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Honey, its Quality and Composition and their Responsible Factors

Rohini Sharma, Meena Thakur, Kiran Rana, Diksha Devi and Mangla Ram Bajiya

Dept. of Entomology, Dr. Yashwant Singh Parmar University of Horticulture and Forestry, Himachal Pradesh (173 230), India

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Corresponding rohiniyspu43@gmail.com

0000-0003-1653-1474

ABSTRACT

Honey is a sweet viscous food substance made by honey bees. It contains more than 180 nutritional substances, due to which it is known for boosting immunity. Honey is comprised of easily digestible honey sugars and energy providing nutrients. It is used as an ingredient in hundreds of manufactured foods for its sweetness, colour, flavor, caramelization and viscosity. It's various physicochemical constituents, moisture content (<25%), reducing sugars (>65%), acidity (<0.2%), electrical conductivity (<0.8 mS/cm for blossom honey), sucrose content (5%), hydroxy methyl furfuraldehyde (HMF) (<40 mg kg^{-1}) etc. contribute to its nutritional quality, granulation, flavor, texture, fermentation and storage quality. Other properties viz. diastase content, proline content, phenols, pollen density etc. are important indicator of quality and origin of honey. Honey is the reservoir of different minerals, with potassium being most abundant element. Owing to its chemical composition, honey has been reported to have significant impact on human nutrition and health. Its quality is evaluated based on standard quality parameters standardized by different authorities. The quality and composition of honey highly depends on the type of flowers utilized by the bees, climatic conditions and various other factors like floral sources, seasons, beekeeper activities, harvesting of honey, processing, storage, agrochemicals, adulteration and geographical origin. The demand of honey is also increasing in upward trend; thus, the analysis of these quality parameters helps us to ensure that the honey we are consuming fulfills the required parameters.

KEYWORDS: Immunity, *Apis cerana*, *Apis mellifera*, physicochemical, honey, quality

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1. INTRODUCTION

Honey also known as Madhu; is a sweet viscous food substance which is made by honey bees. The foraging worker bees visit flowers and collect nectars in their honey stomach and pollen in pollen basket on their legs. The bees return to hive and regurgitate the nectar onto the mouth of other worker bees which chew the nectar for about half an hour, breaking complex sugars into simple sugars enzymatically and finally capping the cells with bee wax, so that enzyme rich bee barf can complete its transformation into honey (Anonymous, 2021). In Ancient Indian holy books and in the Vedas, honey has been mentioned several times and depicted in many wall drawings. The cultivation of bee honey documented as early as 26th or 25th century BC as a scene from the Sun Temple of Ny-woser-Re at Abusir (Middle Egypt) depicts it (Crane, 1983). Religiously, Hindus consider honey an important part of Panchamruta -Madhu abhisheka and plays important role in festival of Budhism (Madhu Purnima).

The world's total honey production is around 1,850,868 tonnes per year. In 2019, global production of honey was 1.9 mt; led by China with 24% (457.203 t) of the world total. Other major producers were Turkey (114.113 Tons), Argentina (79.468 Tons), and Iran (67.442 Tons) (Anonymous, 2020a). India, with 67,442 tonnes of production per year is ranked at 7th position. Global honey food market size is projected to reach USD 8214.9 million by 2026 from USD 6447.6 million in 2020 at a CAGR of 4.1% during 2020-2026 (Anonymous, 2020b). Among the Indian states maximum production of honey is in Uttar Pradesh (22,000 t) followed by West Bengal (18,000 tonnes) and Punjab (16,000 t) (Anonymous, 2021). India has exported 59,536.75 MT of natural honey to the world for the worth of Rs. 6.3 billions/ 88.65 USD Millions during the year of 2019-2020 as compared to 28,378.42 MTs (2013–14) which shows 109.80% increase in honey export (Anonymous, 2020c).

Honey has various bioactive compounds such as vitamins (E, A, K, B1, B2, B6, C), phenolics, flavonoids, amino acids, and fatty acids which contribute to pharmacologic properties such as wound healing, antimicrobial activity, anti-inflammatory, antidiabetic, antioxidant, and anti-tumoral effects (Bouhlali et al., 2019). Honey is also known as functional food, is a complex mixture of sugars (carbohydrates such as monosaccharides, glucose, and fructose) and other components, such as proteins, minerals, vitamins, organic acids, and enzymes (Guerzou, 2021). Honey has attracted great attention as a natural sweetener that promotes health (Nguyen, 2021). Honey is also associated with decreases in inflammation *in vitro* through the simultaneous effects of its antioxidant and antibacterial properties (Ruiz-Ruiz et al., 2017). According to a study conducted by Hossain (2020), the antiviral activity of honey may be beneficial for patients with COVID-19 as it boosts the host immune system and improves comorbid conditions. It has been also proved effective against several viruses such as HIV, influenza virus, herpes simplex, and varicella-zoster virus. Honey helps in treatment of cardiovascular diseases, cancer, cataract, and several inflammatory diseases (Azonwade et al., 2018).

In India, 16 Integrated Beekeeping Development Centers (IDBCs) as a role model of beekeeping have been commissioned. Also, two world class state of the Art Honey Testing Laboratories at NDDB, Anand, Gujrat and one at IIHR, Bengaluru, Karnataka have been setup. Laboratory at Anand has been accredited by NABL and has been inaugurated by Union Minister of Agriculture and Farmers Welfare, Govt. of India on 24thJuly 2020, which has started testing of honey samples for all the parameters notified by FSSAI (Anonymous, 2020d).

2. CLASSIFICATION OF HONEY

Honey has been classified on the basis of origin, processing system and grades as depicted.

2.1. Based on origin

2.1.1. Unifloral honey (Monofloral)

Honey from a single plant is known as unifloral honey. When pollen content of the particular plant species is more than 45 percent of the total pollen content (>45% pollen of one plant) (Alvarez-Suarez, 2010).

2.1.2. Multifloral honey (Polyfloral)

Honey that comes from multiple plant species is known as multiforal honey. When pollen content of different plant species does not exceed 45 percent of the total pollen content (<45% of pollen from different plant species) (Ling Chin and Sowndhararajan, 2020).

2.1.3. Blossom honey

Honey made from the nectar gathered by bees from flowering plants. E.g. Manuka honey (made from nectar gathered from the pink flowers of *Leptospermum scoparium* shrub), Tupelo honey (made from nectar gathered by bees from the White Tupelo tree).

2.1.4. Honeydew honey

It is produced by bees from secretion of living parts of plants or excretions of plant sucking insects (European Commission, 2002). E.g. a sticky liquid produced by aphids and some other insects that feed on plant sap or harvested by bees from the bark or leaves or stems of trees and other sap-producing plants. Honeydew honey usually presents higher values of pH, electrical conductivity, ash content higher

concentration of disaccharides, trisaccharide's, and lower level of monosaccharides, besides darer color and peculiar sensory features compared to blossom honeys (Pita-Calvo and Vazquez, 2016). According to European Legislation (European Economic Community), blossom honeys have electric conductivity values below 0.80ms/cm, while honeydew honey exceeds this value (Anonymous, 2020e)

2.2. Based on processing system

2.2.1. Comb honey

Pieces of honeycomb, as produced by the bees, are sold without processing which fetches good price. It is one of the easiest forms of honey to harvest and prepare for sale.

2.2.2. Strained hone

Honey produced by just straining honeycombs, to separate the honey from beeswax, pollen or other particulate material.

2.2.3. Chunk honey

A piece of comb honey is placed inside the jar of liquid honey, which looks very attractive e.g. honey from Acacia and *Robinia psudoacacia*.

2.2.4. Extracted honey

It is the honey obtained by centrifuging honeycombs.

2.2.5. Crystallized or granulated honey

It is the strained honey that is crystallized and safe to eat as granulation is a natural process, and easily restored to its natural state. Granulation tendency is determined through the ratio between the monosaccharide content and the water content of honey. Honey with more glucose content and less water content granulates more (Singh, 2018).

2.2.6. Creamed honey

It is a strained honey which has been seeded to start crystallization and then stirred to produce a honey of uniform, soft consistency. Honey is creamed by the 'Dyce method' (Dyce, 1975). About 20 percent of fine crystallized honey is mixed with liquid honey and the crystals are allowed to grow at 14 degree Celsius. This procedure stabilizes the honey consistency, and does not affect the honey authenticity, as no foreign matter has been added or removed (Pavlova et al., 2019).

2.3. Based on grading

2.3.1. Grade A

Highest quality of extracted honey with percent soluble solids (min.- 81.4), best flavor and clarity (may contain a trace of pollen grains or other finely divided particles in suspension that do not affect appearance), practically free from any defects, free from caramelization, smoke, fermentation, chemicals and other causes.

2.3.2. Grade B

Second best quality of honey, with a minimum total score of 80 points, percent soluble solids (min.- 81.4), reasonably free from defects, caramelization, smoke, fermentation, chemicals and reasonably clear from suspended particles.

2.3.3. Grade C

Lowest grade of extracted honey, with a minimum total score of 70 points, percent soluble solids (min.- 80.0), fairly free from defects, fairly good in flavor and aroma and reasonably clear from suspended particles. Substandard honey has fewer than 70 points and does not meet the requirements of U.S. Grade C has poor flavor and aroma. (Anonymous, 2019).

India certifies honey grades based on additional factors, such as the Fiehe's test (Detect adulteration of honey with commercial or inverted sugars and overheating or aged honey), and other empirical measurements. Invert sugar and jaggery has high level of HMF content. A positive result (pink or cherry colour) indicates the presence of a high level of HMF (Anonymous, 2020e). According to CSE report (2020), out of 22 honey samples more than 77% were adulterated with sugar syrup and only 5 were unadulterated (Anonymous, 2020f). Top brands including Patanjali, Zandu, Baidyanath, Dabour, Apis- Himalaya and Hitkari failed in NMR (Nuclear Magnetic Resonance) test. Only 3 brands viz. Saffola, Markfed Sohna, Nature's nectar were of standard quality (Table 1).

3. COMPOSITION OF HONEY

Honey contains approximately 80% carbohydrates (35% glucose, 40% fructose, and 5% sucrose) and 20% water; serving as an excellent source of energy (Da Silva et al., 2016). It also contains least 200 substances including amino acids, vitamins, minerals, enzymes and pH is approximately 4.0 (Abselami et al., 2018). The composition and properties of honey are influenced by a number of factors such as geographical origin, botanical (flower) sources of nectar, environmental and climatic conditions as well as its harvesting, processing, and storage techniques (Veena et al., 2020). Honey bees are exposed to industrial pollutants and pesticides. Pesticide residues in raw honeys are an indicator of environment pollution and possibility of using as biomarker has been highlighted (Oryan et al., 2016) (Table 2).

4. PHYSICOCHEMICAL CHARACTERISTICS OF HONEY

Physicochemical constituents of honey influence its storage quality, granulation, texture, flavor, nutritional and medicinal quality and are of great importance to honey industry. As honey is widely used in the formation of certain drugs and cosmetics, as antiseptic and for other therapeutic values (Burlando et al., 2013), standardization of its quality

Table 1: Haney standards by different organizations

S1. No.	Characteristics	FSSAI	BIS			CODEX	AGMARK		
			Superior	Standard	General	Others	Special	A-Grade	B-Grade
1.	SG at 27 C, min.	1.35	1.40	1.37			1.4	1.4	1.35
2.	Moisture (%), max.	20.0	18.4	22.0	20	23 heather honey and 21 clover honey	20	22	25
3.	Total reducing sugars (%), min.	65.0	70.0	65.0	65.0	45 (blend of honeydew and blossom, honeydew honey) and 53 (grass tree honey)	65.0	65.0	65.0
4.	Sucrose (%), min.	5.0	5.0	5..0	5.0	10 (honeydew honey, blend of honeydew and blossom, robinia, citrus, alfalfa, acacia, red gum, sweet clover leatherwood) and 15 (lavendulan and borage)	5.0	5.0	5.0
5.	Ratio F:G, min.	0.95–1.50	1.0	1.0	1.0	-	1.0	0.95	0.95
6.	Ash (%), max.	0.50	0.5	0.5	0.6	1.2 (honeydew or blends of honeydew and blossom)	0.5	0.5	0.5
7.	Acidity (%) max.	0.20	0.2	0.2	0.2		0.20	0.20	0.20
8.	Free acidity (milliequivalent acid /1000 g), max.	50.0			50.0	50.0			
9.	HMF, mg kg^{-1}, max.	80	40	80	40	80 (honey of declared origin from countries or regions with tropical ambient temperature)	50	50	50
10.	Total count of pollen /g of honey, max.	25,000	50,000	50,000	50,000	50,000			
11.	Diastase acitivity (DN), min.	3.0	8.0	3.0	8.0	3.0 (low natural enzyme honey)			
12.	Fiehe's test	-ve	-ve	-ve	-	-			
13.	OD, at 660 nm, max.	-	0.3	0.3	0.3	0.3			
14.	Electrical conductivity mS / cm	0.8		0.8	1.2				
15.	Proline, mg kg^{-1}, min.	180							
16.	Water insoluble content (g 100 g^{-1}), max.	0.10	0.5 (pressed honey)						

is of great significance both from consumer point and beekeepers to get proper remuneration (Thakur et al., 2021).

4.1. Colour

Colour is an important characteristic of honey which contributes to the appearance of honey and varies from water white to dark amber and contributes quality to the market value. The colour of honey is related to its mineral content and is a characteristic of its floral source. Fellenberg and Rusiecki (1938) separated honey color into two fractions namely water soluble (light coloured) and fat soluble (darker honey). Phadke (1962) recorded absence of tyrosine and tryptophan in light honey in contrast to with dark honey. After granulation, honey appears to be lighter. USDA has classified seven colours with optical density range from 0.0945 to 3.008 for honey. As per Indian standards and Agmark requirements, it varies from light to dark brown with maximum optical density 0.3 at 600 nm. Lighter honey is mild flavored, while darker honey is stronger (National Honey Board). Heat affects the colour of honey and also the amount of suspended particles such as pollen. Natural honey become darker in colour when it is heated (Nakov et al., 2019). Based on optical density USDA, there are 7 colour classes of honey (White, 1975) as given in Table 3.

Table 2: Average composition of honey

S l . No.	Components	Value 100^{-1} ghoney	Minerals	Quantity	Vitamins	Quantity	Elements	mg 100 g^{-1}
1.	Total carbohydrates	82.4 g	Calcium	6 mg	Vitamin C (ascorbic acid)	0.5 mg	Aluminium (Al)	0.01-2.4
2.	Glucose	35.8 g (20-44)	Iron	0.42 mg	Riboflavin B2	0.038 mg	Arsenic (As)	0.014-0.026
3.	Maltose	1.44 g	Magnesium	2 mg	Niacin B3	0.121 mg	Barium (Ba)	0.01-0.08
4.	Fructose	40.9 g (21-53)	Phosphorus	4 mg	Pantothenic acid B5	0.068 mg	Boron (B)	0.05-0.3
5.	Sucrose	0.89 g (0.1-7.6)	Potassium	52 mg	Vitamin B6	0.024 mg	Cadmium (Cd)	0-0.001
6.	Galactose	3.1 g	Sodium	4 mg	Folate B9	2 µg	Chlorine (Cl)	0.4-56
7.	Amino acids	0.3 g	Zinc	0.22 mg	Choline	2,2 mg	Cobalt (Co)	0.1-0.35
8.	Total acids	0.57 g	Copper	0.036 mg			Silicon (Si)	0.05-24
9.	Moisture content	17.1 g	Manganese	0.08 mg			Lead (Pb)	0.001-0.03
10.	Nitrogen	0.041 g	Selenium	0.8 µg			Molybdenum (Mo)	0-0.004
11.	Ash	0.2 g	fluoride	7 µg			Nickle (Ni)	0-0.051
12.	Fiber, total dietary	0.2 g					Sulfur (S)	0.7-26
13.	Energy	304 kcal					Vanadium (V)	0-0.013
14.	Total lipid (fat)	0 g					Lithium (Li)	0.225-1.56
15.							Iodine (I)	10-100
16.							Zirconium (Zr)	0.05-0.08

4.2. Viscosity

Viscosity is an important physical property of honey; depends on many factors viz. chemical composition, moisture content and temperature during processing and storage (Yanniotis et al., 2006; Kang and Yoo, 2008, Nayik et al., 2015, Rao, 2016). It is measured by using Modular Compact Rheometer. The viscosity value of honey depends on water content (14–24%) and temperature (Travnicek et al., 2012). Viscosity is the main rheological

Table 3: Colour designation of honey and range for each colour

Sl. No.	Colour Name	Pfund Scale, millimeters	Optical density
1.	Water White	<9	0.0945
2.	Extra White	9 – 17	0.189
3.	White	18 – 34	0.378
4.	Extra Light Amber	35 – 50	0.595
5.	Light Amber	51 – 85	1.389
6.	Amber	86 – 114	3.008
7.	Dark Amber	>114	-

Source: USDA, 2020

properties of honey. Honey is usually used in liquid form with high viscosity. Viscosity is simply correlated to the easiness to flow, the higher the viscosity the more difficult the fluid to flow (Navaza et al., 2009). Honey has viscosity several times (thousand times) of water viscosity and makes it difficult to flow. Honey viscosity is mainly affected by water content, temperature and honey composition (Bambang, 2019).

4.3. pH

Honey is naturally acidic irrespective of its geographical origin, due to presence of organic acids (particularly gluconic, pyruvic, malic and citric acids), lactones, esters, and some inorganic ions, such as phosphate and chloride that contribute to its flavor and stability against microbial spoilage (Acquarone et al., 2007, Bruneau et al., 2017). Variation in pH may be due to floristic composition and floral diversity, salivary secretion of bees, enzymatic process and fermentative conversion of raw material, inappropriate method of harvesting. Honey with pH range from 3.5 to 4.5 are considered to be blossom honey, while honey with a pH above 5 to be of low quality (Chefrour and coworkers., 2009). It is measured by pH meter.

4.4. Pollen density

Bee pollen is mixture of pollen and digestive enzymes from bees. Pollen density is one of the essential physical characteristics of honey. Raw honey contains slightly more pollen than processed honey. Pollen analysis of honey is also of great importance for quality control, ascertains honey adulteration and useful in determination of geographical and botanical origin of particular type of honey by Melissopalynology (Ohe et al., 2004 and Jones and Bryant, 2004). Pollen count should be 25,000 per gram (Anonymous, 2020e; Mattu and Saklani., 2017).

4.5. Moisture content

Moisture content of honey is a limiting factor in determination of its quality, stability and spoilage, resistance against yeast fermentation and granulation (Sigh and Bath, 1997). Extraction of unripe honey, unprocessed honey, collection at time of rainy seasons leads to high moisture content and fermentation during storage. It depends on temperature and relative humidity, geographical origin during. It is determined by measuring the refractive index at 20 degree Celsius by Refractometer or by oven drying method given by Ranganna (2007).

4.6. Electrical conductivity

Mineral substances, amino acids, and organic acids (e.g. Citric acid), present in bee honey, form ions in honey aqueous solutions, affect the conduction of electrical current. EC of honey depends on the content of inorganic salts, organic acids, proteins, complex sugars and mineral content. Minerals, after burning honey, remain as ash. Honey produced from colonies fed with sugar syrup is reported to have low ash content. Higher the content of ions and organic acids, higher the EC (Adenekan et al., 2010). EC is used to discriminate between honeydew and blossom honey and for characterization of unifloral honey. Honey with EC >0.8 mS/cm is considered as honeydew honey and <0.8 mS/cm as blossom honey (Codex Alimentations, 2001). Further conductivity is also based on the colour, dark honey is more conductive (Feas et al., 2010).

4.7. Sugars (Carbohydrates)

The key sugars present in honey are the monosaccharides fructose and glucose. It also contains 25 different oligosaccharides viz., sucrose, maltose, trehalose, turanose, panose, 1- ketose, 6-ketose, palatinose etc. (Bogdanov, 2008). During digestion of honey, principal carbohydrates fructose and glucose are quickly transported into the blood and can be consumed for energy requirements by the human body. A daily dose of 20% of honey will be equivalent to about 3% of the required daily energy (Saha, 2015). Honey can be differentiated on the basis of types of sugars and their content for e.g., Metcalfa honey, a new honeydew honey type, produced mainly in Italy, can be distinguished from other honeydew honeys as it is rich in maltotriose and contains particularly high amounts of oligomers called dextrins. Honey adulteration with syrups (e.g., maple syrup, sugar cane syrup) should be detected as recommended by CAC, 2001. Crystallization of honey can be detected by measuring glucose to fructose ratio since crystallization is accompanied with lower content of fructose and higher content of glucose (Ratiu et al., 2020). Adulterated or overheated honey samples are characterized by higher sucrose content of 8%. Sugars are determined by-Fehlings test and HPLC Carbohydrates profile (Maher et al., 2018). The fructose to glucose ratio indicates the crystallization state of honey. At high ratio, the honey is fluid (Ouchemoukh et al., 2010). The ratio equal to or less

than 1.14 leads to fast growth of honey crystals, whereas ratio greater than 1.3 results in slow growth of honey crystals but proportion higher than 1.58 decreases its value (Parihar et al., 2021).

4.8. Hydroxy methyl furfuraldehyde (HMF)

HMF content of honey is an indicator of improper heating, long storage or adulteration of honey with invert sugars (White and Doner, 1980). Higher the HMF content, lower the quality of Honey. HMF is an aldehyde which is generated by the decomposition of fructose in acidic conditions (Miaillard reaction-a non-enzymatic browning). It occurs naturally over time in most honeys, high levels of HMF is the result of inadequate storage, adulteration with sugar additives, or severe heat treatment (Saxena et al., 2010). Freshly extracted honey displays HMF levels lower than 5 mg kg^{-1}. Honey stored at low temperature has low or minimal HMF concentrations, while aged honey stored at comparatively higher or medium temperatures has high HMF concentrations (Shapla et al., 2018). In addition to storage conditions, the use of metallic containers and honey floral sources are critical factors affecting HMF levels. Codex Alimentarius Standard Commission has set the maximum limit for HMF in honey at 40 mg kg^{-1} (with a higher limit of 80 mg kg^{-1} for honey originating from tropical regions) to ensure that product has not undergone extensive heating during processing and is safe for consumption. HMF content is measured by-Fiehe's test: HMF reaction with resorcinol results in the formation of cherry red color, which indicate a positive reaction. Whereas, a negative reaction is indicated by the appearance of a slight pink colour which disappears after a short time or a yellow to salmon pink colour. Measurement is done only when test is positive by measuring absorbance at 540 nm in spectrophotometer using 3ml of distilled water as a blank. An increase in temperature by 10–C cause the reaction to occur 5 times faster.

4.9. Acidity

The acidity of honey is caused by organic acids (tartaric, citric, oxalic, acetic etc.), nectar or bees secretions an increased value of acidity denotes the beginning of the fermentation process, through which the produced alcohols are transformed into organic acids. Acidity contributes to honey flavor, stability against microorganisms, enhancement of chemical reactions, antibacterial and antioxidant activity. Higher acidity is an indicator of sugar fermentation which is converted into organic acid (Gomes et al., 2011) while low acidity value indicates the freshness of honey samples. The acidity of honey can be measured by titration against sodium hydroxide equivalents. Natural acidity of honey may increase when they grow older, when it is extracted from combs with propolis, and especially when it deteriorates due to fermentation. Moreover, honey adulterated with sugar syrup has a very low acidity (less than 1) while that adulterated with inverted sugar syrup has a higher acidity.

4.10. Amino acids

Proteins and amino acids in honey are derived from animal or vegetal sources, fluids and nectar secretions of the salivary glands of honeybees, but pollen represent the main source of proteins. Therefore, amino acid profile of honey could be characteristic of its botanical origin, and geographical origin. Protein content of floral honey varies from 0.1 to 1.5%, while in honeydew honey this quantity is 3.0%. Proline is the dominant amino acid in honey with 50-80% of the total amino acids (Samira, 2016) which is produced by salivary secretion of honeybees during conversion of nectar into honey. Its content in honey constantly decreases during storage and therefore proline might be an indicator of honey ripeness. Other amino acids present in honey are glutamic acid, alanine, phenylalanine, tyrosine, leucine and isoleucine being the most common (Jovanov et al., 2019). Other amino acids glutamine, histidine, glycine, threonine, arginine, valine, methionine, cysteine, tryptophan, lysine and serine are also present but in lower amounts.

4.11. Vitamin-C

Vitamin B complex and vitamin C represent a minor portion of honey. vitamin C is found in almost all types of honey, and its concentration and antioxidant capacity depend on the processing and storage of honey as well as on its botanical origin. A low acidity value indicates the freshness of honey sample, while high acidity indicates the fermentation of sugars into organic acids. Variations due to botanical sources in Vit. C are also reported (Thakur et al., 2021). Vit. C is very vulnerable to chemical and enzymatic oxidation and has an accelerated rate of change due to various factors such as light, oxygen or heat therefore determination of vitamin C is an unstable indicator

4.12. Phenols

Total phenolic content is a good criterion to determine the quality and responsible for antimicrobial and antioxidant activity of honey (free radicals scavengers) (Cianciosi et al., 2018) . Phenols can be determined by Calorimetric method using Folin Ciocalteu as the reagent. The reaction between Folin- Ciocalteu and phenolic compounds results in formation of blue colour which allows quantification using gallic acid as standard (Gupta et al., 2021). Variations in phenolic content may be due to beekeeping practices, climate conditions and biochemical changes in honey constituents. (Parihar et al., 2021).

4.13. Diastase content

Diastase (alpha and beta amylase) is one of the predominant enzymes in honey, which is added to honey by the bee during

the collection and ripening of flower nectar. Diastase in honey converts starch to short-chain sugars and its activity indicated possible heating and/or poor storage conditions. Diastase activity decreases with increase in temperature and heating time and depends upon geographic and floral origins of the product, also on its freshness (Huang et al., 2019). One unit of diastase activity is defined as that amount of a- amylase, which will convert 0.01 gm of starch to the prescribed point in 1 hour at 40 degree Celsius and expressed in Schade units per gram of honey (Diastase number DN). It can be measured by spectrophotometer at 620nm. In honey with low natural enzyme content e.g. lemon honey, the minimum activity must be at least 3 DN.

4.14. Mineral compositions

Honey is rich in macro and microelements minerals such as potassium, magnesium, calcium, iron, phosphorous, sodium, manganese, iodine zinc, lithium, cobalt, nickel, cadmium, copper, barium, chromium, selenium, arsenic, and silver that are found in different honeys indicating the botanical origin of specific honey (Alqarni et al., 2012). Potassium is the most abundant element, corresponding generally to one third of the total mineral content found in honey (Solayman et al., 2016). Heavy metals (arsenic, mercury, lead and cadmium) are toxic if the maximum limit is exceeded. WHO and the FAO proposed acceptable levels of 15 μg kg^{-1} for arsenic, 25 μg kg^{-1} for lead, 5 μg kg^{-1} for mercury and 7 μg kg^{-1} for cadmium. Increased concentration of minerals near industrial areas has been observed, therefore, quantification of toxic mineral elements in honey is important w.r.t human health, safety and environment.

5. FACTORS AFFECTING HONEY QUALITY AND COMPOSITION

5.1. Influence of geographical origin on honey quality and composition

Chemical composition of honey strongly depends on the kind of nectar flow. Honey with same floral origin but from different locations may have a different composition (Kaskoniene and Venkutonis, 2010). Properties and compositions of honey depend on its geographical floral origin, season, environmental factors and treatment of beekeepers (E L-Metwally 2015). Study of Thakur et. al., 2021 from different agroclimatic zones of Himachal Pradesh, concluded that honey from zone 4 (high hills, Temperate dry zone >2200 m amsl) had statistically highest fructose, F:G ratio, acidity, vitamin C and diastase, whereas pollen density, pH sucrose, HMF, amino acid, phenols, Ca and K were statistically highest for zone 2(mid hills, Sub humid Zone). Studies concluded that honey from different locations of four agro-climatic zones of Himachal Pradesh was well within limits and had positive correlation between pH and electrical conductivity; moisture and pollen density.

5.2. Influence of the different botanical origin of honey plant on quality and quality of honey

Botanical origin is one of the principle factors influencing the composition and quality of honey (Ahmed et al., 2012). Bees mix the collected plant compounds with salivary gland secretions and store the mixed product in honeycomb for maturation. Different flora honey have different composition and varies in quality depend upon different botanical origin (uniflora, multiflora and honeydew honey) (Yadav and Satyajeet, 2014). Schiassi et al., 2020 evaluated different single-flower assa-peixe (*Vernonia polysphaera*), bracatings (*Mimosa scabrella*), coffee (coffee spp.), Laranjeira (*Citrus sinensis*), silvestre and vassourinha (*Baccharis* spp.), polyfloral, extrafloral and honeydew honeys (brazil) with regard to their physico-chemical characteristics. All honey complied with quality parameter standards. Sugarcane honey had more orange colour when compare to others. Assa-peixe, laranjeira and coffee monoflower honey exhibited the highest viscosity, thus confirming the influence of moisture on this parameter. Sugarcane had highest antioxidant property and bracatinga had highest phenolic content. Honey dew honey had bitter, alcoholic, and astringent tastes and extrafloral honey had burned smell.

5.3. *Effect of different seasons on quality and composition of honey*

Foraging activity of bees depend on the season, the biological type of plants and the selective behavior of bees (Nguemo, 2016). Seasonal management of bee colonies can be based on pollen and nectar analysis from flowers which constitute practically the only food source for bees from larval through adult phases (Bastos et al., 2001). Honey color varied with season. Honey from rainy season were more diversified with five colors (amber, black red, light brown and black) compared to those from dry season (amber, black amber, dark brown).

5.4. Beehive technology as a factor affecting quality and composition

Modern and traditional bee hives are commonly used by bee keepers for honey production (Morad, 2008). Low-technology hives are developed for obtaining the advantages of movable frame hives (Nyau et al., 2013) (no need to break combs, standardization, manageability, efficient honey harvest) without the disadvantage of high cost manufacture (Bett, 2017) and can be kept near home and examined and honey harvested is of good quality and free of contamination (pollen or brood) (Beslo, 2004).

5.5. *Effect of harvesting on honey quality and composition*

There are broadly two methods of harvesting honey viz., traditional (honey hunting) and modern methods which

effects the quality and acceptability of the honey and create impression of adulteration when on shelves (Babarinde et al., 2011). The product from honey hunting is a mixture of ripe and unripe honey, beeswax, dead bees and other debris but it does not conclude that the product is of low value (Bradbear, 2009). It is therefore essential to investigate the actual effects of these harvesting methods on quality of honey and its acceptability.

5.6. Effect of processing and storage on honey quality and composition

Heat or thermal processing of honey eliminates the microorganisms, retards fermentation process (Eshete et al., 2019), eases processing and bottling, reduces water content, dissolves the sugar crystal nuclei to retard granulation, homogenizes honey color. HMF content which is an indicator for honey freshness increase and diastase activity decreases for overheated, aged and poorly stored honey. Heating honey to higher temperatures of more than 70 degree Celsius is not suitable as it causes alteration of flavor, colour and granulation of honey, degrades bioactive compounds and oxidants deteriorating the honey (Hasan, 2013).

5.7. Influence of agrochemicals on honey quality

Honeybees are used to monitor environmental pollution, since accumulations of Pesticides residues viz acaricides, organic acids, insecticides, fungicides, herbicides and bactericides certain metals and other substances are measured in hive products (Choudhary et al., 2008). These insecticides, herbicides and fungicides are applied to crops, but reach the bees through pollen, nectar and through air, water and soil (Yadav, 1995). Many of these contaminants are banned because of their effect on health (carcinogenic). This occurs when bees are on the flowers at the time of application of pesticides and the bees die instantly. Some other types of insecticides allow the bees to return home and then they die. worker bees of all ages are susceptible to pesticide exposure but bee wax contamination affects broods. the main reason for toxicity is grayanotoxins, also known as andromedotoxins. (Irungu et al., 2016) reviewed the problem of residues of pesticides and concluded that over the history of use of 75 years of inorganic insecticides, arsenic residues occurred in the range of traces to 0.2 ppm in a few honeys. residues in honey drift from adjoining areas. Insecticide spray were responsible for a number of fatal incidents with bees and also continuous contamination of honey which leads to production of low quality of honey. All pesticides are toxic and several of them are potential carcinogens which may cause chromosomal abrasions. Pesticides are also known to cause changes in the endocrine, the reproductive and the nervous system. Therefore, monitoring of pesticides residues in the commodity is essential to ensure its quality and safety. All the pesticide residues had concentration lower than the recommended EU maximum residual limits, limit of quantification (LOQ) (0.05 mg kg^{-1} in honey) (0.01 PPM).

5.8. Effect of adulteration on quality and composition of honey

Adulteration is a complex process which has been performed directly via addition of commercial sugar syrups to the honey or indirectly via overfeeding honey bee colonies with these commercial industrial sugars during the main nectar flow period. Many researchers reported that the use of excessive sugar adversely affect sugar content, mineral matter content and proline content of honey (El-Bialee and Sorour, 2011).

6. CONCLUSION

Honey has innumerable nutritional properties make it must, especially during the ongoing pandemic as it is known for boosting immunity. Physicochemical constituents influence quality of honey thus great importance in honey industry. Improvement will be achieved through building capacity and awareness of beekeepers. The demand of honey is also witnessing in an upward trend, thus the analysis of these quality parameters help us to ensure that the honey we are consuming fulfills the required parameters.

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