CHAPTER 2

REVIEW OF LITERATURE
Review of literature is undertaken to focus upon the analytical studies of honey in different parts of the world and with specific reference to India. The physical and chemical properties of honey are related to other analytical studies in order to achieve the main objectives of study.

2.1 Analytical studies on honeys

Physico-chemical analysis of honey provides information regarding the various physical and chemical properties which are governed by its major and minor constituents. Several of these properties are of great economic importance in the honey industry as they are essential for storage and marketing purposes.

Many investigators have studied the different physico-chemical characteristics of honey from Europe, America and Australia (Browne, 1908; Lothrop and Paine, 1931; Chataway, 1933; Fulmer et al., 1934; Schuette and Huenink, 1937; Schuette and Triller, 1938; Lothrop, 1939; Schuette and Woessner, 1939; Eckert and Millinger, 1939; Munro, 1943; Pryce-Jones, 1950; Hanson, 1951; Dean, 1953; Helvey, 1954; Fini, 1966, 1967; Martin, 1958; Chaika, 1961; Gamero et al., 1966; Langridge, 1966; White, 1975a, b, c; Bogdanov, 1987; Anderson and Perold, 1964; Aganin, 1965; Brande et al., 1967; Smith, 1967; Vorwohl et al., 1968; Petrov, 1970; Townsend, 1970; Ivanov, 1978; Battaglini and Bosi, 1973; Doull et al., 1977; Bianchi, 1979; Citrilli et al., 1973; Crane,
Similarly work on the physico-chemical characteristics of Asian and African honeys has been reported by many investigators (Latif et al., 1956; Phadke, 1962, Phadke and Nair, 1967a, 1967b; Phadke, 1968; Phadke and Nair, 1970, 1973; Iwaida et al., 1969; Rajebhonsale and Kapadnis, 1970; Echigo et al., 1975; Lin et al. 1977; Fernando, 1978; Nair, 1980; Chung et al. 1984; Mahajan, 1984; Hussein, 1988; Kassaye and Gadegaba, 1988; Ibrahim et al. 1977; Ebrahimzadeh et al., 1979; El-Sherbiny, 1980; Hepburn, 1982; Maishihah and Kiew, 1989; Vorwohl et al., 1989; Brar et al., 1992; Gupta et al., 1992; Tilde and Payawal, 1992; Wakhle, 1997; Mishra, 1997)

An attempt has been made to review the analytical studies done on various aspects physical and chemical characteristics of honey analysis by different investigators in the following sections.

2.2 Physical properties of honey and its analysis

Physical analysis of honey reveals that it is an aqueous dispersion of materials of varying particle sizes. Although sugars are the major constituents of honey, yet various physical characteristics of honey such as refractive index, viscosity, density and conductivity differ somewhat from an invert sugar solution of same moisture content because of the presence of other minor constituents as well as different ratios of different sugars in various honey samples (White, 1975 a, b, c). Each of the properties mentioned above has been used as the means of measuring moisture content of honey which is very important for knowing the likelihood of fermentation. The determination
of water content of honey is very important since, except for sugars, water is the major constituent.

Browne and Young (1908) made a direct gravimetric determination, using a vacuum oven. The procedure is included in association of analytical chemists in 1970. However, based upon the research of Chataway (1932), in which the viscosity, refractive index and total water (determined gravimetrically by drying) were compared, charts were prepared which enabled water content to be determined by measuring either viscosity or refractive index, and consulting tables referred to as Chataway charts. The use of refractive index and Chataway's table is an alternative official method for determination of water in honey. Minh et al. (1971) used a temperature-compensated hand refractometer to determine total solids and water in diluted honey samples. The studies on techniques and determination of refractive index, specific gravity, viscosity, surface tension, electrical conductivity and pH are reviewed in separate sections.

2.2.1 Moisture content and refractive index

One of the important optical properties of honey is its refractive index which provides as easy way of estimating the water or moisture content of honey, a value which determines whether and when honey will ferment at a given temperature. Moisture content in honey varies independently of the amounts of honey constituents. Moisture content of honey samples can be determined either by direct method comprising of direct heating/drying the sample and then measuring water content, or by indirect method of refractometry at 20° C or 40° C, density and viscosity determination of moisture content of honey. Hanson (1951) on analysing 17 Swedish honeys found
that their average water content was 18.5 percent as compared with 19.5 percent for
summer honeys, however, both these types contained almost the same amounts of
sugars, proteins and ash as other honeys, but slightly more diastase. Hanson (1951)
determined water content of Swedish honeys by drying in vacuum, Karl Fisher method
and refractometric method. Experimental errors were the lowest in refractometric
method and maximum in drying in vacuum method. Examination of 21 honey samples
from different floral sources by a new selective adsorption method in USA showed
average moisture content of 16.72 per cent (White and Maher, 1954). Sacchi (1955)
analysed 72 samples of Umbrian honeys from Italy and moisture percentage was
calculated according to the formula of Fulmer et al. (1934). He showed that if a value of
0.32 was subtracted from water content, the results were in close agreement with the
figures found in weighing method. Latif et al. (1956) measured water content in honey
samples from Pakistan, and it ranged from 14.3 to 18.6 per cent (average 16.4). Aso et
al. (1960) and Arai et al. (1960) reported moisture content in Japanese honey as 20.5% and 20.4% respectively. Dean (1963) suggested determination of water content by using
refractometer. He concluded that within normal limits applicable to honey there is a
direct relationship between refractive index and water content of honey, and tables,
originally worked out by Chataway (1933) and revised by Wedmore (1955).

According to Anderson and Perold (1964) the average water content of 66
South African honeys was found to be 15.76 percent and the one derived from
refractive index using Chataway table was 16.23 percent and refractive index at 25°C
ranged from 1.488 to 1.500.
White (1967) with the help of Eichhorn type hydrometer determined moisture content in honeys from USA and concluded that this instrument can be used with the accuracy of a hand refractometer. Gamero et al. (1969) surveyed 169 samples of Argentina honeys during 1968-1969. By dividing them into three groups corresponding to spring, midsummer and latter season honeys, the water content was found to be ranging between 14 to 25 percent (17.8% ± 1.48 S.E.), 15 to 21 percent (18.04% ± 1.31 S.E.) and 15 to 21 percent (18.07% ± 1.36 S.E.) respectively. Iwaida et al. (1969) recommended that in Japanese honey, moisture content of 23 per cent for domestic and 21 per cent for imported honey was considered adequate. In Taiwan honeys, the water content varied from 20 to 24 per cent (Lin et al., 1977). Hussein (1989) estimated that the water content of honey samples from Dhofar (Oman) ranged from 15 to 23 percent with an average of 18.7% ± 0.158 S.E. Refractometric method provided relatively higher water content than the method of direct drying at 70° C under reduced pressure, in 29 commercial and 3 extracted honeys from Japan (Iwaida et al., 1969). Refractometric method was also used along with vacuum drying to determine moisture content in 20 samples from Japan by Hase et al. (1972). Refractometric methods gave 1.5 to 1.8 percent higher moisture content than the first method. Therefore, either method could be employed for simple and rapid moisture determination of honey. Kassaye and Gadegaba (1988) investigated the moisture content of 342 honey samples from Ethiopia and found to be ranging from 15.25 to 30.45 percent. Fernando (1978) reported an average of 18-20% moisture in honey from hived colonies while honey from extra floral nectaries contain 25% moisture from samples from Sri Lanka.
A number of investigators have reported moisture content of Indian honeys, 19.2 percent in Madras honey (Giri, 1938). Nair et al. (1950) reported moisture content of 12.5-28.4 percent from Travancore honey samples obtained from *Apis dorsata*, *Apis indica*, *Apis florea* and *Trigona irridipennis*. *Apis dorsata* honey showed much lower water content and dextrin, while the protein contents and glucose/fructose ratio were higher than those of *A. indica* honey. Moisture content was 16.6 to 26.4 percent in sample from all over India (Mallick, 1958), 7.2 to 19.1 percent in Mahableshwar honey (Phadke, 1962, 1967a), 20.9 percent in samples from all over India (Phadke, 1967b), Perti and Pandey (1967) reported average moisture content of summer and rainy season honeys from *Apis dorsata* colonies as 17.0 and 25.9 percent respectively, 20.5 percent in Calcutta honey (Mitra and Mathew, 1968) and Narayana (1970) reported that Indian honeys have a higher moisture content and acidity than foreign honeys. Moisture content of 40 samples of honey from rubber estates in Kerala, Tamil Nadu and Karnataka were determined by Nair (1983). Mahajan (1984) determined moisture content of 15 honey samples from the Shimla hills of Himachal Pradesh which ranged from 16.45 to 20.45 percent (average 18.85 percent) and refractive index ranged from 1.486 to 1.496 (average 1.490). These values were lower than those observed from other parts of India. The average moisture contents in multifloral, *Plectranthus* and *Eucalyptus* honeys were 18.33, 19.45 and 19.25 percent. Six samples of honey stored from one year did not show a significant increase in moisture content. The moisture content of summer samples (18.65 percent) also did not differ significantly from those of autumn (18.57 percent). Ghoshdastidar and Chakrabarti (1992) found the moisture content ranging from 16.0 to 23.4 percent in honey samples from CBRI Pune.
Krell (1994) reported that many honeys from the tropics have very high moisture content, even when utmost care is taken to harvest only sealed honey. In South India and Sri Lanka moisture content can be up to 22 percent in rubber honey.

2.2.2 Density and relative density/ specific gravity

Relative density of a liquid is determined either by direct weighing of a known volume or by use of a calibrated hydrometer floating partially immersed in liquid. Although, the use of hydrometer is potentially much easier and less expensive than pycnometry but on account of nature of honey, both procedure become comparable.

The earlier work was done by Snyder (1933) who compared density (in pounds/gallon) by direct weighing of 1/4 to 1/2 pint, pycnometer using undiluted honey and refractometer using related conversion and found the average values for 18 samples ranging from 11.859 to 11.867 pounds/gallon respectively. Similarly Marvin (1933) reported average values for 37 floral honeys from different parts of Europe as 11.838 and 11.845 pounds/gallon respectively. Chataway (1933) determined relative density using a large sensitive Baume hydrometer. Marvin (1933) also made use of hydrometric method for determining weight/ gallon for honeys of USA.

Pryce-Jones (1950) determined density ranging from 1.396 to 1.452 g/cc in samples from South Carolina. Dean (1953) determined specific gravity of 89 samples of English and Scottish honeys and showed that model class had a specific gravity (at 60°F) ranging between 1.411 and 1.420 with an average value of 1.414. 115 English, Scottish and Irish honey samples for the next year had a mean specific gravity of 1.4162. Deans (1963) suggested to determine specific gravity and water content by
means of hydrometer. The hydrometer consists of a floating glass tube, weighted at one end. When inserted into a container of honey, the instrument comes to rest at a level corresponding to the specific gravity of the sample, and the hydrometer can be calibrated for use at specific temperatures in order to note the temperature of honey samples.

Studies on some Japanese honey samples showed that specific gravity ranged from 1.310 to 1.410, but generally its value was higher than 1.333 (Watanabe and Gota, 1956). Anderson and Perold (1964) analysed 66 South African honey samples and showed that specific gravity at 20°C varied from 1.417 to 1.445. Aganin (1965) used the suspended drop method in which the specific gravity of honey samples is compared with a solution of Calcium perchlorate which has the same specific gravity as honey containing the 22 percent value. Fini (1966, 1967) revealed that specific gravity ranged from 1.39 to 1.44 in Italian samples. Pertl and Pandey (1967) studied some honey sample from Nainital region of Uttar Pradesh and found that specific gravity ranged from 1.349 to 1.440 (mean value 1.404) and it could not be correlated with moisture content. Nair (1983) studied the specific gravity of 40 honey samples from rubber estates of Kerala in South India. It was estimated that 50 percent of honey produced in India is derived from extrafloral nectar of rubber (*Hevea brasiliensis*). Mahajan (1984) analysed some honey samples from Shimla hills for their physico-chemical characteristics. She showed that specific gravity of these honey samples from Shimla hills for their physico-chemical characteristics. She showed that specific gravity of these honey samples varied from 1.408 to 1.436 with a mean value of 1.422. Specific gravity decreased with the increase in temperature in all the honey samples. Physical properties of citrus, clover and cotton honeys from Egypt and Dokhla oasis showed that citrus
honey samples had the minimum specific gravity (Ibrahim et al., 1977). Specific gravity of honey samples is Dhofar (Oman) ranged from 1.387 to 1.446 with a mean value of 1.420 ± 0.001 (Hussein, 1989).

Helvey (1954) determined several heat related properties in honey samples of United States. Using conventional methods, he reported that the specific heat of honey containing 17.4 percent moisture is 0.54 at 20°C and temperature coefficient is 0.02 cal/°C. He also determined the thermal conductivity of honey solutions over the range from 0 to 90 percent water content at various temperatures and reported that finely crystallised honey at 20°C had thermal conductivity of $129 \times 10^{-5}$ cal/cm sec°C. Heated thermocouple method for thermal conductivity determinations had been used by Artecka et al. (1975) to study honey samples in USA.

2.2.3 Viscosity

Viscosity in technical is used to designate the flow of liquid. Viscosity of honey influences its characteristics that are important in its utilization for specific industrial purposes. High viscosity is important in considering when honey is to be used for consumption. Viscosity is an important factor in extracting, blending, processing and controlling speed of crystallization. Lothrop (1939) studied the effect of composition of honey on viscosity. Even when adjusted to equal moisture content, the viscosity (at 40°C) varied from 3.10 poise for Alfalfa honey to 4.11 poise for Sumac honey. He concluded that viscosity variations in honey samples were due to non sugar materials such as dextrin and colloidal materials. It was concluded by Lothrop (1939) that honeys showing highest viscosity contain lowest water content. Also higher viscosities were
shown by samples having higher proportion of non-sugar substances. He also said that variation in the proportion of dextrose and levulose that occur in honey produce a corresponding influence on the viscosity.

Earlier studies on viscosity measurements was made by Munro (1943) who used a viscometer for determining viscosity of sweet clover (Melilotus) honey, sage (Eriogonum) honey, white clover (Trifolium) honey, at three, six and nine moisture contents respectively. He noted that one percent moisture is equivalent to about 3.5° C rise in temperature in its effect on viscosity, and that viscosity changed most rapidly as the temperature rose to room temperature and above that effect was slow. Analysis of South African samples showed that viscosity values in these ranged from 538 to 3452 centistokes (Anderson and Perold, 1964).

Rajebhonsale and Kapadnis (1970) determined viscosity of some unifloral honey samples from Mahabaleshwar (Maharashtra) in India. They observed that the viscosity of Pisi, Hrida, Jambul and Gela honeys were respectively in the increasing order of magnitude in temperature of 26° C to 66° C. A temperature below 45° C, the viscosity increased very rapidly, but this gradient was low above 45° C. Mahajan (1984) analysed some multifloral, Plectranthus and Eucalyptus honeys from A. cerana colonies located in Shimla hills during summer and autumn seasons. She found that viscosity ranged from 9.601 to 111.807 poise with an average of 32.316 at 30° C which was suggested as the optimum temperature for viscosity determinations.
2.2.4 Colloidal properties and surface tension

Lothrop and Paine (1931) and Paine et al. (1934) conducted earlier studies on colloidal properties of honey and reported that colloidal dispersed material in honey showed an isoelectric point of 4.3 on the examination of 25 per cent solutions of floral honeys and honeydew honeys. Paine et al. (1934) found that 20 ultrafiltration produced an average change in surface tension from 47 to 60.2 dynes/cm and noted the accompanying changes in foaming and retention of air bubbles. Mahajan (1984) analysed some honey samples from Shimla hills and concluded that surface tension in all the samples ranged from 101.737 to 121.737 dynes/cm with a mean value of 106.178 dynes/cm.

2.2.5 Electrical conductivity

Electrical conductivity depends upon various mineral constituents as well as upon ash content of honey. Stitz and Szigvart (1931) showed that values of electrical conductivity measurements of some honeys at 50 percent concentration showed the values ranging between 0.868 to 3.64×10^-4 mho/cm. Vorwohl (1964) analysed some samples from Germany and found maximum values at 20 to 25 percent solids, with values for undiluted honey around 10^-6 to 10^-7 mho/cm. Electrical conductivity values for 20 percent solution ranged from 0.85 to 8.47×10^-4 mho/cm. In floral honey samples, it ranged from 0.6 to 1.46×10^-4 and 6.3 to 6.41×10^-4 mho/cm for honeydew honeys. He proposed the use of conductivity with pollen analysis for identifying honey sources and for determining proportions of honeydew honey. Andrelowicz and Kotlarek (1968) found specific conductance of 5 percent solution of Polish honeys as 100×10^-6 mho/cm.
Studies on electrical conductivity of 279 Italian monofloral honey showed that it could replace measurement of ash content in official analytical methods (Accorti et al., 1986).

Aganin (1971) analysed some Russian lime, sunflower and buckwheat honeys and found that electrical conductivity in these varied from 0.20 to \(1.34 \times 10^{-4}\) mho/cm. Electrical conductivity was found to decrease with decrease in acidity and mineral content of honey. It was also established that electrical conductivity determination can be used as a supplementary method for determining botanical origin of honey. Electrical conductivity of honey produced at different times of year, in various areas of Argentina ranged from 1.73 to \(13.8 \times 10^{-4}\) mho/cm (Bianchi, 1978). Mahajan (1984) found that electrical conductivity ranged from 0.029 to \(0.394 \times 10^{-4}\) mho/cm with a mean value of \(0.139 \times 10^{-4}\) mho/cm in some honey samples from Shimla hills of Himachal Pradesh.

Sancho et al. (1991) studied electrical conductivity in honeys from Basque country (Spain) of 115 samples and analysis of electrical conductivity ranged from 0.01 to 0.16 mho/cm\(^{-4}\).

2.2.6 pH

Anderson & Perold (1964) found the pH ranging between 3.36 to 4.62 in South African honeys. Hoang et al. (1971) determine an average of 3.4 pH on samples of *Apis dorsata* honey and one composite sample of *Apis mellifera* honey all collected in the Phillipines. Detailed physico-chemical analysis of Italian honeys by Cirilli et al., (1973) showed that the pH of the different samples was in acidic range (4.37). Lin et al., (1977) studied 13 honeys from different parts of Taiwan and pH in these samples ranged from 3.8 to 5.9. pH was found to be 3.9 in 158 honey samples from several areas of Finland.
(Varis et al., 1983) Salashinski et al. (1980) studied honeys from Primorki, Krai, Bashkir and Ukrainian regions and pH in these samples was 4.57 ± 0.30 S.E. Ponicini and Wimmer (1983) analysed six honey samples from different areas of Fiji and found a variation from 3.8 to 4.0 in their pH values. Chung et al. (1984) analysed 5 samples from different parts of Taiwan and their pH ranged between 3.23 to 4.32. Persano et al. (1986) studied 737 samples of Italian honeys for their pH determination. Highest pH values were found in Castanea honeys and honeydew honeys (usually > 5) and lowest in *Hedysarum* honeys. Hussein (1989) studied honey production in Dhofar (Oman) region and pH values varied from 3.8 to 8.3 with a mean of 5.34 ± 0.082 S.E. Bonvehi and Coll (1993) studied French Lavender honey from Spain, the pH of all the honey samples analysed ranged from 3.31 to 4.03.

### 2.2.7 Crystallisation

Pryce-Jones (1950) stated that levulose / dextrose ratio, along with many physical characteristics within wide limits, were related to the differences in rate of granulation of honeys. Analysis of 500 honey samples in United states suggested that granulating tendency of honey is related to its composition (White et al., 1962). The role of water activity in honey crystallisation showed that the tendency of honey crystallisation can be predicted from the ratio of glucose to water, but this was not applicable to honeys containing less than 4 percent water. The tendency of honeys to crystallise, which contained less than 8 to 16 percent water was found to be universally related to hygroscopicity while studying the changes in colour during crystallisation in samples from France it was seen that during controlled granulation its luminance.
changed though the dominant colour was not affected, slow granulation caused a
decrease in luminance, after a few days honey became clear, but its 'colour purity' was lower (Gonnet et al., 1986). Granulated 8 samples from same source (water content 18.2 %) were taken, creamed honey was produced after seeding with 5-10% starter for 2.3 hours and then 3 times daily. After 2 days, the consistency became thick (Bogdanov, 1987).

2.2.8 Hygroscopicity

Depending upon the relative humidity and temperature, honey absorbs moisture, thus becoming diluted and has a tendency to ferment. As the viscosity of honey is high, so the moisture absorbed at the surface can diffuse very slowly to the surface. Martin (1958) showed that within seven days a honey sample had 22.5 percent moisture at surface, while 2 cms below the surface, no change was found. Borukh and Panchenko (1973) studied hygroscopic characteristics of honey in relation to humidity equilibrium. Similarly, Doull and Mew (1977) studied Australian honey samples at 34.5° C for hygroscopic properties at different dilution. Liquid honey with 82.5 percent solids was in equilibrium with atmospheric water vapour at relative humidity of 54.4 percent. Only dilution containing less than 40 percent solids maintained humidity close to optimum range for hatching of eggs and survival of young larvae of honeybees. It was suggested that bees were exhibiting a specific response to humidity within cells.

The composition of honey determines its value as a nutritional and medicinal product. The major components of honey are the sugars (about 80 per cent) and water (17 to 20 per cent) in which sugars are dissolved. In addition, so far 181 different
substances have been identified in honey and some of them are unique and do not exist anywhere else. These substances make up only a small part of total components of honey. Important minor elements are: minerals, enzymes, lipids, amino acids, proteins, organic acids etc. (Verma, 1990). These minor components of honey determine its aroma, flavours and colour. Physico-chemical properties of honey are determined by its major and minor elements (Crane, 1975).

2.3 Chemical analysis

2.3.1 Mineral and ash content analysis

The mineral composition influences the various characteristics of honey to some extent. The amount of dry matter present in honey is represented by its ash. Honey ash is further analysed to determine minerals separately. Thus determination of ash and minerals is inseparable from each other and is being carried out extensively.

Browne and Young (1908) found that the ash content of honey averaged about 0.18% but that in some samples it was as high as 0.90%. Schuette and Remy (1932) reported on the silica, iron, copper and manganese contents of light and dark honeys, and their possible relationship to colour. Schuette and Huenink (1937), Schuette and Triller (1938) and Schuette and Woessner (1939) made additional studies on the mineral constituents of honey. They also made a comprehensive survey of US honeys to separate the mineral elements and provided the percentage ash content as well a part per million mineral elements. Various minerals reported were: Potassium, Sodium, Calcium, Calcium as lime, Magnesium, Iron, Copper, Manganese, Chlorine, Phosphorus, Sulphur, Silica and Silica crude. Analysis of honey samples from England
was conducted by Pryce-Jones (1950) who concluded that Iron, Copper, Manganese besides affecting various physical characteristics are largely responsible for honey colour. Root (1950) reported that the minerals in the ash included Silicon, Iron, Copper, Manganese, Calcium, Potassium, Sodium, Phosphorous, Sulphur, Aluminium and Magnesium. Monikowski (1961) analysed 13 samples of honeys calorimetrically at different times of year in Poland. He found the Iron content to be 3.4 to 7.3 ppm whereas, in honeys collected during August-September it was 10.5 to 4.5 ppm.

Chudakov (1964) reported a correlation between the ash content and pH of honey. He derived the following formula from experimental data: \( y = 0.47x - 1.49 \)
where \( y \) is percentage of ash content of honey and \( x \) is pH. The data further supported the buffer mechanism of hydrogen ion formation with participation of organic acids and mineral elements. A high correlation was also found between the volume of a precipitate obtained with Calcium hydroxide and the ash content of honey.

Studies conducted by Vermeulen and Pelerents (1965) on samples of Belgian honey suggested that Phosphorous and Iron contents of honey are related to plant origin. While another set of analysis conducted by Brande et al. (1967) on Belgian honeys of grasslands and Campine origin honey samples showed that they were rich in minerals. Honeys from the grasslands were characterised by high content of Aluminium, Boron, Copper, Magnesium, Zinc whereas those of Campine origin had high content of Magnesium, Zinc, Manganese and ash. It was suggested that dark honeys were rich in minerals and had a high ash content. Studies in some samples from Italy showed a mineral content ranging from 0.1 to 0.35 per cent and comprised of following elements; Potassium, Sodium, Calcium, Manganese, Copper and Silica (Fini, 1966, 1967).
Similarly trace elements found by Mladinov (1968) in 39 samples of Bulgarian honeys were Aluminium, Beryllium, Boron, Iron, Gold, Stannous, Potassium, Cobalt, Calcium, Lithium, Magnesium, Copper, Manganese, Molybdenum, Nickel, Sodium, Lead, silver, Silica, Strontium, Tellurium, Phosphate, Zinc and Zirconium.

Petrov (1970) used method of atomic absorption spectrophotometer in order to determine mineral contents of some light and dark honeys collected from Australia, which differed considerably in their constituents, especially in Cobalt, Aluminium, Manganese, Sodium and Silica contents. Phosphate, Copper, Chromium, Zinc, Nickel, Calcium, Antimony, and Lead were present in almost same quantities. Spectrographic determination of mineral contents from 12 different areas in Hungary by Varju (1976) revealed that following ash content was typical for Acacia honey that is Phosphate, Calcium, Magnesium, Iron, Boron, Copper, Manganese, Zinc, Stannous, Lead were 129, 178, 17, 2.8, 3.5, 0.29, 0.30, 5.17, 0.21, 0.05 ppm respectively.

For light coloured honey, Potassium content together with alkalinity of ash, gave some differentiation between honey from nectar and that produced by sugar fed bees of Poland (Andrelowicz and Kotlarek, 1970). Zinc content of honeys extracted directly from comb, was less than 5 mg/kg but it was up to 30 mg/kg in commercial centrifuged samples from Poland (Fedorwska et al., 1972).

Makarochkin (1972) subjected honeys from Tula Kazakhstan, Kherkov, Ukraine, South Urals and Gorno region of Russia to semi-quantitative spectral analysis. All the samples contained Silica, Aluminium, Magnesium, Calcium, Iron, Manganese, Nickel, Tellurium, Copper, Lead, Phosphorous, Sodium and Potassium. It was observed that micro element content of honey varied according to geographical areas. Rape
honey from Warsaw (Poland) analysed by Poszwinski (1972) contained 0.063 to 0.114 per cent dry weight of mineral contents. Small amounts of Iron, Manganese and Copper and large amount of Sodium, Potassium, Calcium, Magnesium, Phosphorous along with an ash content of 0.38% were reported in Italian honeys by Cirilli et al. (1973).

Investigations on some honey samples from forest zone of Ukrainian Carpathian mountains in Russia showed the ash percentage as 0.186 to 0.578 depending on the origin of honey. In these samples, Aluminium, Calcium, Potassium, Magnesium, Sodium, Phosphorous and Silica were present in high amounts, whereas, only traces of Cobalt and Zinc were detected. However, levels of Iron, Chromium, Copper, Manganese and Zinc were variable (Borukh and Panchenko, 1973).

Mineral level showed following variation in different honey samples of Bulgaria: Mn (6.87 to 67.92), Co (0.10 to 2.71), Zn (45.43 to 163.57), Fe (4.19 to 45.25), Ca (0.0 to 366.0), Na (0.0 to 44.0) and K (0.0 to 14.0) ppm (Shabanov and Ibrishimov, 1975). McLellan (1975) reported more potassium in honeys collected from different places of United Kingdom. Cirutti and Mannino (1979) found in some samples of Italian and imported honeys (1.97 to 12.31 ppm) Iron, (0.29 to 7.61 ppm) Manganese and (0.34 to 0.89 ppm) Copper. No correlation was found between sample colour and contents of these metals.

Atomic absorption spectrophotometric studies by Ivanov and Chevenakova (1984) on Bulgarian honey samples showed that Calcium ranged from 3.052 to 20.165 mg/100 gm and Potassium from 10.57 to 559.38 mg/100g. Aluminium, Ferrous, Zinc, and Manganese were present in much smaller quantities (<1 mg/100 gm) and amounts of Copper, Cobalt, Lead, Strontium were insignificant. Bogdanov et al. (1986) showed
the contents of 3 heavy metals for honeydew honeys and 18 honeys from different nectar sources in Switzerland and other European countries. Mean values (µg/g) for Lead, Cadmium and Zinc were 0.21, 0.019 and 4.53 in honeydew honey, whereas, these values were 0.09, 0.005 and 2.78 for nectar honeys respectively. Some samples taken from areas polluted with heavy metals such as Lead contents were twice as high as from other areas.

Many workers have also investigated ash and mineral contents of several Asian honey samples. Latif et al. (1956) showed that ash content in samples of Pakistani honey ranged from 0.11 to 0.32 per cent while 40 Indian honey samples analysed by Malik (1958) showed ash content ranging from 0.03 to 0.75 per cent. Paper chromatographic studies on Indian honeys revealed that amount of Calcium, Phosphorous, Iron and Magnesium ranged from 1.82 to 4.54, 1.72 to 3.42, 16.90 to 38.89 and 4.68 to 12.77 mg/100 gm respectively (Kalimi and Schonie, 1964). Indian honey samples were also analysed by Singh et al. (1975) by passing aqueous solution of honey through a column of cationic exchange resin and they determined the quantity of acid liberated. The ash values calculated by formula method were in close agreement with the sulphated ash content of samples determined by conventional methods. In Indian honeys, the ash content ranged from 0.03 to 0.52, 0.014 to 0.048, 0.03 to 0.46 and 0.11 to 0.25 in *Apis cerana* honey samples from Calcutta, Mahabaleshwar, Madras and all India representative samples.

Lin et al. (1977) evaluated 13 honey samples collected from different parts of Taiwan which showed an ash content of 0.1 to 0.3 percent. Ebrahimzadeh and Hagchenasse (1979) investigated 17 samples collected from different parts of Taiwan
which showed an ash content ranging from 0.07 to 0.60 percent. Levels of Nitrogen, Phosphorous, Sulphur, Sodium, Potassium, Calcium, Magnesium and Silicon were high, whereas, Iron, Aluminium, Zinc, Copper, Lead, Cobalt, Nickel and Cadmium were also fairly high, as in one sample Lead content was as high as 131.3 mg/100 gm of honey.

It was estimated by Nair (1983) that nearly 50 percent of honey produced in India is derived from the extrafloral nectar of rubber (*Hevea brasiliensis*). The mineral contents of these honeys were low, varying from 0.15 ppm for Copper to 97.42 ppm for Potassium. Mahajan (1984) studied honey samples collected in summer and autumn from *A. cerana* colonies located in Shimla hills and found a mean ash content of 0.27 to 0.36 percent.

According to Mahajan (1984), white or light coloured honey samples of *Apis cerana* from Shimla hills showed lower ash content (0.06-0.9 percent) whereas, dark coloured honey samples were higher (0.33-0.76 percent) in ash content. The total ash content in *Apis dorsata* honeys from Philippines, Pakistan and India averaged 0.17, 0.26 and 0.39 percent respectively (Minh et al., 1971; Latif et al., 1956; Phadke, 1968). In *Apis florea* honey samples, ash content varied from 0.48-0.54 percent with an average of 0.52 percent (Phadke, 1968). Mahajan (1984) reported an average ash content of 0.04 percent in *Apis cerana* honey samples from the Shimla hills. The average concentrations of Phosphate, Potassium, Calcium, Magnesium, Iron and Zinc in these samples were 118, 1432, 265, 109, 5.3, 3.71 and 11.9 ppm respectively. It was also found that electrical conductivity showed significant positive correlation with most of the minerals such as Potassium, Magnesium, Iron and Manganese content.
Bonvehi and Coll (1993) analysed French lavender honey found in Spain. It contained ash content ranging from 0.06 to 0.39 percent. Potassium content was the highest amongst all minerals 84.80 ppm to 1079.50 ppm while Manganese was just found in traces 0.48 to 1.90 ppm. Rodrigues et al. (1994) on analysing the mineral content of the honeys produced in Galacia (Spain) found the mean ash content to be 0.408 percent and concluded that Potassium was the most abundant of the elements with an average content of 1572 mg/kg.

2.3.2 Honey sugars

The average sugar composition was found to be glucose (32.29 %), fructose (39.28 %), sucrose (1.62 %), maltose (7.11 %) and higher sugars (1.03 %). Maltose, isomaltose, nigerose, turanose and maltose were identified by White and Hoban (1959) in American honey samples using infrared spectrum of free sugar and acetate. Pryce-Jones (1950) stated that levulose to dextrose ratio, many physical characteristics and reaction between sugars and amino acids are the main causes which darken honey with age. They concluded this by studying a large number of European samples. The honey sugars dextrose and levulose are monosaccharides; maltose, sucrose, isomaltose, turanose, maltulose, nigerose, kojibiose are disaccharides and melezitose, erlose, ketose, raffinose, dextranriose are trisaccharides or higher sugars. Deans (1963) said that any given sample of honey will not contain all the sugars and is extremely variable according to local flora and season. In an average composition of American honey sample the reducing sugars, levulose (37.92 %) and dextrose (31.15 %)
(monosaccharides) form the main sugars found in honey followed by sucrose (1.32 %), maltose (7.44 %) and higher sugars (1.69 %) (White, 1961).

Honey has the property of rotating the plane of polarised light, which is due to presence of sugars in the honey, their types and relative proportions. The overall effect is produced by concentration of one or other type of sugar. White et al. (1952) used this property to determine sugar content of honey, however, later this method was abandoned due to many shortcomings.

Johnson (1967) studied optical properties of some honeys and honeydew samples in Canada and formulated a generalisation that floral honeys are laevorotatory, while honeydew honeys are dextro-rotatory. White and Maher (1954) used selective adsorption method to analyse 21 samples from 19 different floral sources of USA. Similarly, Battaglini and Bosi (1973) concluded after studying Italian honeys, that specific rotation of each honey sample served as complementary parameter for differentiating the nectar honey from honeydew honey and individual honeydew honeys. Earlier similar studies been made by Browne (1908) who had observed mutarotation in 92 samples of USA honeys however, the cause and mechanism were unknown.

### 2.3.2.1 Procedures for determination of sugars in honey

Different physical properties of honey such as its viscosity, hygroscopicity and granulation depend on its sugar constitution and which form by far the largest portion of dry matter in honey. VanDine and Thomson (1908) analysed 54 samples of Hawaiian honey, some derived from honeydew. They used the procedures of Brown and Young (1908), Shaffer and Hartmann (1921) and Somoyogi (1926) and later Shaffer and
Somoyogi (1933) developed important methods for the accurate determination of reducing sugars in honey and other products. Eckert and Allinger (1939) analysed California honeys. Ellegood and Fischer (1939) also analysed fireweed honey using official A.O.A.C. methods. Lothrop and Holmes (1931) studied methods for determining D-glucose and D-fructose in honey by use of the iodine oxidation method, and showed that D-glucose/D-fructose ratios ranged from 1.02 to 1.70. They found rather close agreement between the high and low temperature polarization methods of Browne (1908) and the iodine-oxidation procedures.

White and Maher (1954) published a new method for determination of the sugars in honey, using a chromatographic column containing activated carbon in which different concentrations of alcohol were used to elute the individual sugars. It was necessary to use a fairly high air pressure on the column to produce sufficient rate of flow through the column. The carbon column adsorption procedures separated the sugars into monosaccharides, disaccharides and higher sugars used with suitable analytical methods for example Marshall and Norman (1938) for D-fructose, Lothrop-Holman (1931) for D-glucose, Shaffer-Somoyogi (1933) for reducing disaccharides such as maltose, honey sugars were determined with greater accuracy than previously. Recoveries of sugars added to honey ranged from 96.28% for sucrose to 100.46% for D-glucose. The method has been subjected to collaborative testing and accepted as first action by the Association of official Agricultural Chemists (White and Hoban, 1959).

Latif, Qayyum and Haq (1956) used the A.O.A.C. methods in their analyses of Pakistan honeys produced by Apis dorsata, A. indica and A. florea.
Austin (1958) analysed 36 Canadian honeys by A.O.A.C. polarimetric methods and by the White and Maher (1954) carbon column method. They reported that latter procedure indicated relatively large amounts of reducing disaccharides (as maltose) in all the honeys.

White and Hoban (1959) using the carbon column method separated the disaccharide fraction from honey and then separated the disaccharides present using paper chromatography. They found and isolated six disaccharides using paper chromatography from honey namely; isomaltose, maltulose, turanose, nigerose, maltose and sucrose. White and Hoban (1959) reported further refinements of the carbon column method.

Watanabe and Aso (1959) using a carbon-celite column followed by paper chromatography, isolated kojibiose from Japanese honey. In addition they found D-glucose, D-fructose, sucrose, maltose, melezitose, 8 di- or trisaccharides, and 9 higher oligosaccharides.

White et al. (1962) analysed 516 samples of honey, using carbon column chromatography to separate the sugars. Anderson and Perold (1964) studied 66 South African honeys using the method of White and Maher (1954) and Phosphorous, Iron, Manganese and Copper were determined by chemical methods.

Minh et al. (1971) analysed 7 samples of Philippine honey produced by Apis dorsata and one pooled sample of Apis mellifera honey using methods of White et al. (1962) with minor modifications.
2.3.2.2 Sugar contents in Honey

Chaika (1961) calculated the mean percentage of dry matter of 368 samples of honey from mixed European sources as was found to be 80.9 per cent. Invert sugar contributed 74.7 per cent of the mean percentage of dry matter. Baculinschi (1961) identified invert sugar, glucose, fructose, sucrose, dextrin in Rumanian honeys collected from Acacia, Lime, Clover, Sunflower, Raspberry, Marshland honeys and for mixed honeys. Anderson and Perold (1964) analysed 66 South African samples and identified fructose, glucose, maltose, sucrose and high sugars in it.

Poutallier (1968) determined sugars in honeys of France using gas chromatography and identified fructose, maltose, αβ-glucose, sucrose, trehalose, melebiose, raffinose and melezitose in a sample of honeydew honey. Analysis of 175 Bulgarian honeys by Ivanov and Mitev (1972) showed that amount of invert sugar varied from 62.20 to 77.76 per cent and total sugar content ranged from 68.98 to 79 to 87 per cent. Cirilli et al. (1973) reported total amount of glucose plus fructose as 72.3 per cent, sucrose as 5.55 and dextrin as 2.37 per cent in Italian honeys. Serrano and Kopecky (1975) analysed fructose and sucrose contents of Russian honey samples quantitatively. Sugar composition of 19 types of Danish honeys was determined and arranged according to their oligosaccharide content by Ravn et al. (1975). Most of the rape honeys and heather honeys contained about 5 per cent; honeydew honey 10 per cent and other unifloral honeys had 6.5 to 7.5 per cent oligosaccharides. 14 types of Yugoslavian honeys were reported to have 31.09 to 38.00 per cent glucose and 35.43
to 41.37 per cent fructose (Murko et al., 1976). Galal et al. (1979) analysed samples of Clover and Cotton honey from A R.E. Levulose (fructose) was found in a range of 38.2 to 42.5% in Clover honey and 36.1 to 40.3% in Cotton honey. Glucose (dextrose) was 31.1 to 35.8% in Clover and 32.0 to 36.7% in Cotton honey. The clover honey with an average of 2.078 L/D ratio was partially granulated while cotton honey with L/D ratio 2.180 was completely granulated. Varis et al. (1983) reported an L/D ratio of 1.1, average 47.3% fructose and 43.9% glucose in Finnish honeys. They also reported that glucose is the crystallizing constituent of honey. Low fructose/glucose ratio caused crystallization.

Shanon et al. (1979) studied the mean values of sugar contents of 4 samples from Iowa and Texas. Sucrose was found to be 1.5 per cent, glucose 36.8 per cent and fructose 46.1 per cent. Dozo (1984) analysed 66 samples of honey from different localities of Buenos Aires. The reducing sugars were in excess of 65.5 per cent the lowest value being 71.1 per cent. The samples contained 4.5 per cent sucrose and only one was found to contain more than 8% of sucrose. White et al. (1986) studied 87 USA honey samples and showed that the average value for 81 honeys was 3.1 mg (as galactose) per 100 g honey, raffinose is a commonly occurring sugar.

Similar investigations were carried out in Asia to determine the sugar content of various honey samples. Latif et al. (1956) investigated samples of Pakistan honey which had sugar percentages as follows; reducing sugars (71.1-76.9), fructose (39.01-53.81), glucose (21.7-34.2) and sucrose (1.9-2.75). The total reducing sugars, glucose, and fructose in honey samples extracted from Apis florea nests in India averaged 71.2, 32.3
and 38.9 per cent, respectively. Latif et al. (1956) reported lower total reducing sugars but higher fructose content in *Apis florea* honey samples from Pakistan.

Sugar content as well as effect of storage in 40 samples of Indian honeys was determined by Mallick (1958). Reducing sugar varied from 53.9 to 78.4 per cent. Five samples possibly from a sugar producing area, had more than statutory 10% sucrose. Phadke (1962) reported that 9 samples of unifloral honeys from *Carvia, Actinodaphna, Leucas, Eugenia, Randia, Terminalia,* three *Stroblanthus* species and *Carvia* sugars contained usually high amounts of non reducing sugars and unlike 8 others, it was dextrorotatry. Indian multifloral samples examined by Phadke (1967) had an average value of non reducing sugars, fructose and glucose as 3.4, 36.8 and 33.4 per cent respectively. Minh et al. (1971) studied chemical composition of 7 samples of *A. dorsata* and one sample of *A. mellifera* honey from Philippines. Total solids of *A. dorsata* honey, monosaccharides were the predominant sugars and there was more D-sucrose but less D-glucose than in *A. mellifera* honeys. Melezitose was present in only one sample, this all showed that *A. dorsata* honey contained less polysaccharides than *A. mellifera* honeys. 13 honey samples produced in Taiwan showed that invert sugar ranged from 66 to 77, glucose 30 to 36, fructose 34 to 40 and sucrose 0.1 to 5.7 per cent (Lin et al., 1977).

According to Mallick (1958) reducing sugars in Indian honeys varied from 53.9 to 78.4 percent, and five samples, possibly from a sugar producing area, had more than 10 percent of sucrose. Kalimi and Schonie (1964) confirmed the increase of higher sugars and decrease of monosaccharides during honey storage at 20° to 28° C for six to 12 months. These authors also reported that the total sugar content of unifloral honeys
varied from 74.7 to 80 percent and reducing sugars content of unifloral honeys varied from 72 to 78 percent of the total.

Other authors also analysed honey samples for their sugar content from Calcutta (Mitra and Matthew, 1968); Travancore (Nair et al., 1950) and Madras (Giri, 1938). These authors showed regional differences in percentages of total sugars, glucose, fructose, and sucrose, and in other properties. Phadke (1967a, b) found that the average value of non-reducing sugars, glucose, fructose as 70.2, 33.4 and 36.5 per cent, respectively in Indian multifloral honeys, whereas, in Mahabaleshwar (India), these percentages of glucose and fructose varied from 31.2 to 38.3 and 35.2 to 43.3, respectively (Phadke, 1967b). Ghoshdastidar and Chakrabarti (1992) analysed honey samples from C.B.R.I. Pune and concluded that they contained reducing sugars ranging from 68.6 to 75.1 percent. Total sucrose was between 0.8 to 6.2% and levulose/dextrose ratio ranged from 1.0 to 1.3.

Tzu-Chang et al. (1994) analysed samples from Taiwan. The data indicated that fructose content in honey collected from kept and wild honeybees in winter, were 38 percent and 45 percent respectively. The fructose to glucose ratios were between 1.1 - 1.2 in honey from kept honey bees and 1.4-1.6 in wild honey. The total sugar content in winter honey was 67 percent in other was 75 percent.

2.3.3 Hydroxymethyl furfural aldehyde (HMF) in honey

Hydroxymethylfurfuraldehyde are the compounds formed during commercial conversion of sucrose to form invert sugar. Its presence or absence has been used as test for determining purity of honey. Winkler (1955) showed that fresh honey contained
small amounts of HMF (0.06 to 0.2 mg/100 gm honey). Romann and Staub (1961) analysed European honey samples for HMF using the quantitative spectrophotometric method and got similar results. Piette (1965) related presence of HMF in honey to age. After studying 75 samples from different countries, different plants and seven years old samples from Belgium, he concluded that HMF values did not change greatly in honey. However, honey heated at 40°C for 48 to 120 hours led to the formation of significant amounts of HMF. Invert sugar (adulteration) can contain variable amounts of HMF and techniques reported were not capable of revealing its presence.

Similar studies were carried out for determining HMF values from honey samples in India. Deodikar and Phadke (1966) concluded that the Fiehe's test for presence of hydroxymethylfurfural (HMF) in honey should be regarded as quantitative rather than qualitative. According to HMF contents honeys may be graded as follows:

<table>
<thead>
<tr>
<th>HMF contents (µg/m of honey)</th>
<th>Grade</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>I</td>
</tr>
<tr>
<td>11-20</td>
<td>II</td>
</tr>
<tr>
<td>21-30</td>
<td>III</td>
</tr>
</tbody>
</table>

Phadke and Nair (1968) further suggested that 30 µg/m of 5-hydroxyethyl furfural per gram would be reasonable value for honeys produced in tropical climate. HMF content can serve as an indicator showing the influence of heat on honey during bottling and storage. This test is applied qualitatively in India at present. HMF levels above 40 ppm indicated either deterioration of honey through faulty or prolonged storage or presence of invert sugar as an adulterant in honey samples from West Bengal in India (Dhar et al., 1973). When honey was heated for 4 hours at 50°C or for ten
hours at 60°C, its HMF content increased only slightly. There was no change in HMF content of honey stored below 10°C, while addition of citric acid to samples enhanced the increase in HMF content. Addition of proline or Ferric compound had no such effect (Hase et al., 1973). Similarly, Japanese samples were analysed quickly and accurately by Motegi et al. (1987) by the HPLC technique for determination of HMF in honey.

Out of the 1724 samples of honey from Germany analysed by Vorwohl (1969), it was found that samples lying in range between 0 to 15 ppm and 5.4 per cent had greater than 40 ppm HMF. HMF content in 110 Polish honeys, which had been stored for upto 14 hours at 18° C to 25° C had been heated at 52°, 75° or 100° C for 540 minutes before storage were analysed by Krauze et al. (1970) who found that light honeys had less HMF than dark ones. White (1980) reported that HMF in honey samples from USA rose from heating, which could affect the validity of test for HMF as indicator of adulteration with invert syrup. It was suggested that a level of 20 mg HMF/100g honey would allow differentiation of honeys possibly adulterated with invert syrup (HMF content 170-650 mg/100 gm) from normally stored and processed 94 mg/100 g). Ghoshdastidar and Chakrabarti (1992) studied the formation of HMF formation during storage of honey at room temperature and found that HMF was correlated with temperature and increased more at higher temperature. Gupta et al. (1992) studied storage of honey at different temperatures for different time periods and concluded that in sensory qualities unheated honey stored at 5° C was found to be the best. Sancho et al. (1992) determined HMF content for 115 honeys at 4, 16 and 28 months after their extraction. The HMF content is influenced by the effect of heat and
storage which affects the heat and storage of honey. Abutarboush et al. (1993) reported H.M.F. higher than 40 mg/kg for Saudi Arabian honeys.

Zondel (1960) determined quantitatively very small amounts of fluorine i.e. 2.5 mg, for total quantities of fluorine up to 50 mg.

2.3.4 Acids

Generally, honey is slightly acidic, its pH values varying from 3.5 to 4.8. However, the acidity of honey is largely masked by its great sweetness. The acids contribute to the honey flavor complex. Until recently, it had been thought that citric acid was the predominant acid in honey.

The earlier work on free acids in honey involved titration of the total acid and its calculation as formic acid. Nelson and Mottern (1931) adopted procedures for separating the volatile and non volatile acids by steam distillation, and the formic acid and acetic acids were determined in the volatile fraction by selective destruction of the formic acid. Succinic, citric and malic acids were also determined in the non-volatile fractions by precipitation of the lead salts. It was found that the acidity of honey is primarily due to gluconic acid, which can be formed from D-glucose by certain enzymes. Giri (1938) studied 12 samples of Indian honeys for acid phosphates activity and reported that this enzyme was effective over pH ranges from 3.5 to 6.5, and at temperature of 35°C.

Phadke (1962) analysed honeys from Mahabaleshwar, the acidity in the samples in terms of formic acid varied from 0.037 to 0.173 percent which was within the maximum limit 0.2 percent prescribed in “Agmark specifications”. Phadke (1968) said
that acidity and ash content was higher in all wild Indian honeys. Acidity, as formic acid was 0.251 % for *A. dorsata* honey and 0.188 % for *Apis cerana indica*.

Hoang et al. (1971) determined that all honeys from Phillipines were acidic. The average free acid was 22.03 meq/kg while the lactone content was 1.44 meq/kg in *Apis dorsata* and 7.11 meq/kg in *A. mellifera* honeys.

El-Sherbiny et al. (1980) determined an acidity of 2.912 meq/100 gm for citrus honey from A.R.E. He also explained that acidity levels in honey seem to influence both flavour and stability. European standard honey have a maximum of 5 meq/100 gm whereas FAO standard is 4 meq/100gm.

Vit et al. (1994) reported 25.5 percent acidity in Venezuelan honeys. The high values for acidity suggested adulteration.

### 2.3.5 Flavour and aroma of honey

An infinite number of aroma and flavour variations can occur in honey, although there seems a definite and characteristics honey flavour. Generally the total aromatic effect is considered due to the presence of volatile compounds. The literature on aromatic materials in honey, before the introduction of gas liquid chromatography is scanty. Earlier studies were made identified diacetyl from German heather honey, while Nelson (1930) and Lothrop (1932) suggested methyl anthranilate in organic honeys.

The presence of fructose, glucose, gluconic acid and proline attributed to the flavour, was found by Maeda et al. (1962) in Japanese honey while 5 HMF was found to be the chief aromatic component by Merz (1963) using gas liquid chromatography. Cremer and Riedmann (1965) also used the same method for identifying aroma
components of different honey samples of Australia. It was found that phenylalanine is a precursor of specific aroma component in honey. Tsuneya et al. (1974) identified 27 aroma constituents in Japanese honeys, including 4 isopropullidine-2-cyclohexene-1-one.

Watanabe and Gota (1980) analysed ether extracts of unifloral honeys by gas liquid chromatography and mass spectroscopy. The compounds identified in some or all of the extracts were: ethyl acetate; 2-butanol, acetic acid, lactic acid, 2,3-butanediol, 3-hexyne, benzyl alcohol, 2-phenyl ethanol, methyl furvate, acetoin, benzoic acid, 5-hydroxy methyl furfural and the hydrocarbons C_{23}H_{48}, C_{25}H_{52} and C_{27}H_{56}. Buseta et al. (1996) analysed lavender and eucalyptus honeys and explained that unifloral honeys have highly characteristics flavour due to various volatiles - linear aldehydes, n-hexanol, coumarin, diketones, phenylacetaldehyde, hydroxyketones and sulphur compounds.

2.3.6 Lipid in honey

Thin layer and gas chromatography were used by Smith (1967) to show qualitatively, the presence of extractable lipids in cotton honey from Arizona. He reported that the acids were Palmitic acid (27%), Oleic acid (60%) with small amounts of lauric, myristoleic, stearic and linoleic acid. Kim et al. (1991) reported that the major volatile organic acids were acetic acid, formic acid, valeric acid and major non volatile acids were gluconic acid, maleic acid, malic acid, quinine acid and citric acid. The principle myristic acid, linolenic acid and palmitic acid.
2.3.7 Protein and amino acids

The presence of proteins in honey has been known for many years and it is one of the important constituents of honey. Kalimi and Schonie (1964) used paper chromatography to study honey samples from Mahableshwar (Maharashtra) in India. The amino acids reported were lysine, arginine, proline, valine, methionine, isoleucine and leucine along with aspartic acid, glutamine, serine, glycine, histidine and alanine. The amino acid composition of Indian honey from *Apis cerana* colonies revealed that lysine, arginine, proline, valine, methionine, isoleucine and leucine along with asparitic acid, glutamine, serine, glycine, histidine and alanine were present (Kalimi and Schonie, 1964).

Curti and Riganti (1966) examined 19 floral honeys and one honeydew honey from Italy. Thin layer chromatography helped in identification of 14 free amino acids, out of which Proline and Phenylalanine were most abundant in all samples. Amino acid composition varied according to botanical origin of samples.

Modi and Biffoli (1967) used thin layer chromatography following ion exchange to examine amino acids and protein content in undiluted and commercial samples. The first 11 samples contained 0.115 to 0.23 per cent acids and 0.34 to 0.57 per cent proteins. Lysine, glutamic acid, glycine, alanine, histidine, leucine/isoleucine, tryptophan/phenylalanine and proline were found in all 11 unadulterated samples.

Cirrilli et al (1973) reported crude protein content as 0.20 per cent in Italian honey. The most important amino acids were: glutamic acid, alanine, aspartic acid, arginine, glycine, leucine, isoleucine, valine, histidine and lysine.

Petrov (1974) using an automatic amino acid analysis studied 7 samples of honey quantitatively. The same method was used by Davies (1975) to study honeys.
from eleven different countries. Both these workers observed differences in amino acid spectrum of honeys collected from different areas of Australia.

To determine the protein content in honey, other methods were also used besides thin layer chromatography. Bianchi (1979) used photo-calorimetric method to determine the total protein content in honey samples from Argentina. El-Sayed (1982) studied amino acids in honey produced by Carniolar bees foraging on *Trifolium alexandrium* in four areas of Egypt. Total amino acids ranged from 12.7 to 18.7 mg/100 gm honey. The main amino acids ranged from proline 3.15 to 8.73; aspartic acid 1.29 to 2.27, glutamic acid 1.15 to 1.18 (in mg/100 g). One honey contained 1.88 mg/100g of lysine, while others had 0.584 to 0.837 mg/100g. Same honeys contained 1.41 and 0.392 to 0.553 mg/100g of arginine. Analysis of monofloral honeys from Russia revealed the main amino acids for each type of honey (in mg %): Alanine, valine, leucine, proline, histidine, serine, threonine, methionine, phenylalanine, glutamic acid, glutamine, lysine, tryosine, asparagine. Threonine the main free amino acid in light honeys ranged from 54.8 to 68.7% (Chepurnoi, 1983). In dark honeys it ranged from 30 to 33.4 per cent in buckwheat and was 40.7 per cent in *Phacilia*. Proline was more evident in darker than in lighter honeys. 26 samples of Sardinian honeys studied by Campus et al. (1983) contained on an average 0.6 percent proteins 73.42 µmg amino acids/100 g dry matter. Proline constituted on an average 69 percent of total amino acids; systine, methionine and tryptophan were present in small amounts.

The content of vitamins such as riboflavin, niacin, thiamine and ascorbic acid in Indian honeys from Apis cerana colonies varied from 12 to 54; 442 to 798, 8 to 22, 2000 to 2400 µgm per 100 gm of honey. These authors also found that after storage of
honey at 28°-30° C for one year, 29 per cent of the vitamins were lost (Kalimi and Schonie, 1964). Buseta et al. (1996) studied the flavour and free amino acid composition of lavender and eucalyptus honeys. The highly characteristics flavours were due to various volatiles probably derived from original plant sources. Major compounds were linear aldehydes, n-hexanol, diketones, hydroxyketones, 3-hexanol.

2.3.8 Honey enzymes

One of the most important components of honey are enzymes. Besides being nutritionally significant, these are responsible for changing the sugar spectrum of original raw material into forms, characteristics of ripe honey. Goethe (1914) observed that honey does not contain lactase, diastase and invertase. Gauhe (1941) reported that it was a glucose oxidising enzyme which formed gluconic acid and peroxide. He suggested that the organic acids formed acted as a preservative for honey, however, its presence could not be confirmed. When nectar is ripened to honey, invertase is the enzyme responsible for the most of the chemical changes that take place. Olaerts (1956) reported that activity of honey invertase depended upon a number of factors. Maximum activity of the enzyme occurred at the pH of 5.6 and at 20 to 30 percent substrate concentration, however, enzyme activity was inhibited by hydroelectric products. Among the 15 different samples studied by him, maximum content was almost the double of the lowest. Ratio of invertase to diastase was almost constant. Further he reported that the activity of honey diastase and shape of acidity curve depended to a greater extent on presence of sodium chloride.
The optimum pH for the enzyme diastase was about 5. Honey diastase includes starch digesting enzymes, which are the amylases. Lampits et al. (1930) studied the effect of pH and temperature on both α-β amylase of honey and found the optimum pH for β amylase at 5.3. Soloveva (1964) suggested that amylase activity was an important criterion for evaluating honey quality. Studying several honey samples from single and multifloral sources studies on the susceptibility of three types of honeys to diastase was under heat treatment showed that honey could be safely heated up to 54°C for a period of 16 hours without loss of any activity, however it may be lost if the temperature exceeded 66°C even for short period (Langridge, 1966). Vorwohl (1969) reported that the diastase activity of A. cerana honeys was significantly lower than those of A. mellifera. He also observed that low diastase activity was specific for A. cerana honeys although they had comparatively high invertase activity. Kerkvilliet (1976) discussed low diastase content in relation to nectar supply, age, race and nutrition of bees. Honey samples that may be low in diastase included Citrus, Lavender and some Eucalyptus honeys from Europe.

White et al. (1962, 1963) showed the production of hydrogen peroxide and gluconol acetone by the action of glucose oxidise on hydrogen peroxide and gluconol acetone. They further observed that the cause of antibacterial effect (inhibine) was due to accumulation of hydrogen peroxide, formed by the action of enzyme. Ivanov (1978) studied activity of invertase, acid and alkaline phosphates and esterase collectively in 43 samples of honeys of different plant origins in Bulgaria. The invertase activity ranged from 54 to 261 mg glucose/gm honey. Invertase number (10.3 to 445) was the highest for the honeydew honey and its activity was highest at pH of 6.0 to 6.2, at temperature
of 23 to 25° C and substrate concentration of 10 to 20 per cent. He also concluded the phosphates activity was from 130-644 µg p-nitrophenol/gm honey. Alkaline phosphates showed negligible activity. Esterase activity towards β-naphthyl acetate ranged from 84 to 424 gm naphthol/gm honey. Duttman et al. (1984) reported that by Gontarski method, invertase activity should be 10 units. Standard deviation was calculated to be 14.2% for Gontarski method and 0.6% for Siegenthaler’s method (40 units of Siegenthaler’s method = 10 units of Gontarski method). 11 honey samples from Edmonton area were found to have significant amounts of a new enzyme β-glucosidase which was detected by modifying Siegenthaler’s spectrophotometric method (Low et al., 1986).

Giri (1938) studied 12 samples of Indian honeys and showed that they contained an acid phosphates, which was effective over the pH range from 3.5 to 6.5, being most effective at 35° C. Takenaka (1980) analysed honey samples from Japan having molecular weight equal to 75,000 by electrophoresis method. The activity of the enzyme was greater on sucrose than on maltose and without any activity on starch.

According to Mahajan (1984), the average specific gravity, viscosity, surface tension and electrical conductivity at 30° C for honey samples of Apis cerana colonies from the Shimla hills were 1.425, 37.764, 106.219 and 0.219 respectively. These physico-chemical characteristics did not show significant differences in their values for both major honey flow seasons (May-June and October-November). After a storage period of one year at room temperature (15-20° C), there was an increase in moisture content, surface tension, electrical conductivity, and a decrease in refractive index, specific gravity and viscosity due to the hygroscopic nature of honey.
2.4 Antimicrobial characteristics

Antimicrobial affect of honey is of great biological significance. Sacket (1919) showed that certain bacteria perished quickly on heat sterilised honey. Diluted honeys were more effective than undiluted ones. Dold et al. (1937) ascribed the antibacterial effect of honey due to the presence of a thermolabile and light sensitive substance known as ‘inhibine’. Khristove and Mladenov (1961) tested antimicrobial properties of 27 honey samples of Russia and found that 26 samples were active against Staphylococcus aurens, one at 1/160 dilution and others at higher concentrations. Similar results were obtained with Staphylococcus haemolyticus and minimum active concentration was 1/40. No sample was found to be active against E. coli or Proteus vulgaris at less than 1/5 concentration and none had any effect on Monilia albicans.

Lindner (1962) found that inhibitory activity was maximum in forest honey of European samples, while in honey sample from sugar fed bees it was minimum. Honey was effective against both gram +ve and gram -ve bacteria and also against some spore forming and penicillin resistant bacteria. Action was bacteriostatic rather than bactericidal, and heating of honey at 70° to 80° C reduced activity to a minimum. The antibacterial values of diluted American honeys were correlated to their hydrogen peroxide values. Hydrogen peroxide was produced when a crude enzyme preparation from honey was incubated with glucose. It was, therefore, concluded by White et al. (1962) that the inhibine activity of honey is due to enzymatic production of hydrogen peroxide.
Sedova and Usmanov (1973) analysed some honey samples from Uzbekistan and concluded that these acted more strongly against *Coccus* bacteria than *Bacillus* type. Similar studies were made by Monakhova and Monakhov (1974) who denied the bactericidal effect on *Mycobacterium tuberculosis* in Russian samples. Seven unifloral samples from Australia were analysed, concentrations of 4, 8, 12, 16, 20 per cent against *Staphylococcus aureus, Eucalyptus meullerana* honey at 4 per cent and four other Eucalyptus honeys at 16 per cent concentration and one at 20 per cent concentration were inhibitory, but *Eichivum lycopsis* and *Eucalyptus microtheca*. Honeys had no inhibitory properties even at 20 percent concentration. At 60°C or above, antimicrobial activity was rapidly destroyed and it was found to be reduced by storage at temperatures above 42°C (Wootton et al., 1978).

The inhibitory action of unprocessed honey on toxigenic strains of *Asperillus flavus* and *A. parasitices* was evaluated by Wellford et al. (1978) on European samples. The fungi grew and sporulated in varying concentrations of honey dilution, but none of the cultures produced detectable levels of aflavotoxin. This confirmed the earlier observations that pure honey inhibited fungal growth and suggested that even diluted honey was capable of inhibiting toxin production and neutralising it.

Popekovic et al. (1985) showed that in Yugoslavian honey samples, inhibitory effect of thistle honey (*Cirum arvense*) on *Staphylococcus aurens* was twice as high, and on *Streococcus faecalis* 8 times as high as that of pear honey.

Analysis of honey samples from Japan showed that the antibacterial property is attributed to its high sugar concentration, low pH value and to the presence of H$_2$O$_2$ produced from glucose by action of glucose oxidase. Jimenez et al. (1994) isolated
yeasts belonging to the genera Saccharomyces and filamentous mould were of the general *Aspergillus, Penicillium, Fusarium* and *Alternaria* in the honey samples.