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# Influence of temperature on the inhibitory potency of Eucalyptus honey against *Candida albicans*

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## ABSTRACT

**Objective:** To evaluate the effects of heat processing on the antifungal activity of honey.

**Methods:** A sample of the honey of eucalyptus was divided into four portions of 250 g each. One of the four portions obtained from studied honey was not heated (not heated fraction 25°C), the other portions were placed in water bath during 24 hours at 40 °C, 60 °C and 80°C temperatures. The HMF rates, Acidity, pH and the index of refraction were determined by harmonized methods. The antifungal tests (Minimum Inhibitory Concentration) were carried out on Sabouraud agar medium embedded with honey according to dilution test. **Results:** The moisture shows values of 15.65% and 15.83%, pH between 4.10 and 4.24, the free acidity ranges between 33.8 and 38.36 meq kg<sup>-1</sup>, hydroxymethylfurfural (HMF) content shows values between 28.8 and 103.44 mg kg<sup>-1</sup>. The antifungal action of the non-heated fraction (Fc) of honey *in vitro* was marked 40 % (vol/vol) than heated fractions of honey (42%, 44%, and 45%) vol/vol. respectively The antifungal activity of each fraction decreased in the following order: Fct<sub>25</sub> > Fct<sub>40</sub> > Fct<sub>60</sub> > Fct<sub>80</sub>. **Conclusion:** our findings indicate that different levels of parameters physical-chemical properties of honey to different temperatures showed inhibitory activity against *C. albicans* with variable degrees.

## 1. Introduction

Candidosis is a mycosis that is currently increasingly affecting the population in consequence of its frequency and the severity of its complications, especially among immunocompromised hosts [1]. The *Candida* species most frequently isolated from clinical sources is *Candida albicans* [2]. *C. albicans* is a commensal yeast on the mucosal surfaces in most healthy individuals, but it becomes an opportunistic pathogen under conditions that permit it to adhere and colonize epithelial tissues, causing superficial, as well as, life-threatening disseminated infections [3].

The increase in resistance to antifungals and the slow delivery of new therapeutic options from the pharmaceutical industry have lead to various studies being carried out with the aim of examining the activity of natural products against fungi that cause infections, mainly in immunocompromised individuals [4,5].

Honey forms part of traditional medicine in many cultures [6], although it is most widely used as sweetener. It is

composed of at least 181 components and is basically a solution supersaturated in sugars, the fructose (38%) and glucose (31%) are the most important [7], the moisture content is about 17.7%, total acidity 0.08%, and ashes constitute 0.18% [8].

Hydroxymethylfurfural (HMF) content is one of the most important quality parameters of the quality and health safety of honey. HMF is formed during acid-catalyzed dehydration of hexoses. [9] It is used as an indicator of honey freshness [10] Codex Alimentarius (CA) [11] proposed two quality indicators for honey, namely, 5-hydroxymethylfurfuraldehyde and amylase (diastase) activity to measure the freshness of honey. Many countries have set the national limit for HMF content in honey to 40 mg/kg.

Several factors influence the formation of HMF in honey: temperature and time of heating [12], and the chemical properties of honey, which are related to the floral source from which the honey has been extracted, these indicate pH, total acidity, and mineral content [13].

Several types of honey are produced in Algeria, where honey production is a traditional practice, well implanted in several regions. The Tiaret region is located in the west of Algeria, where, due to its edaphoclimatic conditions and flora diversity, *Hedysarum coronarium*, *Eucalyptus camaldulensis* and *E. globuluss*, *Pimpinella anisum*, and *Trifolium alexandrinum* are the principal honey types

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produced, being Eucalyptus honey the most important unifloral one.

The present work had an aim; it was to detect which level of parameter physico–chemical properties value of honey had a potential effect on *Candida albicans*

## 2. Materials and methods

### 2.1. Honey sample

A sample of eucalyptus honey was divided into four portions of 250 g each. One of the four portions obtained for honey has not been studied heated (unheated portion 25 °C), but the other three were placed in a water bath for 24 hours at 40 °C, 60 °C, 80 °C. The four fractions of honey were examined immediately after heating for their moisture content, pH, acidity, HMF and antifungal Activity.

### 2.2. Physico–chemical analyses

All physicochemical tests were performed in duplicate.

#### 2.2.1. pH

Honey pH was measured, with a combined pH glass electrode connected to pH meter Basic 20, in a solution prepared with 10 g of honey in 75 mL of distilled water [14]

#### 2.2.2. Moisture content

The moisture content was determined based on the refractometric method. In general, the refractive index increases with the increase in the solid content. The refractive indices of honey samples were measured at ambient temperature using an Atago hand refractometer and the readings were further corrected for a standard temperature of 20 °C by adding the correction factor of 0.00023/°C. Moisture content was determined in duplicate and the % moisture content values corresponding to the corrected refractive index values were calculated using Wedmore's table [14]

#### 2.2.3. Free acidity

Free acidity was determined by potentiometric titration [14]. Honey samples were homogenized in a water bath and filtered through gauze, prior to analysis. Ten grams of honey were then dissolved in 75 mL of distilled water, and alcoholic solution of phenolphthalein added. The solution was titrated with 0.1 N NaOH. Acidity (milliequivalent of acid per kg of honey) was determined as 10 times the volume of NaOH used in titration.

#### 2.2.4. HMF

Hydroxymethyl furfural (HMF) was detected using a technique based on the method described by Winkler [15]. Five grams of honey were dissolved, without heating, in oxygen free distilled water and transferred to a 125 ml graduated flask and diluted to volume with oxygen free distilled water. Two millilitres of honey solution was pipetted into two tubes and 5 ml of P–tuidine solution was added to each. Into one test tube, 1 ml of water was pipetted and into the other 1 ml of barbituric acid solution was added; both mixtures were then shaken. Absorbance was read using a spectrophotometer against a blank at a wave length

of 550 nm. Calculation:  $Mg/100\text{ g hydroxymethyl furfural} = \text{absorbance/test} \times 192$  [16].

### 2.3. Yeast strain and growth conditions

*Candida albicans* (Institut Pasteur of Algiers) was maintained by subculture in specific media Sabouraud agar. The inoculum suspensions was obtained by taking five colonies (>1 mm diameter) from 24 old cultures grown on Sabouraud agar. The colonies were suspended in 5 mL of sterile saline water (0.85%). The inoculum suspensions were shaken for 15 s and density adjusted to the turbidity of a 0.5 McFarland Standard (equivalent to  $1-5 \times 10^6$  cfu/mL).

### 2.4. Minimum inhibitory concentration measurement (MIC)

Increased concentrations of honey (10–50 % vol/vol) were incorporated into media to test their efficiency against *Candida albicans*. Each plate with final volume of honey and media of 5 ml was inoculated and incubated at 37°C for 48 h. The MIC was determined by finding the plates with the lowest concentration of honey on which the strain would not grow. All MIC values are expressed in % (vol/vol). Were added to a range of honey concentrations lower than the MIC

## 3. Results

### 3.1. Physicochemical parameters

#### 3.1.1. pH

All fractions of honeys analyzed were found to be acidic in character their pH values ranged from 4.10 to 4.24 (Table1). In general, honey is acidic in nature irrespective of its variable geographical origin. The pH values of Algerian, Morocco and Portugal honeys have been found to vary between 3.49 to 4.53, 3.52 to 5.13, and 3.45 to 4.70, respectively [17–19]

#### 3.1.2. Moisture content

Honey moisture content depends on various factors such as harvesting season, degree of maturity reached in the hive and climatic factors [20]. Values between 15.56 g/100 g and 15.83 g/100 g were obtained. All fractions tested of honey had moisture contents below 20 g/100 g, which is the maximum prescribed limit as per the Codex standard for honey [21].

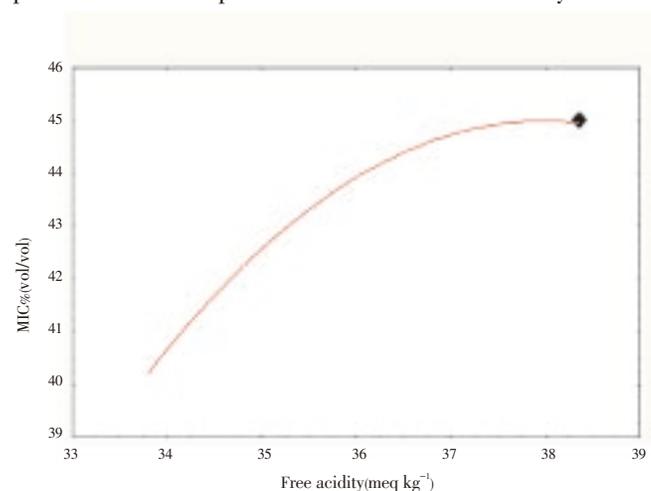
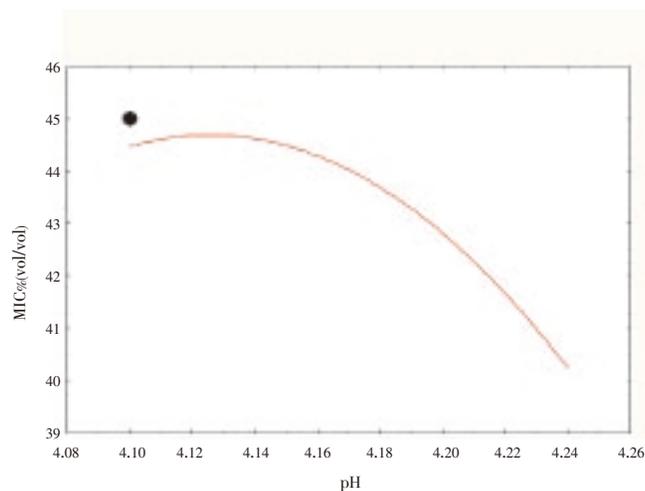


Figure 3. Variation of Free acidity / MIC % (vol/vol)

**Table 1.**Heat treatment, acidity, pH, and HMF and growth inhibition of *C. albicans* in different honey fractions.

Honey	pH	Moisture content%	Free acidity meq kg <sup>-1</sup>	HMF(mg /kg)	MIC% (vol/vol)
Ht25	4.24	15.83	33.8	28.8	40
Ht40	4.22	15.83	34.49	43.29	42
Ht60	4.11	15.65	36.25	78.32	44
Ht80	4.10	15.65	38.36	103.44	45

**Figure 1.** Variation of pH / MIC % (vol/vol)

### 3.1.3. Free acidity

Acidity affects the flavor and aroma of honey and is due to the presence of organic acids, particularly gluconic, pyruvic, malic and citric, in equilibrium with lactones or esters and inorganic ions [22]. The free acidity ranged between 33.8 meq kg<sup>-1</sup> and 38.36 meq kg<sup>-1</sup> were obtained. Values for free acidity were below the allowed limits (50 meq kg<sup>-1</sup>) [23].

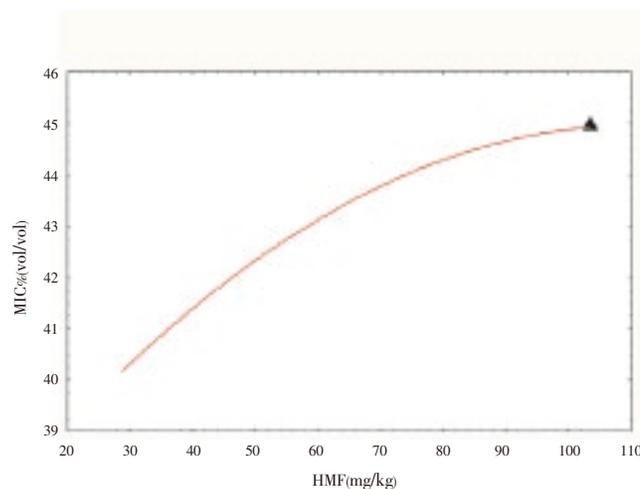
### 3.1.4. HMF values

The HMF content is widely recognized as a parameter of honey samples freshness, because it is absent in fresh honeys and tends to increase during processing and/or aging of the product. Several factors influence the levels of HMF, such as temperature and time of heating, storage conditions, pH and floral source, thus it provides an indication of overheating and storage in poor conditions [24]. HMF shows values between 28.8 and 43.29 mg kg<sup>-1</sup>, fractions 3 and 4 with values between 78.32 and 103.44 mg kg<sup>-1</sup> exceeded the limits set by European Community legislation [21] due to overheating.

## 3.2. Assay for inhibitory activity of honey on *C. albicans* growth

The different level of value for the four fractions of honey showed antifungal activity against *C. albicans* to varying degrees, the table (1).

HMF value of honey (28.8 mg kg) showed the highest inhibitory effect on yeast growth compared to treated fractions by heat (Figure 2). Similarly, free acidity value of honey (33.8 meq kg<sup>-1</sup>) showed the highest inhibitory effect on yeast growth compared to treated fractions by heat (Figure 3). Furthermore, pH value of honey 4.24 showed the highest inhibitory effect on yeast growth compared to treated fractions by heat (Figure 1).

**Figure 2.** Variation of HMF content / MIC % (vol/vol)

## 4. Discussion

In recent years, drug-resistance to antifungal agents and optimizing therapy of candidal infections has been broadly focused [22]. Honey is a natural product that is used for its antifungal activity [23–25]. Several factors may influence the antifungal activity of honey. For example, DeMera and Angert [26] reported that honey from different phytoecographic regions varied in their ability to inhibit the growth of yeasts, suggesting that botanical origin plays an important role in influencing the antifungal activity.

The high sugar concentration, hydrogen peroxide, and the low pH are well-known antibacterial factors in honey and more recently, methylglyoxal and the antimicrobial peptide bee defensin-1 were identified as important antibacterial compounds in honey. [27]. Hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects [28]. Factors known to affect H<sub>2</sub>O<sub>2</sub> accumulation are inactivation of the H<sub>2</sub>O<sub>2</sub>-producing enzyme glucose oxidase by exposure to heat or light [29,30] or degradation of H<sub>2</sub>O<sub>2</sub> by honey [31,32].

Mulu et al [33] studied the antifungal activity of honey in sensitivity tests on 25 strains of *Candida* yeasts and showed clear antifungal activity against yeasts tested. Furthermore, Khosravi et al [34] reported that honey had antifungal activity against *Candida* species such as *Candida albicans*, *C. parapsilosis*, *C. tropicalis*, *Candida kefyr*, *C. glabrata*, and *C. dubliniensis*. Al-Waili [35] found that honey concentration ranging from 30% to 50% inhibited the growth of several pathogenic microorganisms, including *C. albicans*. Ahmed et al [36–38] reported antifungal efficacy of various honeys against clinical isolates of *C. albicans*, *Rhodotorula sp* and *Aspergillus niger*. Collectively, our findings indicate that different levels of parameters physical-chemical properties of honey to different temperatures showed inhibitory activity

against *C. albicans* with variable degrees.

### Conflict of interest statement

We declare that we have no conflict of interest.

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