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Biological, physicochemical and antibacterial properties of pure honey harvested at the municipality of Seraïdi (Annaba, north east of Algeria)

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Abstract

The aim of our work is to assess physicochemical and antibacterial potential of two local honeys compared with two imported honeys. A carbohydrate profile was carried out by HPLC. All honeys are acid and the free acidity of Zriba (36 ± 13 méq.kg^{-1}), Sidi Achour (36.66 ± 0.57 méq.kg-1) were in standards well above those of imported honey: San Francisco (7 méq.kg^{-1}), Elshifa (20 méq.kg-1). Refractive index complied with the standards. Sidi Achour honey was denser (1.4206) than the others. Ash content of local honey (0.76 ± 0.07) and imported honey San Francisco (0.72 ± 0.57) were higher than those of Zriba honey (0.41 ± 0.08) and Elshifa (0.25 ± 0.038). All honeys had a sucrose content within the standard. Local honey contained Trehalose and melezitose, and richer in fructose and raffinose, F & G and maltose levels were consistent with standards. Sidi Achour contained the highest turanose content (2.15 ± 0.49%) relative to the studied honey samples. Antibacterial activity showed that all honeys have antibacterial potential when they are pure. Sidi Achour was active against *E.coli* (24 ± 6.08 mm), *S.enteritidis* (26.33 ± 1.15 mm), *S.aureus* (19.66 ± 0.57 mm), *B.cereus* (13.33 ± 8.73 mm) and *E.faecalis* (15 ± 1 mm). Zriba honey showed the same antibacterial honey except for *B.cereus* (7.66 ± 2.88 mm). The imported honeys were active on the growth of only three bacteria: *E.coli*, *S.enteritidis* and *S.aureus*.

Keywords: local honey; carbohydrate profile; antibacterial activity; Séraïdi; Annaba; Algeria.

Practical Application: This study shows the importance of consuming organic mountain honeys as they provide better protection against certain strains of bacteria and therefore have better biological activities.

1 Introduction

Honey is a natural substance produced out of honeydew or nectar from flowers (Buba et al., 2013), that bees are foraging, convert by combining them with specific substances that they secrete themselves, lay, dehydrate, store and let it improve and ripen within the hive combs (Codex Alimentarius, 2001a). Honey is a supersaturated sugar solution, its content is complex and variable and contains at least 181 different substances (El Sohaimy et al., 2015; Bucekova et al., 2019), this solution is made up of approximately 83% sugars, chiefly glucose and fructose and about 17% water at an average 3.9 pH (Almasaudi et al., 2017). Honey derived through nectar have a pH between 3.5 and 4.5 whereas those produced from honeydew range from 5 to 5.5 (Mbogning et al., 2011). The proportion of the various sugars depends on the kind of flowers foraged by the bees (Feknous et al., 2022). Honey also contains minor components like protein, enzymes, amino acids, lipids, vitamins, phenolic acids, flavonoids and minerals (Zhou et al., 2013; Feknous & Boumendjel, 2022). Based on Brudzynski & Lannigan (2012) and Gregório et al. (2021), the composition the quality of honey varies according to the vegetal species and the environmental conditions. Under the Codex Alimentarius (2001b), the study of the physicochemical parameters makes it possible to verify its quality and its botanical origin. Several physicochemical parameters are checked: The sugar and moisture content, the ash content, electrical conductivity, hydroxy methyl furfural content (HMF); acidity and diastase activity (Silva et al., 2016). Honey is an outstanding antibacterial agent with a high biotechnological potential (Gregório et al., 2020) and is a great dietary supplement (Pasupuleti et al., 2017). It is also used as a natural sweetener in probiotic food since it allows driving growth of lactic bacteria while concomitantly blocking growth of pathogenic bacteria like *Shigella, Listeria monocytogenes* and *Staphylococcus aureus* (Feknous et al., 2022). For millennia, popular medicine uses honey to relieve aches (Desmoulière, 2013). Its structure gives it - in addition to nutritional and energy attributes- some antioxidant, antibacterial properties (Missio da Silva et al., 2016) as well as an anti-hypertensive potential (Erejuwa et al., 2012) and hepatoprotective (Al-Waili et al., 2006). This natural

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product has an inhibitory effect against a broad spectrum of Gram+, Gram- bacteria and against antibiotic-resistant bacteria (Hammond et al., 2016). The antibacterial potential of honey arise from its physicochemical properties (Hegazi et al., 2017): low pH, high viscosity, high osmotic pressure (Kwakman and Zaat, 2012), hydrogen peroxide (Nolan et al., 2019), organic acids, enzymes (amylase, catalase, glucose-oxydase and lysozyme), phenols, fatty acids, acids (ascorbic acid, benzoic acid), flavonoids, vitamins, carotenoids (Wasihun & Kasa, 2016;Oluwapelumi et al., 2017; Dinkov, 2017) and to the presence of defensin and methylglyoxal (Martinotti and Ranzato, 2018). For this purpose, we have studied the biological, physicochemical quality and the antimicrobial potential of four (04) honey samples: two (02) local honeys (called ***Zriba*** honey and ***Sidi Achour*** honey) and two (02) imported honeys (called ***San Francisco*** and ***Elshifa*** honeys). Both local ones come from the mountain municipality of Séraïdi located in the city of Annaba (Algeria). Both imported honeys are available in the Algerian market.

2 Material and methods

2.1 Sampling

The two local honey (***Zriba*** and ***Sidi Achour***), have been harvested in Edough Mountains in the municipality of Séraïdi, city of Annaba at the north east of Algeria. One was collected at high altitude (***Zriba*** honey), in autumn of year 2018, the other one in low altitude (***Sidi Achour*** honey in the summer of that same year. As to the two honeys available in the market, they are imported from King Saoudi Arabia (***Elshifa***) and Spain (***San Francisco***). The collected samples were stored in sterile polyethylene bottles at + 4 °C.

2.2 Locating apiaries

The beekeeping operator has two sites in the municipality of Séraïdi as shown in the map below (Figure 1).

- The first site at high altitude, ***Zriba*** (36°56'7.51" N; 7°40'53.41" E), is located in the forest in the massif of Edough far from any major urban area, at 05 km by road from the village of Séraïdi on a local winding road i.e. at more than 2 km as the crow flies from the nearest urban community, away from any industrial area.
- The second site is at low altitude, ***Sidi Achour*** (36°52'16.71" N ; 7°42'7.58" E), at the foothills of Mountain Edough, in a forest zone of Eucalyptus at around 850 m west of the urban area of Sidi Achour.

2.3 Plant inventory

A plant inventory has been drawn up on from April 2021 to March 2022 for the region surrounding the bees' foraging area

Figure 1. Apiaries localization in Seraïdi.

over a radius of 2 km. A listing was set up chiefly for flowering plants known for their melliferous power. To do so, identification of plants was done based on the Herbarium of de Bélair (2019).

2.4 Analysis of physicochemical quality of honey

Dosage of sugars

Determining sugars of the two local honeys was achieved by high-performance liquid chromatography (HPLC) at the analyses and beekeeping ecology laboratory C.E.T.A.M in France. The study of the other physicochemical parameters of honey was achieved at the Quality control and fraud prevention laboratory. All of the physicochemical parameters were performed in three (03) repetitions.

Free acidity

A few drops of phenolphtalein ($C_{20}H_{14}O_4$ à 1%) were added to 10 gram of honey previously dissolved in 75 mL of distilled water in a beaker. We fill the burette with a sodium hydroxide solution NaOH at 0.1 N and adjust itto zero. The sample is titrated with NaOH at 0.1 N until we obtain a permanent pink color. The result is expressed in milliequivanlents per kilogram of honey and determined by the following Formula 1:

$$Free\ acidity\ =\ \left(Volume\ of\ 0.1\ N\ NaOH\ in\ ml\right)\ x\ 10 \quad (1)$$

pH

pH of honey was measured with a pH-meter with an immersion probe.

Electrical conductivity at 20 °C

Electrical conductivity of honey is measured by way of a conductivity meter. The result of which is expressed in millisiemens per centimeter ($mS.cm^{-1}$).

Brix level

Brix and refractive index were measured using ABBE universal refractometer.

Water content

Water content is established by referring to the official journal (Journal Officiel de la République Algérienne, 2017), which spells out the relationship between the refractive index and the water content.

Ash content

Ash contents were sought according to Journal Officiel de la République Algérienne (2017) in the following way: In a capsule, we drop 5 Gram of honey and a quantity of alcohol. We put the assays in a muffle furnace during 2hours at 500°C, then we cool them down in the desiccator (capsules containing ashes are weighed). The total ash content (W_{TA}) is expressed in weight percent in line with the Equation 2:

$$W_{TA} = (m3 - m_1 / m_2 - m_{1)} x\ 100\% \quad (2)$$

Where $\mathbf{m_1}$: weight in Grams of empty capsule; $\mathbf{m_2}$**:** weight in Grams of the capsule and obtained residues after steaming.

In order to find out on a moisture-free basis, the result is multiplied by [100% / 100% - **C**] where **C** is water content expressed in percentage.

Density

Honey density was calculated according to the following equation [**D=D2/D1**] where **D1** = the weight of pycnometer with distilled water – the weight of empty pycnometer; **D2** = The weight of pycnometer with honey – the weight of empty pycnometer.

2.5 Analyzing the antibacterial power of honey

The antibacterial activity of four honey samples was analyzed by the diffusion method. Gram+ and Gram- pathogenic bacteria were tested, two (02) food-borne bacteria: *Salmonella enteritidis and Enterococcus feacalis* supplied by the regional veterinary laboratory of El-Tarf along with three (03) reference strains supplied by The Pasteur Institute in Algiers: *Staphylococcus aureus* ATCC 25923, *Bacillus cereus* ATCC 11778 and *Escherichia coli* ATCC 25922.

Preparing honey dilutions

For each type of honey, four (04) dilutions were prepared with sterile distilled water in order to get the following concentrations: 25% honey, 50% honey, 75% honey and 100% honey.

Diffusion method in agar medium

Preparing inoculums and seeding

In an effort to prepare a bacterial suspension, we extract some well-isolated and identical colonies from each 24 h pure culture that we seed in 5 to 10 mL of sterile physiological water at 0,9%. The prepared homogenized bacterial suspension must have an opacity that equals 05 Mac Farland or an OD of 0.08 to 0.10 read at 625 nm. Seeding must be achieved within the 15 min following the preparation of inoculums. We dip a sterile cotton swab in the bacterial suspension. We wring it out by firmly pressing it against the inner wall of the tube so as to dump as much as possible and we rub out all of the dry agar surface up and down while streaking down. We repeat this operation twice, by rotating the box each time at 60°. In case we seed several Petri dishes, the cotton swab must be reloaded every time. We apply four (04) sterile discs on each seeded agar. We add 25 µL of each honey dilution to every disc and incubate the boxes at 37 °C during 24 hours in a bacteriological incubator.

Interpretation

After incubating, the diameters of inhibition areas were measured using a caliper.

3 Results and discussion

3.1 Plant inventory of the region

The Table 1 below lists the melliferous species discovered.

As to the honey of organic quality, we have carried out a plant inventory of *Edough* where lies the beekeeping operation yard *Zeriba*. We could pinpoint 111 melliferous species; a very significant source of nectar and pollen. These results are identical to our precedent study (Feknous et al., 2022). Our results are very close to Hamel & Boulemtafes (2017) who identified 107 species in the same region. This number of melliferous plants is higher than that of the study performed in the Algerian South-west by Laallam et al. (2011) in the light of which 66 melliferous species were spotted. The obtained results are encouraging. This can be explained by the appropriate location of the beekeeping yard. The latter, thanks to its vegetation criteria complies with the

Table 1. Plant inventory of the sampling area.

Common name	Latin name
Wild leek	*Allium ampeloprasum*
Rosy garlic	*Allium roseum*
Three-cornered leek	*Allium triquetrum*
Mauritania grass	*Ampelodesmos mauritanicus*
	Anchusa aggregate
Garden anchusa	*Anchusa azurea*
Strawberry tree	*Arbatus unedo*
Absinthe wormwood	*Artemisia absinthium*
Wild asparagus	*Asparagus acutifolius*
Common wild oat	*Avena fatua*
Common daisy	*Bellis perennis*
Borage	*Borago officinalis*
Big quaking grass	*Briza maxima*
Quaking grass	*Briza media*
Field marigold	*Calendula arvensis*
Thorny broom	*Calycotome spinosa*
Clustered bellflower	*Campanula glomerata*
Distaff thistle	*Carlina gummifera*
	Catananche coerulea
Honeywort	*Cerinthe major*
Chamomile	*Chamaemelum nobile*
Garland chrysanthemum	*Chrysanthemum coronarium*
Common chicory	*Cichorium intybus*
Montpellier cistus	*Cistus monspeliensis*
Sage-leaved rock-rose	*Cistus salviifolius*
	Clematis cirrhosa
Old man's beard	*Clematis vitalba*
Cleonia	*Cleonia lusitanica*
Mallow bindweed	*Convolvulus althaeoides*
Field bindweed	*Convolvulus arvensis*
Dwarf morning glory	*Convolvulus tricolor*
Azarole	*Crataegus azarolus*
Midland hawthorn	*Crataegus laevigata*
Artichoke thistle	*Cynara cardunculus*
	Cynoglossum creticum
Common broom	*Cytisus scoparius*
Wild carrot	*Daucus carota*
	Dipsacus follonum
Southern globethistle	*Echinops ritro*
	Echium Horridum
Purple viper's-bugloss	*Echium plantagineum*
Couch grass	*Elytrigia repens*
Redstem filaree	*Erodium cicutarium*
African valerian	*Fedia cornucopiae*
Fennel	*Foeniculum vulgare*

Table 1. Continued...

Common name	Latin name
Common fumitory	*Fumaria officinalis*
Purple milk thistle	*Galactites tomentosa*
Aulaga	*Genista scorpius*
Dovesfoot geranium	*Geranium molle*
Wood cranesbill	*Geranium sylvaticum*
Sword lily	*Gladiolus communis*
Spotted rock-rose	*Helianthemum guttatum*
Heliotropium europaeum	*Heliotropium europaeum*
Hare's-tail	*Lagurus ovatus*
Red dead-nettle	*Lamium purpureum*
Topped lavender	*Lavandula stoechas*
Narrowleaf flax	*Linum bienne*
	Lonicera implexa
Birdsfoot deervetch	*Lotus corniculatus*
Gypsywort	*Lycopus europaeus*
Blue pimpernel	*Lysimachia foemina*
Common mallow	*Malva sylvestris*
White horehound	*Marrubium vulgare*
Yellow sweet clover	*Melilotus officinalis*
Pennyroyal	*Mentha pulegium*
Hairy mint	*Mentha villosa*
Common myrtle	*Myrtus communis*
Watercress	*Nasturtium officinale*
Basil	*Ocimum basilicum*
European olive	*Olea europea subsp europea*
Spiny restharrow	*Ononis spinosa*
African wood-sorrel	*Oxalis pes-caprae*
Blindeyes	*Papaver dubium*
Common poppy	*Papaver rhoeas*
Yellow bartsia	*Parentucellia viscose*
Pellitory-of-the-wall	*Parietaria officinalis*
Mastic	*Pistacia lentiscus*
Ribwort plantain	*Plantago lanceolata*
Sea radish	*Raphanus raphanistrum*
White mignonette	*Reseda alba*
Bridal broom	*Retama monosperma*
	Retama raetam
Evergreen rose	*Rosa sempervirens*
Rosemary	*Rosmarinus officinalis*
Blackberry	*Rubus fruticosus*
Wild clary	*Salvia verbenaca*
Big yellow thistle	*Scolymus grandiflorus*
Spanish oyster thistle	*Scolymus hispanicus*
Caterpillar-plant	*Scorpiurus vermiculatus*
	Silene colorata
Common catchfly	*Silene gallica*
Cardus marianus	*Silybum marianum*
Charlock mustard	*Sinapis arvensis*
Sarsaparille	*Smilax aspera*
	Solenopsis bicolor
French honeysuckle	*Sulla coronaria*
Black bryony	*Tamus communis*
Common dandelion	*Taraxacum officinale*
Deadly carrots	*Thapsia garganica*

Table 1. Continued...

Common name	Latin name
Conehead thyme	*Thymus capitatus*
Hop trefoil	*Trifolium campestre*
Red clover	*Trifolium pretense*
White clover	*Trifolium repens*
Roman nettle	*Urtica pilulifera*
Scallop-leaved mullein	*Verbascum sinuatum*
Scallop-leaved mullein	*Verbascum sinuatum*
	Vicia peregrina
Common vetch	*Vicia sativa*
Bush vetch	*Vicia sepium*

requirements put out by European Union (2021) and United State Department of Agriculture (2017a, b) for bee-keeping and the production of honey of organic quality. Indeed, many of these meliferious plants are also considered as aromatic and medicinal one's **(**Hamel et al., 2018**;** Taibi et al., 2018**;** Boumendjel et al., 2021**;** Rekioua et al., 2022**)**, thus adding quality to the honey harvested.

3.2 The carbohydrate Profile of honey

The dosing results of the above-mentioned honey sugars are included in table # 2 below.

Monosaccharides

The average contents of fructose and glucose (Table 2) are higher than those of other identified monosaccharides (isomaltose, maltose, trehalose, turanose, erlose, melezitose and raffinose). Lobreau-Callen et al. (2000) point out that honey contains higher glucose and fructose contents vis-à-vis the other monosaccharides. According to Shin & Ustunol (2005) fructose has a slight prominence than glucose in honey, indeed, fructose was at 33.95 ± 0.21% in ***Zriba*** honey and at 36.6 ± 0.42% in ***Sidi Achour*** honey, at 38.8 ± 0.2% in ***Elshifa*** honey whereas at 40.5 ± 0.5% in ***San Francisco*** honey. These rates are slightly higher than those found in glucose which respectively are at: 33.46 ± 0.42%, 24.1 ± 2.4%, 34.2 ± 2% and at 30.8 ± 0.2%. Total sugars levels (F+G) fluctuated from 59.05 ± 0.45% to 60.7 ± 3.25% for local honey and from 71 ± 0.7% to 73 ± 2.2% as far as imported honey are concerned.

The Codex Alimentarius (2001a) restricts at 60% total fructose and glucose, we notice that imported honey exhibited a higher total on this limit unlike local honey which remained consistent with the international standards.

F/G ratio displays the characteristics of some honeys. This ratio was at 1.35 ± 0.01 for ***Zriba*** honey and 1.52 ± 0.12 for ***Sidi Achour*** honey. The latter is richer in fructose **(**Polus, 2008**)** relative to that of ***Zriba***. As to imported honey, their F/G ratio was lower than that of local honey (1.13 ± 0.017 for the Saudi honey ***Elshifa*** and 1.31 ± 0.01 for the Spanish honey ***San Francisco***).

Disaccharides

The sucrose content in local honey was lower at 1% against 3.9 ± 0.6% in ***Elshifa*** honey and at 4.3 ± 0.3% in ***San Francisco*** honey. All honey (local or imported) meet the recommendations established by the ***Codex Alimentarius*** which sets a maximum limit of 5% for any type of honey and 10% for Eucalyptus honey because a high sucrose rate indicates that bees have been fed with sucrose syrup, or an early honey harvest, in which the sucrose has not been fully transformed into glucose and fructose. According To Cavia et al. (2006), maltose content was considerably greater than sucrose content. Based on the same author, when honey are pure, they often contain 2 to 3 times more (even 10 times more) of maltose than sucrose. The average maltose content found in ***Zriba*** honey was 0.6 ± 0.14% whereas in ***Sidi Achour*** honey it was 0.35 ± 0.21%. These contents are stronger than those of Sucrose which were 1% lower (Table 2). Maltose contents in the two imported honey (***El Shifa*** and ***San Francisco***) were respectively 2.8 ± 0.2% and de 2.4 ± 0.4%, well below sucrose contents.

Zriba and ***Sidi Achour, EL Shifa*** and ***San Fransisco*** honey exhibited respectively different isomaltose rates 1.35 ± 0.07%, 0.95 ± 0.49%, 1.1 ± 0.2% and 1.2 ± 0.3%.

Trehalose was not present in the imported honey but identified in local honey (***Zriba*** and ***Sidi Achour***) at these respective contents 0.55 ± 0.07% and 0.7%. This disaccharide was spotted in Eucalyptus honey by Makhloufi et al. (2010).

Sidi Achour local honey contains in average the highest turanose content 2.15 ± 0.49% in relation with samples of the other studied honeys, then comes ***Elshifa*** honey with an average content of 2.02 ± 0.2%. ***Zriba*** local honey as well as ***San Francisco*'s** contained respectively: 1.8 ± 0.56% and 1.2 ± 0.2%.

Trisaccharids

The international regulation does not provide any standard for trisaccharids like erlose, raffinose and melezitose. The lowest erlose content was found in local honey ***Sidi Achour*** (0.1%), followed by ***Zriba*** honey (0.1%) with a 0,2% content, as to both imported honey, their content was 0,3% (Table 1). The carbohydrate profile showed that local honey ***Zriba*** (1.25 ± 0.77%) and ***Sidi Achour*** (0.7 ± 0.28%) carry higher raffinose contents than that of imported honey ***Elshifa*** (0.2 ± 0.02%) and some traces in ***San Francisco*** honey. Regarding melezitose, it was not detected in imported honey yet this trisaccharid was present in local honey at 0.6% in ***Zriba*** honey while 0.35 ± 0.07% in ***Sidi Achour*** honey (Table 1).

3.3 Physicochemical parameters:

The results of the various studied physicochemical parameters are broken down in Table 3.

pH

According to Mbogning et al. (2011), nectar honey have pH ranging from 3.5 to 4.5 while those of honeydew lie between 5 to 5.5. pH values of all studied honey were acid, the average value was 4.07 (***Elshifa*)**, 4.07 ± 0.12 (***Zriba***), 4.25 (***Sidi Achour***) and 4.06 ± 0.12 (***San Francisco***). Ibrahim Khalil et al. (2012) indicate that honey is naturally acid irrespective of its geographical origin. This is likely related to the presence of organic acids that contribute to its flavor and stability against microbial spoilage.

Free acidity

As per the Codex Alimentarius (2001a) free acidity must be lower than 50 méq.kg^{-1}, our honey samples showed a free acidity within the standards. All values were less than 50 méq. kg^{-1}. ***Sidi Achour*** honey displayed a slightly elevated free acidity (36.66 ± 0.57 méq.kg^{-1}) in comparison to ***Zriba*** honey which was 36 ± 13 méq.kg^{-1}, followed by ***Elshifa*** valued at 20 méq.kg^{-1}. The lowest value was ***San Francisco's*** (7 méq.kg^{-1}). Acidity of local honey was thus not artificially modified **(**Lobreau-Callen et al., 2000**;** Feknous et al., 2021**)**. Honey's natural acidity goes up when honey ages and whenever it is altered by fermentation (Schweitzer, 2004).

Electrical conductivity

According to Zerrouk et al. (2014), honey's electrical conductivity is closely associated with the concentration of mineral salts, organic acids and proteins. It is regarded as a high variability parameter based on the floral engine and one of the best differentiation parameters between all types of flowers honey and honey dew. ***Sidi Achour*** honey displayed a higher electrical conductivity value of (6.9 mS.cm^{-1}) than that of ***Zriba*** (5.66 mS.cm^{-1}). Imported honeys (***Elshifa*** and ***San Francisco***) appeared to present a lower electrical conductivity estimated at 2.74 and at 2.65 mS.cm^{-1}respectively. The maximum limit recommended by the European standards is 0.8 mS/cm (Official Journal of the European Communities, 2001).

The electrical conductivity is all the higher than honey is rich in ionisable substances like mineral matter (Lobreau-Callen et al., 2000).

Brix level

We notice that low altitude honey ***Sidi Achour*** contains a higher dry matter rate (80.7%) than that of the high altitude ***Zriba*** having a value of 78.83%. Both imported honey revealed

Table 2. HPLC profile of glucid percentage (n=3).

Sugars (%)	*Zriba*	*Sidi Achour*	Elshifa (Amri et al., 2007)	San Francisco (Amri et al., 2007)
Fructose	33.95 ± 0.21	36.6 ± 0.42	38.8 ± 0.2	40.5 ± 0.5
Glucose	33.46 ± 0.42	24.1 ± 2.4	34.2 ± 2	30.8 ± 0.2
Sucrose	<1	<1	3.9 ± 0.6	4.3 ± 0.3
Isomaltose	1.35 ± 0.07	0.95 ± 0.49	1.1 ± 0.2	1.2 ± 0.3
Maltose	0.6 ± 0.14	0.35 ± 0.21	2.8 ± 0.2	2.4 ± 0.4
Melibiose	NI	NI	NI	NI
Trehalose	0.55 ± 0.07	0.7 ± 0.0	NI	NI
Turanose	1.8 ± 0.56	2.15 ± 0.49	2.02 ± 0.2	1.2 ± 0.2
Erlose	0.2 ± 0	0.1 ± 0.0	0.3 ± 0.05	0.3 ± 0.03
Melezitose	0.6 ± 0	0.35 ± 0.07	NI	NI
Raffinose	1.25 ± 0.77	0.7 ± 0.28	0.2 ± 0.02	TRACES
F/G	1.35 ± 0.01	1.52 ± 0.12	1.13 ± 0.017	1.31 ± 0.01
F+G	59.05 ± 0.45	60.7 ± 3.25	73 ± 2.2	71 ± 0.7

Table 3. Physicochemical parameters for studied honeys (n=3).

Parameters	*Zriba*	*Sidi Achour*	*Elshifa*	*San Francisco*
Acidity	36 ± 13	36.66 ± 0.57	20 ± 0.0	7 ± 0.0
pH	4.07 ± 0.12	4.25 ± 0.0	4.07 ± 0.0	4.06 ± 0.12
Conductivity 20°C	5.66 ± 0.0	6.9 ± 0.0	2.74 ± 0.0	2.65 ± 0.17
Brix	78.83 ± 0.15	80.7 ± 0.60	80.33 ± 0.15	80.63 ± 0.11
Refractive index	1.48 ± 2.71	1.49 ± 0.001	1.49 ± 0.0003	1.49 ± 0.0002
Moisture content	19.4 ± 0.0	17.86 ± 0.57	17.93 ± 0.2	17.66 ± 0.11
Ash content	0.41 ± 0.08	0.76 ± 0.07	0.25 ± 0.038	0.72 ± 0.57
Density	1.4031	1.4206	1.4179	1.3934

a slightly lower Brix level to local honey: ***Elshifa*** (80.33%) and ***San Francisco*** (80.63%).

Density

Our samples provided varying density values ranging from 1.39 (***San Francisco***) to 1.42 (***Sidi Achour***). These density values are within the standards since for an average moisture content of 17.2% at 20 °C, the average density is 1.42 and usually varies from 1.39 to 1.44 depending on the type of analyzed honey (Lobreau-Callen et al., 2000).

Moisture content

Moisture content is closely linked to the quality of honey, its viscosity, its crystallization, its fermentation and its flavor (Nombré et al., 2010). According to Tchoumboue et al. (2001), high moisture contents arise from a premature harvest or a lack of stabilization of the postharvest produce. The average moisture content of ***Zriba*** honey was at 19.4%; ***Sidi Achour's*** was 18.00%, ***Elshifa*** 17.93% and ***San Francisco*** 17.66%. Moisture contents of our honey are 20% lower than the maximum limit recommended by the Codex Alimentarius (2001a).

Refractive Index

The two local honeys (***Zriba*** and ***Sidi Achour***) had respectively the following refractive indexes: 1.4880 and 1.4918. Imported honey showed the same average value of the refractive index rated at 1.49. These values remain within the standards since as per Lobreau-Callen et al. (2000); the refractive index varies with temperature and moisture content from 1.4915 to 1.5041 for moisture content from 13 to 18%.

Ash content

With regards to local honey, the ash content of the analyzed samples fluctuated from 0.41 (***Zriba***) to 0.76% (***Sidi Achour***). The ash content of the imported honey varied from 0.25% (***Elshifa***) to 0.72% Nanda et al. (2003), report that the standard threshold for ash content of nectar honey is 0.6%. The findings are consistent with the authorized limit set by the Codex Alimentarius (2001a). ***San Francisco*** honey and ***Sidi Achour*** honey registered respectively higher ash contents than the standard.

3.4 Antibacterial activity

The antimicrobial potential of honey depends on several factors like: floral source (Latifa et al., 2020), acid pH (Abdulrhman et al., 2013), high sugar concentration (Belhaj et al., 2015), Hydrogen peroxide (Chua et al., 2015), Methylglyoxal (Daniels et al., 2016), defensin (Ilyasov et al., 2012), phenolic acids (Kwakman & Zaat, 2012), flavonoids (Couquet et al., 2013), lysozyme (Bruneau, 2006) and volatile compounds (Abd El-Moaty, 2010).

The results of *in vitro* evaluation of antibacterial activity for local and imported honey samples in the presence of Gram+ and Gram – bacteria by the diffusion method in agar media are summarized in Table 4 below.

Al-Hasani (2018) have shown that diameters of inhibiting areas in bacteria diminish gradually as honey concentrations drop. ***Sidi Achour*** 100% pure honey had an inhibiting action

Table 4. Antibacterial activity for local and imported honey samples.

Honey at 100%	*Zriba*	*Sidi Achour*	*San Francisco*	*Elshifa*
Escherichia coli ATCC 25922	26.33 ± 1.52	24 ± 6.08	25.66 ± 4.16	26 ± 5.56
Salmonella enteritidis	26.33 ± 7.37	26.33 ± 1.15	29.33 ± 2.51	25.00 ± 2.00
Staphylococcus aureus ATCC 25923	20.33 ± 3.05	19.66 ± 0.57	15.33 ± 9.01	11.33 ± 6.65
Bacillus cereus ATCC 11778	7.66 ± 2.88	13.33 ± 8.73	8.33 ± 1.52	8.66 ± 2.30
Enterococcus faecalis	14.66 ± 3.51	15.00 ± 1	8.00 ± 2.00	7.66 ± 2.08
Honey at 75%	*Zriba*	*Sidi Achour*	*San Francisco*	*Elshifa*
Escherichia coli ATCC 25922	20.66 ± 1.15	17.00 ± 2.16	17.00 ± 2.00	22.00 ± 6.17
Salmonella enteritidis	19.66 ± 4.93	19.00 ± 1.63	21.33 ± 5.13	20.33 ± 6.15
Staphylococcus aureus ATCC 25923	15.00 ± 2.00	11.00 ± 0.81	12.33 ± 5.68	12.00 ± 6.08
Bacillus cereus ATCC 11778	6.33 ± 0.57	9.33 ± 4.71	6.00 ± 0.00	6.33 ± 6.21
Enterococcus faecalis	11.33 ± 4.16	11.33 ± 1.24	7.00 ± 1.73	6.66 ± 00
Honey at 50%	*Zriba*	*Sidi Achour*	*San Francisco*	*Elshifa*
Escherichia coli ATCC 25922	14.66 ± 3.78	11.33 ± 0.57	9.66 ± 3.21	17.66 ± 4.71
Salmonella enteritidis	11.33 ± 5.03	12.33 ± 3.05	13.00 ± 6.24	15.66 ± 2.35
Staphylococcus aureus ATCC 25923	10.66 ± 1.52	6.33 ± 0.57	8.66 ± 4.61	9.00 ± 2.44
Bacillus cereus ATCC 11778	6.00 ± 0.00	7.66 ± 2.88	6.00 ± 0.00	6.00 ± 0.00
Enterococcus faecalis	8.33 ± 4.04	9.00 ± 2.64	6.66 ± 1.15	6.00 ± 0.00
Honey at 25%	*Zriba*	*Sidi Achour*	*San Francisco*	*Elshifa*
Escherichia coli ATCC 25922	10.33 ± 5.13	7.33 ± 2.30	6.00 ± 0.00	12.33 ± 5.50
Salmonella enteritidis	7.00 ± 1.73	6.33 ± 0.57	9.00 ± 3.60	9.33 ± 4.93
Staphylococcus aureus ATCC 25923	6.33 ± 0.57	6.00 ± 0.00	6.00 ± 0.00	7.33 ± 2.30
Bacillus cereus ATCC 11778	6.00 ± 0.00	6.66 ± 1.15	6.00 ± 0.00	6.00 ± 0.00
Enterococcus faecalis	7.33 ± 2.30	7.66 ± 2.88	6.00 ± 0.00	6.00 ± 0.00

on the growth of all the tested strains, this inhibitive action was observed at 75% concentration of the same honey on *Escherichia coli*, *Salmonella enteritidis, Staphylococcus aureus* and *Enterococcus faecalis,* except *Bacillus cereus* (9.33 ± 4.71 mm).

At 50% concentration, this honey has no longer any inhibitive effect on the growth of Gram+ bacteria: *S. aureus, B. cereus* and *E. faecalis* which displayed respectively the following diameters: 6.33 ± 0.57 mm, 7.66 ± 2.88 mm and 9 ± 2.64 mm. At 25% concentration, all Gram- et Gram+ bacteria have given small diameters of around 7.33 ± 2.30 mm, 6.33 ± 0.57 mm, 6 ± 0 mm, 6.66 ± 1.15 mm and 7.66 ± 2.88 mm (Table 4). Overall, the obtained results show that almost all types of honey had an inhibiting effect on the growth of bacterial strains when their concentrations were high at 75% and at 100%. Many studies through the word demonstrated the antibacterial efficiency of honey (Bueno-Costa et al., 2016**)**. This effect is reduced when honey concentrations drop to 50% and at 25%. Indeed, ***Elshifa*** 100% pure honey had an inhibitive action on the growth of three bacteria (Table 4): *E. coli* (26.00 ± 5.56 mm), *Salmonella enteritidis* (25.00 ± 2.00 mm) and *S. aureus* (11.33 ± 6.65 mm). At 75% concentration, the measured diameters were respectively 22.00 ± 6.17 mm, 20.33 ± 6.15 mm and 12.00 ± 6.08mm. The reference strain *S. aureus* along with ***San Fransisco*** honey recorded greater diameters than those posted by ***Elshifa*** honey (15.33 ± 9.01 for 100% and 12.33 ± 5.68 mm for 75%). On ***Elshifa*** honey, these enterotoxigenic bacteria showed a diameter of 9.00 ± 2.44 mm at 50% concentration and a diameter of 7.33 ± 2.30 mm at 25% concentration of the same honey. Many authors outlined in their researches honey's antibacterial activity on staphylococci. According to Nagi et al. (2009), honey is an effective bactericide to fight against resistant bacteria like *S. aureus* methicillin-resistant **(SARM).** Grecka et al. (2018) found that honey produced by Polish apiaries can be used as an alternative agent for treating staphylococcal infections. Wadi & Geregandi (2020) demonstrated that the growth of this Gram+ was strongly inhibited after a topical application of honey on an infected wound by this bacterial pathogen. All of the tested honey (***San Francisco*, *Elshifa, Zriba*** and ***Sidi Achour***) had an inhibitive effect against this pathogenic germ to high concentrations (75% and 100%), and ***Zriba*** honey as well at 50%.

Bacillus cereus is a bacterium involved in food-borne diseases and food poisoning. It secretes several virulence factors like: Enterotoxins, hemolysins and phospholipases (Kilcullen et al., 2016**)**. In addition to the ground, we find microorganisms in the insects'intestines (Swiecicka & Mahillon, 2006), in the meals-ready-to-eat and rice (Altayar & Sutherland, 2006**)**. Based on our results, only the 100% concentrated ***Sidi Achour*** local honey had an inhibiting effect on this food-borne pathogen with a diameter of 13.33 ± 8.73 mm.

Enterococci are responsible of skin infections, soft tissues and surgical wounds in intensive care units (Arias & Murray, 2012**)**. *Enterococcus faecalis* was sensitive to two local honeys **(*Zriba*** and ***Sidi Achour***) at 100% and 75% concentrations.

Imported honey (***San Francisco*** and ***Elshifa***) did not impact its growth regardless of the tested concentrations.

Table 3 shows the inhibiting effect of imported honey (***San Francisco***) on the growth of *Salmonella enteritidis* with diameters 29.33 ± 2.51 mm (100%), 21.33 ± 5.13 mm (75%), 13.00 ± 6.24 mm (50%).

Salmonella is a food-borne bacterium, most involved in food intoxications worldwide (Eng et al., 2015; Rajan et al., 2017).

Food-borne infections caused by this disease-causing agent have not seen their number decrease during the last 15 years (Chen et al., 2017**)**. The two local honeys **(*Zriba* and *Sidi Achour*)** along with imported honey (***Elshifa* and *San Francisco***) had an inhibiting effect on this pathogenic bacterium in the three concentrations 50%, 75% and 100%. Our findings are similar to those that Hussain et al. (2015) came up with when testing Pakistani honey, by Sowa et al. (2017) with Polish honey, by Hegazi Ahmed et al. (2020) with honey coming from Saudi Arabia and by Cilia et al. (2020) with honey from Ukraine.

San Francisco honey concentrated at 25% and at 50% did not have any inhibitive effect on *E. coli* as opposed to the strong 75% and 100% concentrations.

Several researchers like Adebolu (2005), Sherlock et al. (2010), Voidarou et al. (2011), Belhaj et al. (2016), Hegazi et al. (2017), Matzen et al. (2018) and Hegazi Ahmed et al. (2020) revealed in their works the antibacterial potential of several honey against *E. coli.* The same thing was noticed in our study: All types of tested honey had an inhibitive effect on the growth of this Gram- in the three concentrations 100%, 75% and 50%. This germ was inhibited as well by both honey ***Zriba*** and ***Elshifa*** concentrated at 25%.

All of the tested Gram + and Gram- bacteria were sensitive to ***Zriba*** local honey concentrated at 100% and 75%, except *B. cereus* that showed respectively a diameter of 7.66 ± 2.88 mm and 6.33 ± 0.57 mm (Table 3).

At 50% concentration, ***Zriba*** local honey did not inhibit *B. cereus* and *E. faecalis* which disclosed a resistance with respectively diameters 6.00 ± 0.00 mm and 8.33 ± 4.04 mm.

This diluted honey at 25% seems to have an inhibiting effect only on: *E. coli* with a diameter of 10.33 ± 5.13 mm.

This diameter was greater than the other bacteria which recorded 7 ± 1.73 mm for *Salmonella enteritidis,* 6.33 ± 0.57 mm for *S. aureus,* 6.00 ± 0.00 mm for *B. cereus* and 7.33 ± 2.30 mm for *E. faecalis*.

4 Conclusion

Audit results enabled to assert that ***Zriba*** apiaries bring forward a very encouraging overall compliance before the prescriptive regulation of organic production used in reference thanks to its geographical situation and management approach. The physicochemical, organic quality along with an assessment of the antibacterial activity of the two local honeys harvested at the municipality of Séraïdi located at the North-East of Algeria and two imported honey available in the Algerian market were studied. The physicochemical parameters disclosed that all types of honey were acid, that the free acidity of local honey was within the standards and far greater to that of imported honey. The findings also showed that Brix values, refractive index, moisture content in all honey were compliant with the international standards. ***Sidi Achour*** honey was denser compared to the other analyzed honey. Ash content of the same local honey and that of imported honey ***San Fransisco*** were higher than that of ***Zriba*** and ***Elshifa*** honey. The carbohydrate profile showed indicated that all honey had sucrose content within the standards, that local honey contained trehalose and melezitose. These honeys were richer in fructose and raffinose and that the F+G rates and maltose were consistent with the standards relative to imported honey. ***Sidi Achour*** local honey contained the highest average content of turanose vis-à-vis the samples of the other honey. The study of *in vitro* antibacterial activity disclosed that both local honey **(*Zriba*** and ***Sidi Achour*)** together with the two imported honey (***Elshifa*** and ***San Francisco*)** had an inhibitive effect on: *Salmonella enteritidis, E. coli* at three concentrations 50%, 75% and 100%; *S. aureus* in two concentrations 75% and 100%; *E. feacalis* sensitive only to two local honey at 100% and 75% whereas imported honey did not impact its growth at the four tested concentrations. As to *B. cereus*, only 100% pure local honey ***Sidi Achour*** had an inhibitive effect on this food-borne pathogen with a diameter of 13.33 ± 8.73 mm. We can conclude that local honey were richer in sugars with physicochemical parameters within the standards, more active and evidenced an inhibitive effect against the tested germs in comparison with imported honey.

Conflict of interest

Authors declare that they have no conflict of interest.

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