BIOCHEMICAL PROPERTIES OF SOME EGYPTIAN HONEYS IN RELATION TO THEIR BOTANICAL ORIGIN

By

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ABSTRACT

Some of classic chemical and physical properties became useless in classification of honey types, for that reason we need special characteristic as a tool for differentiation of honey type depending on its botanical origin for honey, so we study the phenolic compounds and protein profile as a new tool to track the botanical origin of honeys.

Determination of phenolic compounds in honeys

Gallic acid and pyrogallic were the dominant compounds in all honey samples except for sun flower honey samples which had a high amount of protocetchol and pyrogallic acid, and phenolic compounds like gallic acid, protocetchol, euganol, rutin, caffic acid, syringinc, coumaric, salicylic, ferulic acid, quercetin which can be used as a marker for certain Egyptian honey "citrus, clover, cotton, eucalyptus, banana, fennel, marjoram and sun flower honeys.

Honey protein profile as a marker for its botanical origin

Egyptian citrus honey samples showed a unique band of a molecular weight 225.00KDa. And the molecular weights ~213.00, 186.00, 180.50, 173.00, ~84.00, 63.25, 59.00, 47.00, 35.00, 27.00, 18.00, 12.00 were unique bands for differentiation between botanical origin in clover, cotton, eucalyptus, banana, fennel, marjoram and sun flower honeys, respectively.

Honey as antibacterial agent

Antibacterial activity for all honey samples under investigation at undiluted and eight diluted concentrates against five bacteria strains *Bacillus cereus* 33018, *Staphylococcus aureus* ATCC25923, *E. coli* ATCC25922, *E. coli* 0157:H7 and *Salmonella typhirmurium* ATCC 20231 which showed different sensitivity to honeys dilution and total phenolic content, antioxidant activity and ash which representing in the electrical conductivity are very affecting rules for antibacterial activity in clover and cotton honeys

Key words: ***honey, botanical origin, phenolic compound, protein profile, antibacterial effect.***

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INTRODUCTION

In the Holy Quran, Almighty Allah mentioned the special ability of honey to heal and cure disease. Allah said “And your Lord revealed to the bees: Make hives in the mountains and the trees in what they build, then eat all the fruits and walk in the ways of your lord submissively, there comes forth form their bellies a beverage of many colours, in which there is healing for mankind. Verily in this, sign for those who give thought.” (surah Al-Nahal; verses 68 and 69).

The major component of honey are the sugar, of which the monosaccharides fructose and glucose together make up around percent of the total; disaccharides including sucrose add perhaps 10 percent, and the water in which the sugars are dissolved 17-20 percent. Yet many of the characteristics for which honey is well known-its flavor, aroma and color for instance- are determined not by these major components but by other that are present in quite minute amount. So far, 181 different substances have been identified in honey, some are not known to exist else, where (Crane, 1980).

Honey contains a number of proteins and eighteen free amino acids (Won *et al.*, 2008 and Mohammed and Azim, 2011). The presence of proteins in natural honey has been known for many years in addition to carbohydrates, vitamins and minerals (Taghavizad and Majid, 2010). Honey contains approximately 0.5% proteins (Won *et al*., 2008). Nineteen bands of honey proteins have been detected by silver staining in SDS-PAGE (Marshal and Williams, 1987). Different proteins of diverse molecular weight are found in natural honey depending upon the species of the harvesting honey bees (Won *et al.,*

2008). Most of the enzymes are added by honey bees during the process of natural honey ripening (White, 1975).

Health benefits of honey have been reported in a variety of conditions including microbial infections, wound healing, inflammation, glucose tolerance and analgesia. Honey is a supersaturated sugar solution mainly comprised of D-fructose, D-glucose, sucrose, maltose and higher sugars (~80% of solid mass). While other natural products *i.e.* alkaloids, flavonoids/isoflavones, glycosides, phenolics, peptides/proteins are present in minor quantities. A number of enzymes such as invertase, amylase and glucose oxidase have been found in honey. (Antibacterial and antifungal activities of honey were well documented and characterized). These antimicrobial properties have been related to oligosaccharides, glycopeptides and peptides present in honey. Honey glucose oxidase provides a continuous and slow release of hydrogen peroxide at a level which is antibacterial but not tissue-damaging. Hydrogen peroxide produced by glucose oxidase plays important roles in inflammation, wound healing etc. The antimicrobial properties of honey had great potential for application in medicine as well as in food industry (Aurongzeb and Azim, 2011).

Natural honey has been used as an effective medicine around the world since ancient time. It has had a valued set in traditional remedy for centuries. The ancient Egyptians, Assyrians, Chinese, Greeks and Romans employed honey for wounds and diseases of the gut (Bogdanov *et al.,* 2008).

Honey is rich in phenolic compounds, which act as natural antioxidants, and are becoming increasingly popular because of its

potential role in contributing to human health. These compounds can also be used as indicators in studies into the floral origin of the honey itselves.

The exact composition of honey depends mainly on the plant sources it is derived from, but also on the weather, soil and other factors, and no two honeys are identical. The infinite variety is in fact one of the great attraction of honeys and it can be appreciated best by those who harvest honey direct from the hive (Crane, 1980).

Relative quantity of natural honey proteins was measured as a quality indicator. The proteins in natural honey originate from the nectar, pollen and honeybee.

The aim of this study was, determinate the physical and chemical properties of some Egyptian honeys collected from different locations in Egypt, honey protein profile as a marker for its potential original, antimicrobial activities of Egyptian honeys (26 samples) against positive and negative bacteria organisms, phenolic compounds as indicators into the floral origin of the honey samples.

REVIEW OF LITERATURE

1. Physical and chemical properties of nine Egyptian honeys

a. Water content

To protect honey from microbiological spoilage the water content must not exceed 21% by mass; for clover and heather honey, the value must not be more than 23%. Water content is usually determined by refractive index measurement. In the case of creamy or crystallised honeys, a preheating process at 50°C is necessary. According to another method (AOAC, 1995), all samples are preheated at 40 °C and then measured at 20 °C (Isengard and Schulthei, 2003).

White (1978) found that, the range of water content of 490 samples collected from the United States were 13.4 to 22.9%. The amount of water content in honey is of the major importance to its stability against fermentatiom and granulation. Normally ripened honey has water content below 18.6%; honey of higher water content doesn't quality for the USDA grading classifications.

Al Naji and Hujazy (1982) investigated honey samples from different areas in North Iraq. The samples were stored for three months in steril glass jars. Results showed that, as the moisture content of honey exceeded 18%, the total number of yeast and bacteria increased rapidly, while below this moisture percentage, the physical properties and quality of honey weren't affected through the storded period.

Hassan (1985) found that the water content of 3 citrus samples were 18.4, 18.8 and 20.6%, while the values of 2 clover samples were 18.4 and 18.6%. Two cotton samples showed values of 19.6 and 20.6%.

Tilde and Payawal (1992) reported that, 72 honeys were produced by (A) 'Wild' honeys, (B) *Apis mellifera* honeys and (C) honeys from reputable apiaries. Mean water contents were: A 21.3% (range 16.2-33.1%), B 18.2% (15.0-22.0%), C 18.0% (15.1-22.0%); the Codex maximum was 21%.

Sanz *et al*. (1995) determined water content for 19 samples of honeys produced in the Cantabrain coast. Only 2 honeys contained less than 17.1% water (no risk for fermentation), 4 contained more than 20% permitted by Spainch low, and the rest were intermediate. Those with values above 17.1% water content had a high risk of fermentation.

Russmann (1998) indicated that the honey with relatively high moisture content (> 18%) may undergo yeast fermentation; honeys which are fermented, don't meet German regulations for honey for fresh consumption. Yeast count and glycerol concentration were increased as a result of fermentation of honey.

Bogdanov (1999) reviewed the international regulatory standard for honey quality and reported that the moisture content is the only composition criteria, which as a part of the honey standard has to be fulfilled in world honey trade. Honey having high water content is more likely to fermentate. A maximum value of 21g/100g is suggested in the draft for a new standard. The exception for clover honey is not justified by measurement during recent years. Accordingly the maximum water

content for clover honey should also be 21g/100g in partice, values as high as 21g/100g are very seldom attained. Most of the honey samples had a water content of less than 20g/100g. Also in Switzerland a standard of 20g/100g has been successfully used over the past 20 years, until the last revision of the Swiss Food Ordinance, where the European Union maximum value of 21g/100g had to be accepted. Many national beekeeping organizations (*e.g*. Germany, Belgium, Austria, Italy, Swissland and Spain) have moisture content maximum values of 17.5g to 18.5g to honey for social classes of quality honey.

Conti (2000) conducted a survey of 84 samples of commercial honeys of different botanical origin, collected all over the Lazio region (central Italy) in order to assess their quality, and moisture content which was as a mean value (16.57%).

Anupama *et al.* (2003) studied eleven commercial samples of Indian honey for their sensory and physicochemical properties, and found that moisture content varied from 17 to 22.6%.

Isengard and Schulthei (2003) reported that preheating at 50 °C, was necessary for creamy honeys to render them liquid for determinations. This procedure entails a slight water loss as follows 17.320±033, 17.240±026, 17.120±030 and 17.080±050 for preheating (none, 1 h at 40 °C, 1 h at 50 °C and 2 h at 50 °C, respectively).

Badawy *et al.* (2004) found that, the water content of the fresh sample (collected in 2000) was in the normal range of fresh honey (19.5%). The other samples that had been stored for longer periods (12