Effect of heat treatment on antimycotic activity of Sahara honey

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Objective: To evaluate the influence of the temperature on honey colour, polyphenol contents and antimycotic capacity and to evaluate the correlation between these parameters.

Methods: Sahara honey were heated up to 25, 50, 75 and 100 °C for 15, 30 and 60 min, and their colour intensity, polyphenol contents and antimycotic capacity. The Folin–Ciocalteu test was used to determine the total polyphenol contents (TPC). The antimycotic activity was evaluated both by agar diffusion method and micro wells dilution method against the Candida albicans (C. albicans) and Candida glabrata (C. glabrata).

Results: Initial values for TPC in Sahara honey ranged from 0.55 to 1.14 mg of gallic acid per kg of honey, with the average value of 0.78 mg of gallic acid per kg of honey. The TPC values after heat–treatment were 0.54 to 1.54 with the average value of 1.49 mg. The minimal inhibitory concentrations before heat-treatment of Sahara honey against C. albicans and C. glabrata ranged from 3.06%–12.5% and 50% respectively. After heat-treatment the minimal inhibitory concentrations between 12.5% and 50% for C. albicans and C. glabrata, respectively. The diameters of inhibition zones of Sahara honey with 50% concentration varied from (12.67–15.00) mm by C. albicans to (14.33–15.67) mm by C. glabrata. The diameters of inhibition zones after heat-treatment at 25 and 50 °C for 15, 30 and 60 min ranged from (2.00–18.67) mm by C. albicans to (8.00–16.67) mm by C. glabrata. Statistically significant relations between the TPC and the colour intensity of Sahara honey (r=0.99, P<0.05). Furthermore, the results showed that the TPC and colour is not correlated with the antimycotic capacity.

Conclusions: To our knowledge this is the first report on the antimycotic capacity of Sahara honey.

KEYWORDS
Sahara honey, Antimycotic capacity, Heat treatment

1. Introduction

Yeast infections have emerged during the past two decades as important pathogens causing formidable morbidity and mortality in an increasingly diverse and progressively expanding population of immunocompromised patients. The therapeutic against yeast infections presents limitations: toxicity, high cost and resistance of fungal. The development of new antifungal agents becomes necessary. Therefore, bees products have been traditionally used as therapeutic agents and about half of the drugs that we use today are derived from natural sources. Honey has been used for its medicinal properties in many cultures since ancient times. The antimicrobial of honey have formed the basis of many applications including pharmaceuticals, alternative medicine and natural therapies[1–3]. Honey has been reported to contain about 600 substances and is considered as an important part of traditional medicine[4]. Several bioactive compounds have been identified in honey which contributed to its antimicrobial action[5]. Honey has antimicrobial effects, which are...
attributed to the osmotic effect of the substance’s sugars, its pH, and particularly its peroxidase activity [6]. Furthermore, heating honey inactivates the glucose oxidases. The antimicrobial effects are also due to the presence of non-peroxide substances such as phenolic acids, flavonoids, and lysozymes [7–9]. The non-peroxide antimicrobial activity is insensitive to heat and light [10]. Data concerning the effects of honey against different yeasts is limited from different parts of the world [11–13].

To date, no data is available on the antimycotic activity of Sahara honey samples from Algeria. This study has two goals: 1) to investigate the effects of thermal treatment on color intensity and polyphenols content of Sahara honey; 2) to determine antimycotic capacity of unheated and heat–treated honeys.

2. Materials and methods

2.1. Sample collection

Monofloral Sahara honey samples (n=3) from Apis mellifera were collected in 2012 from separate apiaries in the south of Algeria. The samples were stored at 4 °C until analysis in dark conditions.

2.2. Thermal treatment

Sahara honey samples were heated at 25, 50, 75 and 100 °C for 15, 30 and 60 min. Then they were cooled down to 4 °C by immediately plunging the tubes in an ice bath and analyzed. The samples were filled into watertight tubes.

2.3. Colour intensity: \(\text{ABS}_{720-450}\)

The net absorbance of the honey was determined by the method of Beretta et al [14]. The honeys were diluted to 50% (w/v) with warm (45–50 °C) milli Q water and the solution was filtered through a 0.45 µm filter. There was a complete absence of coarse particles in the honey solutions as all the commercial samples were no crystalline liquid honeys. The absorbance was measured using a spectrophotometer at 450 and 720 nm and the difference in absorbance was expressed as mAU.

2.4. Determination of total phenolic contents (TPC)

TPC was determined spectrophotometrically according to modified Folin–Ciocalteu [15]. A total of 30 µL of honey solution (0.1 g/mL) was mixed with 2.37 mL of milli Q water and 150 µL of 0.2 mol/L Folin–Ciocalteu reagent. The solution was thoroughly mixed by vortexing and incubated for 2 min at ambient temperature. A total of 450 µL of sodium carbonate solution (0.2 g/mL) was added to the reaction mixture and further incubated for 2 h at ambient temperature. The absorbance was measured at 765 nm using a spectrophotometer. The TPC was determined by comparing with a standard curve prepared using gallic acid (0–200 mg/L). The mean of at least three readings was calculated and expressed as mg of gallic acid equivalents (mg GAE)/100 g of honey.

2.5. Strains and growth conditions

Candida glabrata (C. glabrata) and Candida albicans (ATCC 10231) (C. albicans) were cultivated in Sabouraud dextrose agar (bioMerieux, Marcy-l’Etoile, France) and were incubated at 37 °C for 48 h. Colonies from 48 h cultures were suspended in 5 mL of a sterile–saline solution. The count of yeasts was adjusted to yield 1.5×10⁸ CFU/mL using the standard McFarland counting method.

2.6. In vitro antimycotic susceptibility assay

2.6.1. Screening of Sahara honey for antimycotic activity

Agar–well diffusion assay was used to evaluate the antifungal activities of the honey based on Ahmed et al [16]. Briefly, agar plates (90 mm) containing 20 mL of Sabouraud dextrose agar were inoculated using a swab from a suspension of each organism containing 1×10⁶ CFU/mL. An 8–mm diameter well was cut into the agar and 100 µL of 50% and 100% honey solution (w/v) prepared in sterile distilled water was aliquoted into the well. The controls were set up with equivalent quantities of water as controls. The plates were incubated at 37 °C for 48 h. Zones of inhibition were measured using a Vernier caliper (Draper). The antifungal potential of test compound was determined on the basis of mean diameter of zone of inhibition around the wells in millimeters. Each assay was performed in duplicate and repeated twice. The diameter of the inhibition zones: <5.5 mm, inactive; 5.5–9.0 mm, very low activity; 9–12 mm, low activity; 12–15 mm, average activity; and >15 mm, high activity.

2.6.2. Broth microdilution assay

The antimycotic activity of Sahara honey was examined by determining the minimal inhibitory concentration (MIC) using the macro dilution broth technique by Eloff [17]. Briefly, serial 2–fold dilutions of honey were inoculated with 10⁶ CFU/mL (final concentrations) for each of the microorganisms tested. Yeast suspension of approximately 5×10⁶ CFU/mL was inoculated into tubes containing honey.
at different dilutions and incubated at 37 °C for 48 h. All MIC values were expressed in percentage (%) (v/v). Bioassay was performed in duplicate and repeated twice.

2.7. Data analysis

Each honey was analyzed in triplicate. Results were shown as mean±SD. Correlations were established using Pearson’s correlation coefficient (r) in bivariate linear correlations (P<0.05). All statistical analyses were performed with the Statistica 7.0 software for windows.

3. Results

3.1. Total phenolic contents

TPC determined by Folin–Ciocalteu method was expressed as GAE mg/100 g of honey. TPC values of Sahara honey before and after heat-treatment were (0.551±0.002) to (1.140±0.000) GAE mg/100 g and (0.590±0.000) to (1.540±0.000) GAE mg/100 g of honey respectively (Figure 1 and Table 1).

3.2. Color intensity

The color intensity of the Sahara honey before and after heat–treatment ranged from (1.26±0.09) to (1.44±0.20) and (1.01±0.56) to (1.88±0.45) mAU respectively (Figure 1 and Table 2). Honey color depends on various factors for example: mineral content, ash content and botanical origins. Besides the floral and geographical origin, honey colour can also be affected by heat, time of storage.

3.3. Antimycotic capacity

The antimycotic activity results obtained from the macrodilution assays for Sahara honey samples are presented in Figure 2, Tables 3 and 4.

MIC was used to determine the lowest concentration of...
honey–in–water solution (w/v) at which the percentage inhibition is almost 100%[18,19]. The MIC before heat–
treatment of Sahara honey at 25 and 50 °C for 15, 30, 
and 60 min against C. albicans and C. glabrata ranged 
from (3.06% to 12.5%) and 50% respectively. After heat–
treatment the MIC ranging from 12.5% and 50% for C. 
albicans and C. glabrata, respectively. No inhibition of C. 
albicans growth was observed when honey at 75 and 100 
°C, and no inhibition of C. glabrata growth was observed 
when honey at 100 °C.

Table 4
Antimycotic capacity of heat–treated honeys at different temperatures for different heating times against C. glabrata.

| Honeys | Time (min) | 25 °C | 50 °C | 75 °C | 100 °C |
|--------|------------|-------|-------|-------|--------|
| SH1    | 15         | 12.5  | 12.5  | 12.5  | ND     |
|        | 30         | 12.5  | 12.5  | 12.5  | ND     |
|        | 60         | 12.5  | 12.5  | 12.5  | ND     |
| SH2    | 15         | 12.5  | 12.5  | 12.5  | ND     |
|        | 30         | 12.5  | 12.5  | 12.5  | ND     |
|        | 60         | 12.5  | 12.5  | 12.5  | ND     |
| SH3    | 15         | 12.5  | 12.5  | 12.5  | ND     |
|        | 30         | 12.5  | 12.5  | 12.5  | ND     |
|        | 60         | 12.5  | 12.5  | 12.5  | ND     |

Using agar well–diffusion assay, Sahara honey were 
tested for their ability to inhibit growth of C. albicans 
and C. glabrata. The results of the in vitro 
antimycotic capacity of Sahara honey determined by diameters of 
inhibition zones (DIZ) are presented in Figure 3. The DIZ 
produced by the undiluted honeys against C. albicans and 
C. glabrata ranged from (16.67±0.00) to (23.00±0.00) and 0 
mm respectively. The DIZ with 50% concentration varied 
from (12.67–15.00) mm by C. albicans to (14.33–15.67) mm 
by C. glabrata.

Figure 3. Means of zone of growth inhibition of Sahara honey before heat–
treatment.

After heat–treatment (at 25, 50 and 75 °C for 15, 30, 
and 60 min), the DIZ ranged from (2.00–18.67) mm by C. 
albicans to (7.33–16.67) mm by C. glabrata (Tables 5 and 
6).

Table 5
Means of zone of growth inhibition (mm) of Sahara at different temperatures for different heating times, using agar–well diffusion assays against C. albicans.

| Honeys | Time (min) | 25 °C | 50 °C | 75 °C | 100 °C |
|--------|------------|-------|-------|-------|--------|
| SH1    | 15         | 13.00 | 11.67 | 15.33 | ND     |
|        | 30         | 13.33 | 10.00 | 16.33 | ND     |
|        | 60         | 13.00 | 9.67  | 13.67 | ND     |
| SH2    | 15         | 13.33 | 10.67 | 17.33 | 2.00   |
|        | 30         | 18.00 | 11.00 | 16.33 | 6.00   |
|        | 60         | 18.00 | 9.67  | 12.33 | 9.33   |
| SH3    | 15         | 12.67 | ND    | 17.67 | 2.33   |
|        | 30         | 15.00 | ND    | 12.33 | 2.00   |
|        | 60         | 18.67 | ND    | 12.33 | 8.00   |

Table 6
Means of zone of growth inhibition (mm) of Sahara at different temperatures for different heating times, using agar–well diffusion assays against C. glabrata.

| Honeys | Time (min) | 25 °C | 50 °C | 75 °C | 100 °C |
|--------|------------|-------|-------|-------|--------|
| SH1    | 15         | ND    | 10.00 | 16.67 | ND     |
|        | 30         | ND    | 12.67 | 8.33  | ND     |
|        | 60         | ND    | 8.00  | 10.00 | ND     |
| SH2    | 15         | ND    | 8.67  | 14.67 | ND     |
|        | 30         | ND    | 12.33 | 8.33  | ND     |
|        | 60         | ND    | 8.00  | 10.33 | ND     |
| SH3    | 15         | ND    | 10.00 | 14.67 | ND     |
|        | 30         | ND    | 12.00 | 8.33  | ND     |
|        | 60         | ND    | 7.67  | 10.00 | ND     |

ND=not determined. Diameter of the inhibition zones: <5.5 mm, inactive; 5.5–9 mm, very low activity; 9–12 mm, low activity; 12–15 mm, average activity; and >15 mm, high activity.

4. Discussion

In many countries, some fungal infections have been 
treated with natural products remedies and also some 
pathogenic fungi are commonly resistant to many 
antibiotics. So, interests at antifungal properties of natural 
products from bee products particularly honey are very 
important. Several types of honey are produced in Algeria, 
where honey production is a traditional practice, well 
implanted in several regions. The Sahara region is located 
in the south of Algeria, where, due to its edaphoclimatic 
conditions and flora diversity, sidr and euphorbia are 
the principal honey types produced. A great number of 
research studies focused on the pharmacological and 
biological properties present by honey, including anti–
flammatory, antioxidative, antibacterial and antifungal 
activity among others[20]. Honey is an effective antifungal 
activity as shown by several studies. Large number of 
honeys from different geographical locations and different 
botanical sources show growth inhibitory action[21,22]. 
Limited numbers of studies have examined the activity of 
Algerian honey against fungi. In brief, Ahmed et al. found 
that the MIC of six varieties of honey against Rhodotorula 
mucilaginosa ranged between 28% and 36% (v/v)[23].
In our previous studies, Ahmed et al. was the first to report the antifungal effects of heat processing on the antifungal activity of honey (40%-45% C. albicans and 45%-50% Aspergillus niger[24,25]). Molan reported that honey loses its antimicrobial activity when subjected to heat or exposed to light[26]. Radwan et al. showed that heat exposure in a water bath at a temperature that was not clearly specified, but appeared to be 50 °C for 10 min, had an adverse effect on the antibacterial activity of honey[27].

Bogdanov tested the effect of heat on peroxide and non-peroxide antibacterial activity of blossom and honeydew honey against Staphylococcus aureus[28]. Heating at 70 °C for 15 min resulted in a decrease of the initial peroxide activity of blossom honey by 92% and of honeydew honey by 22%, whereas the non-peroxide activity of both honey types was decreased only slightly.

Several factors may influence the antifungal activity of honey, which include its botanical origin, aromatic acids, including benzyl cinnamate, methyl cinnamate, caffeic acid, cinnamyl cinnamate, cinnamoylglycine, terpenoids, and polyphenols[20]. In recent years, a number of studies have been reported on the antifungal activity of phenolic compounds from natural sources[29]. Polyphenols in honey considered a potential source of the antimicrobial properties of honey[30]. In the studied Sahara honeys, only very small amounts of polyphenols contents were present.

The literature data indicate that there is a strong positive correlation between honey color and total phenolic content. In the present study, relationship between phenolic content and colour of Sahara honeys (r=0.99) is possibly due to a highly extended conjugated systems, especially when complexed with minerals. Furthermore, in the present study, no relationship between phenolic content, colour and the antymycotic capacity of Sahara honeys was observed.

Resistance of microorganisms to antifungal drugs is becoming a growing concern worldwide. Natural products have been traditionally used in the treatment of fungal diseases because they are sources of many active compounds. Honey is a honeybee product known for its biological and pharmacological properties for centuries. It has been extensively used in traditional medicine and also in complementary medicine because of its antibacterial and antymycotic activities. This is the first study reporting the results of a representative screening of Sahara honeys for antymycotic capacity, deserving further investigation for clinical applications against fungal infections.

**Conflict of interest statement**

We declare that we have no conflict of interest.

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**Comments**

**Background**

Bees products attracted an increasing interest in many cultures since ancient times because of their medicinal properties. Thus, honey is used for its pharmacological activities, especially as antimicrobial agent. This activity is attributed to the osmotic effect of sugars substances, pH, peroxidase activity, phenolic acids, flavonoids, and lysozymes. The non–peroxide antimicrobial activity is insensitive to heat and light which forced scientists to search the thermal effect on honeys activities.

**Research frontiers**

In this work, authors evaluate the thermal effect on honey colour, polyphenol contents and antimycotic capacity and to determine the correlation between these parameters.

**Related reports**

Large number of honeys from different geographical locations and different botanical sources show growth inhibitory action. But, Ahmed et al. (2012) are the first to report the heat effects on the antifungal activity of honey. In another hand, the literature data indicate that there is a strong positive correlation between honey color and total phenolic content. Authors of this study indicate that there is no relationship observed between phenolic content, colour and the antymycotic capacity of Sahara honeys.

**Innovations & breakthroughs**

This study has explored for the first time of the influence of heat on colour, polyphenol contents and antimycotic capacity of honey Algerian Sahara.

**Applications**

It is very interesting to use honey from Sahara as antibacterial natural agent. The results of the present study is an interesting form of the valorization of local honeys and its possibility use in clinical applications against fungal infections.

**Peer review**

This is an interesting and valuable research work in which authors evaluated for the first time the influence of heat on
colour, polyphenol contents and antimycotic capacity of honey from Algerian Sahara. This article includes interesting scientific results in the valorization of local honeys as antimicrobial agent.

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