HONEY: A FUNCTIONAL FOOD

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Honey, miraculous, unique product of nature, is being created by honeybees from flower nectar. This nectar then mixes with special enzymes in the bees' saliva, a process that turns it into honey. The fluttering of their wings provides the necessary ventilation to reduce the moisture content making it ready for consumption. Among the different food items, honey with its diverse health benefits can be regarded as a functional food. Honey is being used for centuries as a traditional therapeutic. Honey was used as a drug delivery system in Ayurveda. The unique components including minerals and vitamins that are available in honey form an array of micronutrients. These micronutrients in combination, produce a range of health promoting functions. Honey is increasingly valued for its antibacterial activity, but knowledge regarding the mechanism of action is still incomplete. Bee defensin-1, H2O2, methylglyoxal and low pH are some of the factors responsible for the antimicrobial activity. Phenolics present in different unifloral honeys serve as the biomarkers for unifloral honey ex. Sesame lignans in sesame honey. Gallic acid, myricetin, rutin, quercetin and naringenin are some of the phenolic compounds present in nectar and honeys as well. The identification of biological indicators of the floral origins for honey could provide added value to honey commercialization by endorsing the botanical origin of their chemical features and biological attributes.

According to European Union Council Directive, 2002, honey is defined as 'the natural sweet substance produced by *Apis mellifera* bees from the nectar of plants or from secretions of living parts of plants or excretions of plant-sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store and leave in honeycombs to ripen and mature'.

Honey is a supersaturated solution of sugars, in which fructose (38%) and glucose (31%) are the main contributors, containing a wide range of minor constituents like phenolic acids, flavonoids, certain enzymes, carotenoid like substances, amino acids, organic acids, Maillard reaction products, vitamins and minerals. Its composition is rather variable and primarily depends on the botanical and geographical origin of the floral source, although certain external factors also play a role, such as seasonal and environmental factors and its processing.

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Phenolic compounds in honey are mainly flavonoid, aglycones and phenolic acid derivatives like benzoic and cinnamics acids and their respective ester. Phenolic compounds are the phytochemicals, and thus the phenolic composition of honey varies depending on the vegetation area visited by the bee. With this in mind, phenolic compounds have been proposed as potential chemical markers for authenticating the geographical and botanical origin of honey. Flavonoids are the most common phenolics in floral honeys, and characteristic profiles could be expected in unifloral honeys depending on the corresponding plant source. A strong correlation has been observed between the antioxidant activity of honeys and their phenolic composition and especially the total phenolic content. Thus, characterization of phenolics and other components in honey that might have antioxidant properties is essential to improve our knowledge about honey as a source of nutraceuticals and would also be an important tool to contribute to their authentication.

The quality of a honey depends on its chemical composition and botanical origin. The flavonoid profiles of honeys are determined by their botanical and geographical origin(s), and by the climatic conditions of the area. Therefore, identification and quantification of the flavonoids in honey is of great interest. The flavonoids constitute one of the largest groups of naturally occurring phenolic compounds. They are derived from plants, and when the plants are used by bees to collect nectar or honeydew, these bioactive components are transferred into the bee honey. Three subgroups of flavonoids with similar structures are present in honey; namely: the flavones, the flavonois and the flavanones.

Chemical composition of honey

Honey is a natural product produced from the nectar and exudation of plants by the honeybees, *Apis mellifera* (Alvarez-Suarez *et al.* 2010). The natural honey has been reported to contain about 200 substances, which consist of not only highly concentrated solution of sugars, but also the complex mixture of other saccharides, amino acids, peptides, enzymes, proteins, organic acids, polyphenols, carotenoid like substances, vitamins, and minerals (Gheldof *et al.* 2002; White 1975).

Sugars are the main constituents of honey, comprising about 95% of its dry weight (Alvarez-Suarez *et al.* 2010). While glucose (31%) and fructose (38%) are the dominant constituents, about 25 different sugars have been detected. The actual proportion of glucose to fructose in any particular honey depends largely on the source of the nectar. The average ratio of fructose to glucose is 1.2:1 (White 1975). Saccharose (sucrose) is present in honey (approximately 1% of its dry weight). However, this level can be increased if the beekeeper has over-fed the bees with sugar during the spring.

White (1975) has demonstrated that proteins in honey are mainly enzymes. Honey contains roughly 0.5% proteins (Alvarez-Suarez *et al.* 2010) and the protein contents in some honeys can be over 1000 μ g/g (Azeredo *et al.* 2003). Main enzymes include diastase, invertase, glucose oxidase and catalase. Although the content of amino acids in honey is relatively small, it has been found that almost all of physiologically essential amino acids are present in honey. The primary amino acid is proline, contributing 50-85% of the total amino acids (Hermosín *et al.* 2003).

The level of organic acids in honey is relatively low and about 18 organic acids have

been detected (Nanda *et al.* 2003). Most of the acidity present in honey is added by honeybees. Gluconic acid, the predominant honey organic acid, is the product of glucose oxidation, presenting at 50-fold higher levels than other acids (Cherchi *et al.* 1994).

The mineral content varies from about 0.04% in pale honeys to 0.2% in some dark honey samples and is dependent on the type of soil in which the original nectar bearing plant was located. Investigations have shown that a wide range of trace elements are present in honey, including Al, Ba, Bi, Co, Cr, Mo, Ni, Pb, Sn, Ti, as well as macro minerals like Ca, Cu, Fe, K, Na, Mg, Mn, Zn. Among them, the main mineral element is potassium while copper presents lowest amount (Nanda *et al.*, 2003).

Very minute quantities of Vitamins such as thiamin (B1), riboflavin (B2), pyridoxine (B6), and ascorbic acid (C) have also been reported to be present in honey samples (Nanda *et al.*, 2003).

When honey is treated with mild heat or after prolonged storage, a compositional change occurs due to caramelization of the carbohydrates, the Maillard reaction, and decomposition of fructose in the acid medium of honey (Villamiel *et al.* 2001).

Phytochemicals are chemical substances naturally occurring in plants and many of them are now recognized to have health-promoting activity (Liu 2004; Liu 2003). Phenolic substances are the largest group of phytochemicals. The plants containing phytochemicals might be used as a supply of the bees; thereby bioactive compounds can be transferred to honey. Studies have shown that honey contains great variation in content of different phenolic substances particularly flavonoids according to floral sources and climatic conditions, which contribute to different characteristic colors, flavors, aromas, and bioactivities (Molan 1996). As herbal medicines are derived from different plants, which can produce different therapeutic properties (Villegas *et al.*, 1997), some honey derived from these specific plants may provide added value for health promotion.

In the recent years, flavonoid profiles of different types of honey was investigated to identify specific compounds that could be used as markers for the determination of the botanical and geographical origin of a honey (Truchado *et al.*, 2008; Kenjeric' *et al.*, 2007; Tomas-Barberan *et al.*, 2001; Martos *et al.*, 2000). Hesperetin which belong to flavanones is known as a predominant phenolic compound in citrus honey. Flavonols such as 8-hydroxykaempferol and quercetin are the main phenolic compounds in rosemary honey for the former and sunflower honey for the latter. Luteolin, a predominant compound in lavender honey (Baltrusaityte *et al.*, 2007; Yao *et al.*, 2003; Anklam, 1998), Abscisic acid, a non-phenolic compound of heather honey, have been proposed as markers (Ferreres et al., 1996) although has bscisic acid has been reported in other types of honey as well (Tomas-Barberan *et al.*, 2001). Conversely, Pulcini *et al.*, 2006 indicated that for the characterisation of the botanical origin of a honey, differences in the whole composition of the flavonoids might be more suitable than the use of any single specific compound. Moreover, higher antioxidant capacity was found in darker honey samples. (Chen *et al.*, 2002; Nagai *et al.*, 2001).

"Raw" honey contains extraneous matter including pollen, wax and variable levels of sugar-tolerant yeasts and may contain crystals of dextrose hydrate. These substances are removed from honey in order to improve its performance in large-scale markets. Honey is prone to fermentation unless the moisture content is maintained below 17% (White, 1978),

and most honey crystallizes over time unless action is taken to prevent crystallization. Granulated honey is more likely to ferment than liquid honey (White, 1967). Raw honey, without processing, is unsuitable for large-scale marketing. Commercial honey processing includes controlled heating to destroy yeast and to dissolve dextrose crystals, together with fine straining or pressure filtration. Honey is usually warmed to a temperature of 32-40°C in order to lower its viscosity which facilitates extraction, straining or filtration. This processing temperature is similar to that in beehives and does not affect the honey very much during the relatively short processing period. However, some honey samples are heated to higher temperatures for liquefaction or pasteurization reasons.

Health Benefits of Honey

Antimicrobial activity: Since ancient times, honey has been used for treatment and prevention of wound infections. With the advent of antibiotics, the clinical application of honey was abandoned in modern Western medicine, though in many cultures it is still used. For all antibiotic classes, including the major last resort drugs, resistance is increasing worldwide and even more alarming, very few new antibiotics are being developed. The potent activity of honey against antibiotic-resistant bacteria resulted in renewed interest for its application. Several honeys have been approved for clinical application. The incomplete knowledge of the antibacterial compounds involved and the variability of antibacterial activity are however major obstacles for applicability of honey in medicine. Factors affecting antibacterial nature of honey was reported by Molan and Cooper, 2000. The difference in antimicrobial potency among the different honeys can be more than 100-fold, depending on its geographical, seasonal and botanical source as well as harvesting, processing and storage conditions. The antibacterial nature of honey is dependent on various factors working either singularly or synergistically, the most salient of which are H₂O₂, inhibine (White et al., 1963), phenolic compounds, wound pH, pH of honey and osmotic pressure exerted by the honey. The antibacterial activity of honey against several pathogens and its dependence on the floral origin and their phenolics has been widely reported (Al et al., 2009, Finola et al., 2007). Honey has been used in the treatment of and prevention of wound infections. Phenolic compounds originating from plant nectar have been proposed as important factors for the nonperoxide antibacterial activity of honeyand so not all honeys possess similar activity. Several antibacterial phenolic compounds have been identified in honeys (Molan, 1992, Russell, 1990, Weston et al., 2000, Isla, 2011), but their contribution to the overall activity of honey remains unclear. The activity of individual phenolics isolated from honey is too low to substantially contribute to the antibacterial activity ((Molan, 1992, Weston et al., 2000). Hydrogen peroxide is produced by a natural glucose oxidase system in honey; it has antibacterial effects called inhibine (White et al., 1962).

Antioxidant activity

Besides being used as a sweetening agent, honey serves as the natural food preservative. Deteriorative oxidation reactions in foods, such as browning reactions in fruits and vegetables and lipid oxidation in cooked, ground poultry are being prevented by honey. Consumption of honey elevated the antioxidant capacity of human subjects (Gheldof *et al.*, 2003). Honey

is reported to be at its best in terms of flavor and color immediately after extraction (White, 1978). Fermentation starts unless the moisture content is below 17%. The freshness and quality of the honey was evaluated by measuring diastase, invertase and hydroxymethylfurfural (HMF).Mailard reaction products generated during storage of honey have been found to show antioxidant capacity. Caramelization of carbohydrates, Maillard reaction, and decomposition of fructose in the acid medium of honey (Villamiel *et al.*, 2001) lead to the formation of HMF, other furfural compounds, and MRP which eventually function as antioxidant preventing lipid peroxidation. High amounts of total flavonoids were quantified in heather honey, raspberry, black locust and linden honey. A positive correlation between the content of polyphenols, flavonoids and antioxidant activity was observed in honey samples (Dezmirean *et al.*, 2012).

Wound healing property

The wound healing property of honey is due to its antibacterial activity, its property of maintenance of a moist wound condition, and its high viscosity which provides a protective barrier against infection. Its immunomodulatory property is relevant to wound repair too. But, there is a large variation in the antimicrobial activity of some natural honeys, which is due to spatial and temporal variation in sources of nectar. Thus, identification and characterization of the active principle(s) may provide valuable information on the quality and possible therapeutic potential of honeys (against several health disorders of humans). Recently, high concentrations of an antibacterial compound, methylglyoxal (MG), was identified in manuka honeys (Adams et al., 2008, Mavric et al., 2008). MG is a reactive aoxoaldehyde formed both enzymatically and nonenzymatically in mammalian and microbial cells as an intermediate in the glycolytic pathways (Booth et al., 2003, Kalapos, 2008). MG is involved in the formation of advanced glycation end products implicated in growth arrest and cell death (Booth et al., 2003). Clinical studies on the wound healing properties of manuka honey have found that it stimulates healing and promotes the re-growth of healthy tissue with no adverse effects (Molan, 2006). Subjects with Fournier's gangrene (Subrahmanyam et al., 2004), burns (Subrahmanyam, 1991) who were treated with honey dipped gauze to their wounds demonstrated faster clearing of slough and healthy granulation tissue and reduced duration of hospital stay.

Prebiotic property The GI microflora is in a dynamic equilibrium that may be altered by diet, medication, stress, aging and various other environmental factors. The non-digestible carbohydrates, a variety of oligosaccharides that occur naturally in foods such as fruits, vegetables, milk and honey (Shamala *et al.*, 2000) serves as prebiotic (Crittenden,1999). The effect of honey oligosaccharides on the growth of fecal bacteria like bifidobacteria and lactobacilli revealed that honey has potential prebiotic activity.

Apoptosis Honey has been used in palliative care of various cancers. Antineoplastic activity of honey was studied in an experimental bladder cancer implantation model (Swellam *et al.*, 2003). Anti proliferative and proapoptotic effects of honey was studied on human gastric mucosa (Ghaffari *et al.*, 2012).

The comparative physiochemical characterization of different honeys from various regions of the world has been carried out extensively. Although in India, honey is produced and consumed on a large scale, there is a lack of systemic information on the comparative

biochemical properties of Indian honeys, especially the honeys collected from the state of West Bengal. However, the state of West Bengal contributes a major chunk of the ~ 65000 million tones to the honey produced in India (Kumar, 2010).West Bengal harbors a natural and unique ecosystem and consequently is a hub of natural products. Prominent features of this ecosystem includes a part of the world's largest ecosystem i.e., the Sundarban mangrove forests (~ 40% of the forest area is present in West Bengal). Although numerous reports have highlighted the importance of this ecosystem and the natural products from these regions this leaves a huge void in understanding the prophylactic impact of the natural products especially honeys procured from these regions. This can be attributed to the fact that the honey samples from this geographical area and similar ecosystem across West Bengal have not been documented and characterized for their prophylactic and associated impact on health.

CONCLUSION

Honey, the healthiest golden liquid food, processed by honeybees, with various compositions of phytonutrients, enzymes, bioactive peptides, oligosaccharides, is a real gift of nature.

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