



## Evaluation of Polyphenols, Vitamin C Contents and Antioxidant Activity of Two Types of Algerian Honey

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### ABSTRACT

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This study aimed to estimate two different types of Algerian honey: antioxidant contents (total phenols and vitamin C), and levels of antioxidant (DPPH assay) and reducing activity (FRAP assay). Honey samples were characterized by high content of total phenols ( $314.231 \pm 281.346 - 394.231 \pm 155.835$  mg AG  $100\text{ g}^{-1}$ ) and low vitamin C values ( $0.25 \pm 0.05 - 0.35 \pm 0.05$  mg  $100\text{ g}^{-1}$ ). As a result, the antioxidant activity and reducing capacity values were found to be  $9.578 \pm 3.157$  and  $11.255 \pm 2.668\%$  for DPPH and  $15.240 \pm 4.578$  to  $17.794 \pm 8.179$   $\mu\text{g } 100\text{ g}^{-1}$  for the FRAP assay. Our data showed that dark honey contains bioactive compounds with significant antioxidant activity.

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## Introduction

According to da Silva et al. (2016) and Seraglio et al. (2019), honey is a nutritional complex of various molecules (over 200 substances) with varying proportions containing. The main components of honey are carbohydrates (glucose, fructose, sucrose). Also, it contains several trace constituents such as colorants, polyphenolic compounds, amino acids, vitamins (C and E), enzymes, organic acids and minerals (Djebli et al., 2021; Saxena et al., 2010). Some of these compounds are known as antioxidants, which can inhibit chain oxidation reactions that are responsible for various medical conditions such as atherosclerosis, cancer, cardiovascular disease, cataracts, muscle degeneration, impaired wound healing, and inflammatory diseases of the gastrointestinal tract (Al et al., 2009; Aljadi and Kamaruddin, 2004).

Presently, the antioxidant and antimicrobial properties of fungi are important research topics. The studies show various plants exhibits significant antioxidant activities such as *Hohenbuehelia myxotricha*, *Centaurea rigida*,

*Euphorbia eriophora* and *Echium italicum* (Akgül et al., 2022; Kina et al., 2021; Krupodorova et al., 2022; Uysal et al., 2021). Usually, antioxidant activity is assessed by the ability of antioxidants to maximally retard oxidation (Mohammed et al., 2022; Sevindik et al., 2017). To analyze this activity in honey, the analytical methods commonly used refers to the sample's reducing capacity ferric reducing antioxidant power (FRAP) tests as well as antiradical activity with the 2,2-diphenyl-1-picryl-hydrazyl (DPPH) radical scavenging assays (Khalil et al., 2011).

Phenolic compounds are gaining increasing interest given their beneficial functional and nutritional effects, these compounds extinguish the effects of free radicals and thus protect the human body against their damage (Cicerale et al., 2009). Several studies have shown that the antioxidant activity of honey comes mainly from its content of phenolic compounds (Alvarez-Suarez et al., 2013; Olas, 2020).

The appearance of honey is the first attractive attribute of honey, and as such is very important for commercialization. It is an important parameter in the quality, acceptance and preference of consumers (da Silva et al., 2016; Pauliuc et al., 2020). Honey color is an organoleptic parameter that varies by honey type and depends on chemical parameters such as mineral content and polyphenol content (González-Miret et al., 2005). Solayman et al. (2016) was proved that amber and dark honey have a higher content of certain minerals (Na, K, Ca, Mg, Fe, Cu, Zn, Al, Ni, Cd and Mn) by comparison to light-colored honey. According to Pauliuc et al. (2020), color is a parameter that is dependent on the botanical origin of honey and its values can be used to classify honey.

This study aims to evaluate the content of polyphenols, ascorbic acid (Vit. C) of two types amber and dark of Algerian honey from different localities. Also, the antioxidant activities were determined by DPPH and FRAP analyses.

## Methodology

Two different samples of honey from different region: Orange (H<sub>01</sub>) and black (H<sub>02</sub>) were collected in June 2021 from two Algerian regions El-Tarf and Djelfa for the realization of the following analyses:

- **Polyphenols:** Total phenols in honey samples were measured using spectrophotometry using the Folin-Ciocalteu reagent (Singleton, 1999). Absorbance was measured at 765 nm using a UV-Vis spectrophotometer. Levels of total polyphenols were expressed in gallic acid equivalents (GAE) in mg 100g<sup>-1</sup> samples according to the calibration curve.
- **Vitamin C (ascorbic acid):** The AA content was determined by means of Mau et al. (2005) method, the absorbance was measured at 515 nm.
- **DPPH:** To evaluate the antiradical activity, the method described by Brand-Williams et al. (1995) using DPPH as a relatively stable free radical was used. The antioxidant reduces the purple DPPH to a yellow compound. Its color intensity is inversely proportional to the ability of surrounding antioxidants to donate protons (Sánchez-Moreno, 2002). Absorbance readings are taken on blanks prepared for each concentration at 517 nm after incubation for 30 minutes at room temperature in the dark.

$$FRA(\%) = \frac{(Abs\ Control - Abs\ Sample)}{Abs\ Control} \times 100$$

Where:

FRA: Free Radical Activity (%)

Abs Control is the absorbance of the blank sample.

- **Ferric Antioxidant Power (FRAP):** It was determined using the method described by Yildirim et al. (2001). Absorbance is measured spectrophotometrically at 700 nm against a similarly prepared blank.

## Results and Discussion

The Table 1 summarizes all results that were obtained.

### Antioxidant Contents

Pawlowska et al. (2006) noted that the determination of total polyphenols gives us an estimation of the content of different classes of phenolic compounds contained in the samples analyzed.

Our results of polyphenols contents show a high values: 394.231±155.835 mg GA E 100 g<sup>-1</sup> for orange honey and 347.115±281.346 mg GA E 100 g<sup>-1</sup> in the dark honey (Figure 1).

However, Beretta et al. (2005), Djossou et al. (2013) and Bouyahya et al. (2018) find that darker honeys have a higher content of polyphenolic compounds.

Our results are higher than those obtained by Ouchemoukh et al. (2017) with values of 90 and 318 mg GA E 100 g<sup>-1</sup> in Algerian honeys, Bouyahya et al. (2018) find an important content of polyphenols ranges between 12460.00±112.00 and 7514±78.00 mg GA E 100 g<sup>-1</sup> of honey of Morocco.

Boussaid et al. (2018) with Tunisian honeys (32 and 119 mg GA E 100 g<sup>-1</sup>) and Can et al. (2015) with Turkish honeys (16 and 120 mg GA E 100 g<sup>-1</sup>). The results obtained by Doukani et al. (2014) showed that the concentration of polyphenols recorded in the honeys vary considerably from 166.11 to 427.14 mg of GA E 100 g<sup>-1</sup> of honey. Djossou et al. (2013), mentioned that the total phenol content of the honey samples is between 27.88 to 248.80 mg GA E 100 g<sup>-1</sup>.

Studies by different authors have shown that the variation in phenol contents of the honey samples depends mainly on the botanical and geographical origins (Martos et al., 2000; Meda et al., 2005; Rodríguez-Flores et al., 2015).

Phenolic compounds are a chemically heterogeneous group that fall into different classes (non-flavonoids and flavonoids) according to their basic chemical structure (Andersen and Markham, 2005). Plants possess several polyphenol derivatives that are highly structurally diverse and complex, and when honeybees collect nectar, these bioactive compounds may be transferred from plants to honey (Silici et al., 2010). Phenolic compounds found in honey are used as floral markers and have the ability to scavenge or reduce free radical (reactive oxygen species-ROS) formation, so there is also interest in studying the antioxidant activity attributed to these compounds (da Silva et al., 2016).

### Vitamin C contents (Ascorbic acid)

Foods rich in vitamin "C" are popular for consumers because it is associated with health and vitality (Gensler et al., 1995). Vitamin C performs a vital position as an element of enzymes concerned with inside the synthesis of collagen and carnitine. It stimulates the immune system and protects it from damage caused by free radicals released by the body against infections (Dobrinás et al., 2006).

The results show that honey contain low concentrations of vitamin C in our honey samples (H<sub>01</sub>: 0.25±0.05 mg 100 g<sup>-1</sup> and H<sub>02</sub>: 0.35±0.05 mg 100 g<sup>-1</sup>) (Fig.1). The obtained results were in the range reported by Ciulu et al. (2011) with lower values in Italian honeys (0.1-0.6 mg 100 g<sup>-1</sup>). pH-unstable environment could be the reason for this low values amount in honey samples (Sawicki et al., 2020).

Table 1. Results of antioxidant content and antioxidant activity

Parameters Samples	Antioxidants		Antioxidant activity	
	Vitamin C (mg 100 g <sup>-1</sup> )	Polyphenols (mg GA E 100g <sup>-1</sup> )	DPPH (%)	FRAP (µg 100g <sup>-1</sup> )
H <sub>01</sub>	0.25±0.05	394.231±155.835	11.255±2.668	15.240±4.578
H <sub>02</sub>	0.35±0.05	314.231±281.346	9.578±3.157	17.794±8.179

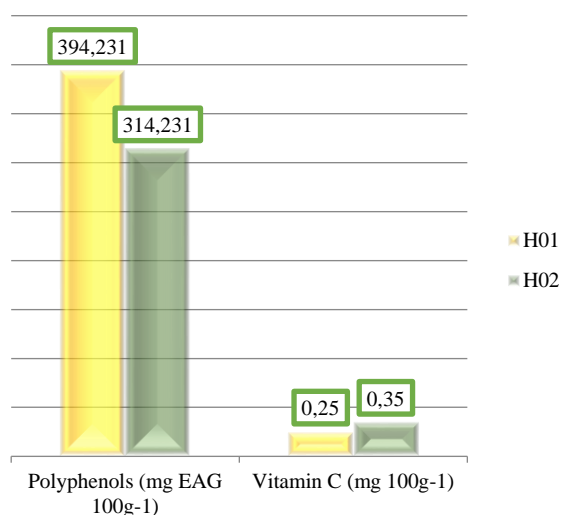


Figure 1. Total polyphenols and vitamin C contents in honey samples

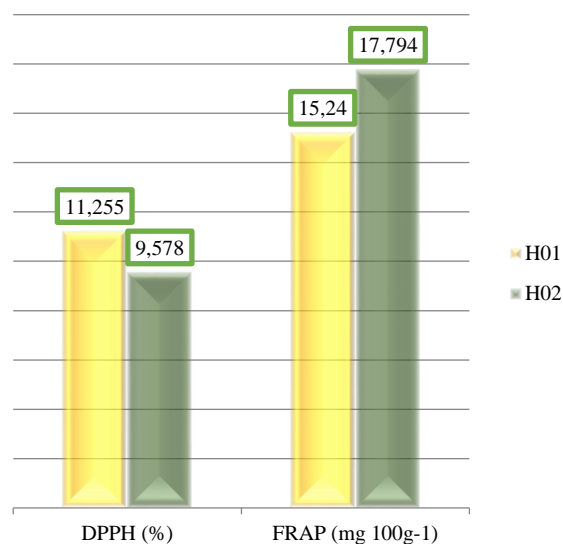


Figure 2. Antioxidant activities in honey samples

However, in Algerian honeys of Mouhoubi-Tafinine et al. (2016) and Otmani et al. (2021) and values were ranged from 0.4 to 3.4 mg 100 g<sup>-1</sup> and from 0.40±0.08 to 2.10±0.2 mg 100 g<sup>-1</sup> respectively. Similar to those results, Dobrinás et al. (2006) findings showed that the examined samples contain an important amount of vitamin C (0.91-2.90), which is necessary for human body. The highest concentration of vitamin C was obtained in commercial honey samples (2.77 and 2.90 mg AA g<sup>-1</sup> honey) that those determined in honey samples collected from beekeepers, probably due to the use of some preservatives or even the use of ascorbic acid in commercial samples.

Vitamin C in honey comes primarily from bee pollen, and its content varies by plant and geographical origin. However, heat treatment and bad storage conditions can lead to loss of vitamin C in this product (Chua et al., 2013). Moreover, León-Ruiz et al. (2013) cited that the evaluation of vitamin C is an unstable indicator, because it is very vulnerable to chemical and enzymatic oxidation and has an accelerated rate of change due to various factors like light, oxygen and, heat. In addition, the place of origin and the harvest period affect the concentration of bioactive substance (as well as vitamin C) (Sawicki et al., 2019), including the extraction and analysis method (Boussaid et al., 2018; Wang et al., 2008).

#### Antioxydant Activities

The DPPH assay was used in order to determine the free radical-scavenging activity of the honey samples.

The values of this assay ranged from 9.578 % (H<sub>02</sub>) to 11.255% (H<sub>01</sub>) (Fig.2). As similar as our results, Doukani et al. (2014) reported low percentage of DPPH radical

inhibition (3.4–22%). However, Zaidi et al. (2019) noted an anti-radical activity ranging from 4.4 to 84% in Algerian honey.

According to Kanoun (2010) and Doukani et al. (2014) the lower percent inhibition value indicates strong free radical scavenging ability. Blasa et al. (2006) and Salonen et al. (2017) argued that light-colored honey possessed lower antioxidant activity by comparison to darker colored honey, an observation that seems to be accurate for our study where the sample H<sub>02</sub> noted a low value, indicated a high antioxidant activity.

DPPH radical scavenging activity is a parameter that varied significantly depending on the botanical origin of the honey samples analyzed. This literature was confirmed by the results of Pauliuc et al. (2020), reported in their study a high DPPH radical scavenging activity which varied from a sample to another according to honey types: for raspberry honey (79.05%) and mint honey (74.03%) and, a low value for thyme (63.77%) and rape honey (55.49%).

The reducing power was low in the samples, where we noted that the value in the dark honey (H<sub>02</sub>: 17.794±8.179 mg 100g<sup>-1</sup>) was superior to the orange honey (H<sub>01</sub>: 15.240±4.578 mg 100g<sup>-1</sup>) (Fig.2). This observation was confirmed by Khalil et al. (2012) and Doukani et al. (2014) where dark honey shows a higher antioxidant activity.

Also, Mouhoubi-Tafinine et al. (2016) reported lower values in Algerian honeys (21-49 mg GAA E 100g<sup>-1</sup>). The values for this antioxidant test reported by Doukani et al. (2014) were 8.3 and 240 mg AA E 100g<sup>-1</sup> of honey. Krpan et al. (2009) obtained an antioxidant activity expressed as FRAP value from 6.95 to 142.43 mg GAA E 100g<sup>-1</sup>.

It looks that such differences are probably due to the geographical origin of honey samples (Al-Mamary et al., 2002; Can et al., 2015).

Several studies on the antioxidant activity of honey have concluded the existence of a positive correlation between phenol levels in samples and antioxidant activity. Bouyahya et al. (2018) showed a positive strong correlation between total polyphenols and antioxidant activity ( $R^2 = 0.821$ ). This conclusion is confirmed by the study of Al-Mamary et al. (2002) who showed that the antioxidant activity of different types of honey from different countries depended mainly on their concentration of phenolic compounds. According to the results obtained by Doukani et al. (2014), there is a considerable correlation between the antioxidant activity measured by the FRAP test, the content of phenolic compounds and the antiradical activity in all the samples of honey. Also, the authors observed that the color intensity is a quick qualitative test that allows us to get an idea of the phenolic content of honey. Frankel et al. (1998) found a significant correlation between the antioxidant capacity determined by the DPPH method and the color of honey. Moreover, a high correlation coefficient ( $r = 0.924$ ) was observed between the antioxidant activity evaluated by the FRAP method and the color of the honey (Bertoncelj et al., 2007). In addition, numerous studies have shown that dark colored honeys have a high content of total phenolic compounds and therefore a high antioxidant capacity (Beretta et al., 2005; Djossou et al., 2013; Otmani et al., 2021; Wilczynska, 2010). On the other hand, Khalil et al. (2012); Doukani et al. (2014) and Otmani et al. (2021) confirmed that TPC and vitamin C from honey are the main factors responsible for its reducing power, especially in dark honey. This was shown by the honeys of Bordj Bouarrerdj (dark amber) and Jijel (dark brown) that had significantly higher TPC, TF, vitamin C and also the most powerful reducing activity. Our results confirmed the observations noted by those authors.

## Conclusion

In this study, the honey samples showed different contents of bioactive compounds (polyphenols and vitamin C) between the orange and dark samples which vary according to the floral source and the area of harvesting. The investigated samples contain an important amount of total polyphenols contents, which are responsible for the significant antioxidant activity measured by DPPH and FRAP assays.

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