens*. International Standards Organization, Switzerland, 2004.
- ISO, 8433-1. (2013). Microbiology of Food and Animal Feeding Stuffs – Horizontal Method for the Detection of SPC. International Standards Organization, Switzerland, 2013.
- IURLINA, M.O., FRITZ, R. (2005). Characterization of microorganisms in Argentinean honey from different sources. *Int. J. Food Microbiol.*, 105 (3), 297-304. <http://dx.doi.org/10.1016/j.ijfoodmicro.2005.03.017>
- KAČANIOVÁ, M., MELICH, M., KŇAZOVICKÁ, V., HAŠČÍK, P., SUDZINOVA, J., PAVLIČOVA, S., ČUBOŇ, J. (2009). The indicator microorganisms value in relations to primary contamination of honey. *S.p.a.s.b.*, 42 (2), 159-163. <http://dx.doi.org/10.1007/bf02931394>
- KAMBA, A.S., HASSAN, L. G. (2010). Phytochemical screening and antimicrobial activities of Euphorbia balsamifera leaves, stems and root against some pathogenic microorganisms, *Afr. J. Pharm. Pharmacol.*; 4(9), 645-652. [Online]. Available in <http://www.academicjournals.org/ajpp>
- KUBO, Y., JINBO, K., KANEKO, S., MATSUMOTO, M. (1984). Prevalence of spore forming bacteria in commercial honey. *Ann. Rep. Tokyo Metr. Res. Lab. Public Health.*, 35, 192-196.
- KUNOVA, S., KAČANIOVÁ, M., HAŠČÍK, P., ČUBOŇ, J. (2015). Microbiological and chemical quality of slovak and european honey. *J Microbiol Biotech Food Sci.*, 4 (1), 41-44. <http://dx.doi.org/10.15414/jmbfs.2015.4.special1.41-44>
- LAMPEL, K. (2001) Shigella. In: Compendium of methods for the microbiological examination of foods. ed. Downes, F.P., Ito, K.. Washington: American Public Health Association. 381-385.
- MAHMOUDI, R, KIYANI, R, MOOSAVI, M, NOORIAN, R. (2016). Survey of Hygienic quality of honey samples collected from Qazvin province during 2011-2012. *Arch Hyg Sci.*, 5(1), 9- 14.
- MALIKA, N., MOHAMMED, F., CHAKIB, E.A. (2005). Microbiological and physico-chemical properties of Moroccan honey. *Int. J. Agr. Biol.*, 07 (5), 773-776.
- MCKEE, B. A., DJORDJEVIC, S. P.; GOODMAN, R D, HORNITZKY, M. A. Z. (2003). The detection of *Melissococcus pluton* in honeybees (*Apis mellifera*) and their products using a hemi-nested PCR. *Apidologie*, 34, 19-27. <http://dx.doi.org/10.1051/apido:2002047>
- MERCOSUL- MERCADO COMUM DO SUL. - Resolução nº 56, de 29 de setembro de 1999. Aprova o Regulamento técnico "Identidade e Qualidade do Mel. Montevidéu, (1999). [Online] Available in [http://www.mercosur.int/msweb/portal%20intermediario/Norma/Normas_web/Resoluciones/PT/Res_056_099_RTM%20Identidade%20Qualidade%20_99.PDF](http://www.mercosur.int/msweb/portal%20intermediario/Norma/Normas_web/Resoluciones/PT/Res_056_099_RTM%20Identidade%20Qualidade%20Ata%20_99.PDF). [In Spanish], [Accessed Novembre, 10, 2016]
- MIGDAL, W., OWCZARCZYK, H.B., KEDZIA, B., HOLDERNA-KEDZIA, E., MADAJCZYK, D. (2000). Microbiological decontamination of natural honey by irradiation. *Radiation Physics and Chemistry*, 57 (3-6), 285-288. [http://dx.doi.org/10.1016/S0969-806X\(99\)00470-3](http://dx.doi.org/10.1016/S0969-806X(99)00470-3)
- MOROCCAN LAW 28-07. (2010). Sécurité sanitaire des produits alimentaires, *Morr. Off. Bull.*, n° 5822, 18/03/2010, p214 [Online] Available in : <http://onssa.gov.ma/fr/images/reglementation/transversale/LOI.28-07.FR.pdf>. [In French], [Accessed Novembre, 10, 2016]
- MOROCCAN NORM (NM) 08.0.000. (2008), Norme Marocaine (NM) relative aux principes généraux d'hygiène alimentaire, homologuée par arrêté du ministre de l'industrie, du commerce et de l'artisanat n° 1774-95 du 23 moharrem 1416 », IMANOR, 2008. *Morr. Off. Bull.*, n°. 6036 [In French].
- MOROCCAN NORM (NM) 08.5.600. (2010). Norme Marocaine (NM) relative au Miel, » IMANOR. *Morr. Off. Bull.*, n°6063, p07. [In French].
- MOROCCAN AGRICULTURE ORDER N°624-04. (2004). Arrêté conjoint du Ministre de l'Agriculture et du Développement Rural, du Ministre de la Santé et du Ministre de l'Industrie, du Commerce et des Télécommunications relatif aux normes microbiologiques auxquelles doivent répondre les denrées animales ou d'origine animale, *Morr. Off. Bull.*, N°5214 du 20/05/2004. 2004[In French]. 727. <http://onssa.gov.ma/fr/images/reglementation/transversale/ARR.624-04.FR.pdf>.
- MOROCCAN ORDER, N°1721-12. (2012). Reconnaissance de l'IGP « Miel d'Euphorbe Tadla-Azilal » et homologation du cahier des charges y afférent (NOMACERT sarl). *Morr. Off. Bull.*, N°6074, 2542-2525.
- FRENCH NORM (FN) V 08 060, (2009). Dénombrement des coliformes thermotolérants par comptage des colonies obtenues à 44°C. 2009. [In French].
- PONTARA, L.P.M., CLEMENTE, E., OLIVEIRA, D.M., KWIATKOWSKI, A., CÁSSIAINÈS LOURENZI FRANCO, R., SAIA, V.E. (2012). Physicochemical and microbiological characterization of cassava flower honey samples produced by africanized honeybees, *Ciênc. Tecnol. Aliment. Campinas.*, 32(3), 547-552. <http://dx.doi.org/10.1590/S0101-20612012005000066>
- PUCCIARELLI, A.B., SCHAPOVAL, O.F.F., KUMMRITZ, S., SEŇUK, I.A., BRUMOVSKY, L.A., DALLAGNOL, M.E. A.M. (2014). Microbiological and physicochemical analysis of yateí (*Tetragonisca angustula*) honey for assessing quality standards and commercialization. *Rev. Argent. Microbiol.*, 46(4), 325-332.
- RAGAZANI, A.V.F., SCHOKEN-ITURRINO, R.P., GARCIA, G.R., DELFINO, T.P.C., POIATTI, M.L., BERCHIELLI, S.P. (2008). Clostridium botulinum spores in honey commercialized in São Paulo and other Brazilian states. *Cienc.Rural.*, 38(2), 396-399. <http://dx.doi.org/10.1590/S0103-84782008000200016>
- RALL, V.L.M., BOMBO A.J., LOPES T.F., CARVALHO L.R., SILVA M.G. (2003). Honey consumption in the state of Sao Paulo: a risk to human health? *Anaerobe*, (9), 299-303. [http://dx.doi.org/10.1016/S1075-9964\(03\)00121-5](http://dx.doi.org/10.1016/S1075-9964(03)00121-5).
- RIBEIRO E.P., SERAVALLI E.A.G. (2004). Química de Alimentos. São Paulo: Edgar Blücher. 184p.
- RÍOS F., SÁNCHEZ A.C., LOBO M., LUPO L., COELHO I., CASTANHEIRA I. (2014). A chemometric approach: characterization of quality and authenticity of artisanal honeys from Argentina, *J. Chemometrics*, 28 (12), 834-843. <http://dx.doi.org/10.1002/cem.2654>.
- RÓŽANŠKA, H., OSEK, J. (2012). Effect of storage on microbiological quality of honey. *Bull. Vet. Inst. Pulawy.*, 56 (2), 161-163. <http://dx.doi.org/10.2478/v10213-012-0029-x>
- SERIEIA, M.J., ANAUD DE TOLEDO, A.V., MARCHNINI, L.C., ALVES, E.M., FAQUINELLE, P., ARNAUD DE TOLEDO S.O. (2010). Microorganisms in organic and non-organic honey samples of Africanized honeybees, *J. Apic. Sci.*, 54 (1), 49-54.
- SHERWANI, S.K., SHAH M. A., ZUBAIR, A., HAROON, A., KAZMI S.U. (2013). Microbiological quality assessment of different commercially available honey products in Karachi, Pakistan, *ijapr*. 4 (3), 1531-1535. [Online] Available in http://ijapronline.org/admin/images/20130307_Muhammad%20Ajmal%20Shah%20%20%20%20et%20al%20IJAPR.pdf
- SNOWDON J.A., CLIVER D.O. Microorganisms in honey. *Int. J. Food Microbiol.*, 1996 (31), 1-26.
- SNOWDON JA. (1999). The microbiology of honey meeting your buyers specifications (Why they do what they do). *Am. Bee J.*, 1(1), 51-60.
- STENFORS, A.L.P., FAGERLUND A., GRANUM P.E. (2002). From Soil to Gut: *Bacillus cereus* and its Food Poisoning Toxins. *FEMS Microbiol. Rev.*, 32, 579-606. <http://dx.doi.org/10.1111/j.1574-6976.2008.00112.x>
- TERRAB, A., MARCONIB, A. BETTAR, I., MSANDAD, F., DÍEZ M.J., (2014). Palynological Characterization of *Euphorbia* Honeys from Morocco, *Palynology*, 38(1), 138-146. <http://dx.doi.org/10.1080/01916122.2013.871797>
- TYSSSET, C., FURANE, C., RROUSSEAU, M. (1980). Microbism and wholesomeness of commercial honey. *In: Apiacta*, (15), 51-60.
- UMMULKHAIR OSHOMAH, M. (2014). Microbiological Evaluation of Commercial Honey from Edo State, Nigeria, *ijser.*, 5(12), 796-799. [Online]. Available in <http://www.ijser.org/researchpaper/Microbiological-Evaluation-of-Commercial-Honey-From-Edo-State.pdf>

WHITE, J.W. (1975).Composition of honeys. In: Crane, E. (ed.), Honeys: A comprehensive survey. Heinemann, London UK,
WHITE, J.W., SUBERS, M.H., SCHEPARTZ A.L. (1962). The identification of inhibine. *American Bee J.*, 102, 430-1.