Honey is natural diet and contains natural antioxidant properties that can destroy biologically destructive chemical agents which have been linked to many diseases such as cancer. Honey contains a variety of phytochemicals (as well as other substances such as organic acids, vitamins, and enzymes) that may serve as sources of dietary antioxidants. The amount and type of these antioxidant compounds depends largely upon the floral source/variety of the honey. In general, darker honeys have been shown to be higher in antioxidant content than lighter honeys (Ghelfod et al, 2002). Because of the large variety of pathologies that have been related to reactive oxygen species (ROS), it is quite important to find new antioxidants that could inhibit or prevent the effects of ROS. Consumption of honey increases the antioxidant activity of human plasma. The antioxidant capacity of honey is comparable to that of uric acid, a potent antioxidant found in living systems (Elizabeth, 2006). In this section some of the recent literature is reviewed under the following headings –

- **Historical Uses of Honey**
- **Production and Consumption of honey**
- **Composition of honey**
- **Free radicals**
- **Antioxidants**
- **Antioxidant Capacity of honey**
- **Phytochemicals in honey**
- **Applications in the food industry**
- **Probiotics and Prebiotics**
- **Health effects of honey**

**Historical Uses of Honey**

Honey has long been documented as having medicinal properties, and its uses as a wound dressing and an antiseptic have been recorded since ancient times. The earliest written records of honey used as medicine is in Egyptian papyri and Sumerian clay tablets dated from 1900 to 1250 BC and in one of these, honey was used in 30% of the prescriptions (Stomfay-Stitz, 1960). Ancient Egyptians also used honey in embalming. They made salves with it for treating diseases of the eyes and skin (Al Waili, 2003). As well, the ancient Greeks are reported to have used honey to treat
fatigue: athletes drank a mixture of honey and water before major athletic events (Wilson and Crane, 1975). Hippocrates (460-357 BC) found that honey cleaned sores and ulcers of the lips and healed buncles and running sores. As well, the healing properties of honey were mentioned in the Holy Quran 1400 years ago (Al Waili, 2003). Honey has continued to be used in folk medicine ever since, with mention of its use in the Middle Ages reported by Daude de Pradas in approximately 1200 AD (Wilson and Crane, 1975). Similarly, honey has been documented as being used as a remedial agent throughout Europe, as well as through areas of Arabia and China. Generally, honey has been used as a remedy for gastric and intestinal complaints, although the sedative and soporific powers of honey have also been mentioned. In addition, the diuretic effect of honey has been recorded, and it has been a favoured remedy for kidney inflammations and stones. Attic honey, in particular, was thought to have special curative powers for eye disorders, and honey, in general, has been documented as having being used for the treatment of skin diseases and smallpox, as well as in surgical dressings. The Hindu people also had great faith in the medical virtues of honey, using it mainly for coughs, pulmonary issues and gastric disorders. Similarly, populations in rural communities from almost all nations have documented the use of honey through time. German women, specifically, believed that a mixture of honey and crushed bees would have a beautifying and strengthening effect, and that it would regulate menstrual flow. In more recent times, honey has played a relatively minor role in medicine, mostly due to it not being accepted by western practitioners in a world where antibiotics and other pharmaceuticals are seen as the remedies of choice. Among the Chinese, Hindu, Arabic and African races, however, honey is still considered to be a valuable internal and external remedy (Beck and Smedley, 1997). Slowly the use of honey in western medicine is gaining recognition; particularly as scientific evidence continues to be produced demonstrating its efficacy, often in situations where more usual remedies are ineffective. The antibacterial properties of honey and its wound-healing capabilities (Molan, 2006), in particular, have gained substantial recognition in the last 10–15 years, although only now are researchers beginning to understand the processes by which this occurs. People have started beginning to understand that honey may indeed be the elixir that the ancient people believed, as research is showing a number of health-related benefits, including a laxative effect, beneficial effects on blood glucose levels, anti-inflammatory and immune stimulating properties and potentially a cancer-preventative action.
Production and Consumption of honey

Apiculture is currently one of the most widespread agricultural activities carried out in India. According to National Bee Board of India (2006–2007), there are about 1.4 million colonies in the country and honey production is about 52,000 tonnes a year (54.15% from domesticated & 45.85% from wild) and Punjab, northwest state of India situated at 290-32° to 320-32° North latitude and 730-55° to 760-50° East longitude, accounts for over one third of 52,000 tones of honey produced in India (Bajaj, 2008).

In India beekeeping is practiced in mountains, foot hills, forest, agricultural lands, mangrove forests etc. The techniques involved in beekeeping vary from region to region. The main harvest is from Apis dorsta, Apis cerana and Apis mellifera. Apis dorsata, (the rock bee or giant bee) is found in foot hills of Himalayas and northern regions of the country. In central India in the forests and plains large number of dorsata colonies is present. The tribals collect large amount of honey and bees wax. The Sunderban forests in West Bengal are rich in Apis dorsata, the organic honey from these forests are of great demand today. The southern part of India is also having large number of Apis dorsata colonies and contribute large share of total honey production in India.

Table 1 and 2 depict the production of honey in different continents and countries, respectively. Apis florea (Little bee), is common in central part of India, occurs in arid and desert region of extreme climates, and also in plains and forests. Large quantity of Apis florea honey is collected from the Kutch area of Gujarat (Soman and Chawda, 1996); very less quantity is being collected in other parts, which are consumed by the hunters itself.

Trigona species (Dammar bee) is common in all parts of the country and remains long periods in the same abode. It is a very small bee and collects nectar from small flowers. Since the quantity of honey produced is small, these bees are not commercially used. It is a very important crop pollinator, and their honey has repute in folk medicine. Apis mellifera has been imported initially to Punjab and has become popular among commercial beekeepers because of its higher honey yield. The
Table 1-World production (continent wise) of honey (data in thousand tons)

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<td>1103</td>
<td>1137</td>
<td>1091</td>
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Source: Bogdanov, S (2009)
Table 2: Major honey producing countries (data in thousand tons)

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Source: Bogdanov, S (2009)
mellifera gradually spread to Himachal, Bihar, Uttar Pradesh, West Bengal and recently in Kerala, Karnataka and Maharashtra. Since Apis mellifera beekeeping need sound financial support it is difficult for the poor farmers to afford (Thomas et al, 2002).

At present the annual world honey production is about 1.2 million tons, which is less than 1% of the total sugar production. Today, honey is one of the last untreated natural foods. The consumption of honey differs strongly from country to country. The major honey exporting countries are China and Argentina. Their annual consumption is as small as 0.1 to 0.2 kg per capita. Honey consumption is higher in developed countries, where the home production does not always cover the market needs. In the European Union, which is both a major honey importer and producer, the annual consumption per capita varies from medium (0.3-0.4 kg) in Italy, France, Great Britain, Denmark, Portugal to high (1-1.8 kg) in Germany, Austria, Switzerland, Portugal, Hungary, Greece, while in overseas countries such as USA, Canada and Australia the average per capita consumption is 0.6 to 0.8 kg/year (www.bee-hexagon.net).

**Composition of honey**

**Carbohydrates**

Sugars are the main constituents of honey, comprising about 95 % of honey dry weight. Main sugars are the monosaccharides hexoses fructose and glucose, which are products of the hydrolysis of the disaccharide sucrose. Besides, about 25 different sugars have been detected (Doner, 1977). The principal oligosaccharides in blossom honeys are disaccharides: sucrose, maltose, turanose, erlose. Honeydew honeys contain besides, also the trisaccharides melezitose and raffinose. Trace amounts of tetra and pentasaccharides have also been isolated. The relative amount of the two monosaccharides fructose and glucose is useful for the classification of unifloral honeys (Bogdanov et al, 2004). On the other hand, the sugar spectra of minor sugars do not differ greatly in different blossom honeys. This is due to the fact, that the oligosaccharides are mainly a product of honey invertase (White, 1975). There are considerable differences between the sugar spectra of blossom and
hones, the latter containing a higher amount of oligosaccharides, mainly the trisaccharides melezitose and raffinose, both absent in blossom honeys. The differentiation between different types of honeydew honeys is difficult. An attempt to differentiate between honeydew honeys from various aphids was made by determination of specific oligosaccharides. 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 (Flori et al, 2000). The sugar composition can be determined by different chromatographic methods (Bogdanov et al, 1997), HPLC being the most widely used one (Bogdanov et al, 2004).

Proteins, enzymes and amino acids

Honey contains roughly 0.5% proteins, mainly enzymes and free amino acids. Protein content has been reported in honey from different floral sources, where high protein contents were considered as over 1000 μg/g (Azeredo et al, 2003). Nevertheless the contribution of that fraction to human protein intake is low. The three main honey enzymes are diastase (amylase), decomposing starch or glycogen into smaller sugar units, invertase (sucrase, α-glucosidase), decomposing sucrose into fructose and glucose, and glucose oxidase, producing hydrogen peroxide and gluconic acid from glucose (Bogdanov et al 2008). Amino acids in honey account for 1% (w/w). The amount of total free amino acids in honey corresponds to between 10 and 200 mg/100g, with proline as their major contributor, corresponding to around 50% of the total free amino acids (Iglesias et al, 2004). Besides proline, there are 26 amino acids in honeys, their relative proportions depending on its origin (nectar or honeydew). Since pollen is the main source of honey amino acids, the amino acid profile of a honey could be characteristic of its botanical origin. The main amino acids identified in honey from different botanical and geographical origin are: glutamic acid, aspartic acid, asparagine+serine, glutamine, histidine , glycine, threonine, b-alanine, arginine, a-alanine, g-amino butyric acid, proline, tyrosine, valine ammonium ion, methionine, cysteine, isoleucine, leucine, tryptophan , phenylalanine, ornithine and lysine (Perez et al, 2007).
Vitamins, minerals and trace compounds

It is known that different trace and mineral element concentrations in honey depend on its botanical and geological origin. Trace elements play a key role in the biomedical activities associated with this food, as these elements have a multitude of known and unknown biological functions. For this reason, the concentrations of minerals and trace elements in honey were investigated. Different trace and mineral elements were systematically investigated in botanically and geologically defined honey. The vitamin content in honey is low. Vitamins such as phyllochinon, thiamin, riboflavin, pyridoxine, and niacin are reported in honey but in general the amount of vitamins and minerals is small and the contribution of honey to the RDI of the different trace substances is small (Jose et al, 2009).

Aroma compounds, taste-building compounds and polyphenols

There is a wide variety of honeys with different tastes and colours, depending on their botanical origin. The sugars are the main taste-building compounds. Generally, honey with high fructose content (e.g. acacia) are sweet compared to those with high glucose concentration (e.g. rape). Beyond sugars the honey aroma depends on the quantity and quality of honey acids and amino acids. In the past decades some research on honey aroma compounds has been carried out and more than 500 different volatile compounds have been identified in different types of honey. Indeed, most aroma building compounds vary in the different types of honey depending on its botanical origin (Bogdanov, 2007). Honey flavour is an important quality for its application in food industry and also a selection criterion for consumer’s choice. Polyphenols are another important group of compounds with respect to appearance and functional properties. 56 to 500 mg/kg total polyphenols were found in different honey types, depending on the honey type (Gheldolf and Engeseth, 2002). Polyphenols in honey are mainly flavonoids (e.g. quercetin, luteolin, kaempferol, apigenin, chrysin, galangin), phenolic acids and phenolic acid derivatives (Tomas-Barberan et al, 2001). The flavonoid content can vary between 2 and 46 mg/kg of honey and was higher in samples produced during dry season with high temperatures. The polyphenols are responsible for the antioxidant properties of honey.
**Free Radicals**

Free Radicals are molecules with an unpaired electron. Due to the presence of a free electron, these molecules are highly reactive. They are important intermediates in natural processes involved in cytotoxicity, control of vascular tone, and neurotransmission. Radiolysis is a powerful method to generate specific free radicals and measure their reactivity (Oakley, 1998).

**Types of long lived radicals**

*Stable radicals*: The prime example of a stable radical is molecular oxygen. Organic radicals can be long lived if they occur in a conjugated π system, such as the radical derived from α-tocopherol & vitamin E. Thiazyl radicals show remarkable kinetic and thermodynamic stability, with only a very limited extent of π resonance stabilization.

*Persistent radicals*: Compounds with persistent radicals are long lived due to steric crowding around the radical center and makes them physically difficult to react with another molecule. Examples of these include Gomberg's triphenylmethyl radical, Fremy's salt (Potassium nitrosodisulfonate, Nitroxides, such as TEMPO (2, 2, 6, 6-Tetramethylpiperidine-1-oxyl), verdazyls, nitronyl nitroxides, azeaphenylenyls, radicals derived from PTM (perchlorophenylmethyl radical) and TTM (tris 2,4,6-trichlorophenylmethyl radical). The longest-lived free radical is melanin, which may persist for millions of years.

*Diradicals*: Molecules containing two radical centers are called diradical. Multiple radical centers can also exist in a molecule. Molecular oxygen naturally (i.e. atmospheric oxygen) exists as a diradical (in its ground state as triplet oxygen). The high reactivity of atmospheric oxygen is owed somewhat to its diradical state (although non-radical states of oxygen are actually less stable). The existence of atmospheric molecular oxygen as a triplet-state radical is the cause of its paramagnetic character, which can be easily demonstrated by attraction of oxygen to an external magnet (Pacher et al, 2007).

**Production route of free radicals**

Production of free radicals in the body is continuous and inescapable. The basic causes include the following -
**The immune system:** Immune system cells deliberately create oxy-radicals and ROS (Reactive oxygen species) as weapons.

**Energy production:** During energy-producing cell generates continuously and abundantly oxy-radicals and ROS as toxic waste. The cell includes a number of metabolic processes, each of which can produce different free radicals. Thus, even a single cell can produce many different kinds of free radicals.

**Stress:** The pressures common in industrial societies can trigger the body's stress response to mass produce free radicals. The stress response races the body's energy-creating apparatus, increasing the number of free radicals as a toxic by-product. Moreover, the hormones that mediate the stress reaction in the body - cortisol and catecholamine - themselves degenerate into particularly destructive free radicals.

**Pollution and other external substances:** Air pollutants such as asbestos, benzene, carbon monoxide, chlorine, formaldehyde, ozone, tobacco smoke, and toluene, Chemical solvents such as cleaning products, glue, paints, and paint thinners, Over-the-counter and prescribed medications, perfumes, pesticides, Water pollutants such as chloroform and other trihalomethanes caused by chlorination, Cosmic radiation, Electromagnetic fields, Medical and dental x-rays, Radon gas, Solar radiation, the food containing farm chemicals, like fertilizers and pesticides, processed foods containing high levels of lipid peroxides, are all potent generator of free radicals.

**General factors:** Aging, Metabolism, Stress

**Dietary factors:** Additives, alcohol, coffee, foods of animal origin, foods that have been barbecued, broiled, fried, grilled, or otherwise cooked at high, temperatures, foods that have been browned or burned, herbicides, hydrogenated vegetable oils, pesticides, sugar.

**Toxins:** Carbon tetrachloride, Paraquat, Benzo (a) pyrene, Aniline dyes,

**Drugs:** Adriamycin, Bleomycin, Mitomycin C, Nitrofurantoin, Chlorpromazine

**Formation of free radicals**

Normally, bonds don’t split to leave a molecule with an odd, unpaired electron. But when weak bonds split, free radicals are formed. Free radicals are very unstable and react quickly with other compounds, trying to capture the needed electron to gain stability. When the "attacked" molecule loses its electron, it becomes a free radical itself, beginning a chain reaction. All this happens in nanoseconds. Once
the process is started, it can cascade, finally resulting in the disruption of a living cell. Some free radicals may arise normally during metabolism and by immune system’s cells purposefully to neutralize viruses and bacteria. Normally, the body can handle free radicals, but if antioxidants are unavailable, or if the free radical production becomes excessive, damage can occur (Sarma et al, 2010). **Figure 1 and 2 represent free radical formation and cellular damage due to free radical, respectively.**

**Reactive oxygen species (ROS)**

Reactive oxygen species (ROS) are very small molecules and are highly reactive due to the presence of unpaired valence shell electrons. ROS is formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling. However, during times of environmental stress ROS levels can increase dramatically, which can result in significant damage to cell structures. Platelets involved in wound repair and blood homeostasis release ROS to recruit additional platelets to sites of injury. Generally, harmful effects of reactive oxygen species on the cell are most often like - Damage of DNA, oxidations of polydesaturated fatty acids in lipids, oxidations of amino acids in proteins, oxidatively inactivates specific enzymes by oxidation of co-factors (Pignatelli et al, 1998 and Guzik et al, 2003). **Figure 3 shows the effects of ROS.**

**Free radicals and human disease**

**Figure 4 depicts the damage caused by free radicals in human body.**

**Cancer:** Like radiation and carcinogens, free-radical oxidation breaks strands of DNA. The breaks are repaired, but some mistakes occurs leading to mutations. These genetic mutations can cause cancers. The age-related increase in cancer rates might have something to do with an age-related rise in oxidative damage to DNA.

**Alzheimer’s disease:** The brain in Alzheimer's disease (AD) is under increased oxidative stress and this may have a role in the pathogenesis of neuron degeneration and death in this disorder. The direct evidence supporting increased oxidative stress in AD is: (1) increased brain iron, aluminum, and mercury in AD, capable of stimulating free radical generation; (2) increased lipid peroxidation and decreased polyunsaturated fatty acids in the AD brain, and increased 4-
Figure 1: Free radical formation (Sarma et al, 2010)

Figure 2: Cellular damage due to free radicals (Sarma et al, 2010)
Figure 3: **Effects of Reactive Oxygen Species** (Sarma et al, 2010)

Figure 4: **Overview of free radical damage** (Sarma et al, 2010)
hydroxynonenal, an aldehyde product of lipid peroxidation in AD ventricular fluid; (3) increased protein and DNA oxidation in the AD brain; (4) diminished energy metabolism and decreased cytochrome c oxidase in the brain in AD; (5) advanced glycation end products (AGE), malondialdehyde, carbonyls, peroxynitrite, heme oxygenase-1 and SOD-1 in neurofibrillary tangles and AGE, heme oxygenase-1, SOD-1 in senile plaques; and (6) that amyloid beta peptide is capable of generating free radicals. So free radicals are possibly involved in the pathogenesis of neuron death in Alzheimer's disease (AD).

**Cardiac Reperfusion Abnormalities:** Oxygen free radicals are highly reactive compounds causing peroxidation of lipids and proteins and are thought to play an important role in the pathogenesis of reperfusion abnormalities including myocardial stunning, irreversible injury, and reperfusion arrhythmias. Free radical accumulation has been measured in ischemic and reperfused myocardium directly using techniques such as electron paramagnetic resonance spectroscopy and tissue chemiluminescence and indirectly using biochemical assays of lipid per oxidation products. Potential sources of free radicals during ischemia and reperfusion have been identified in myocytes, vascular endothelium, and leukocytes. Injury to processes involved in regulation of the intracellular Ca\(^{2+}\) concentration may be a common mechanism underlying both free radical-induced and reperfusion abnormalities.

**Kidney:** Mitochondrial free radical production induces lipid peroxidation during myohemoglobinuria. Iron catalyzed free radical formation and lipid peroxidation are accepted mechanisms of heme protein-induced acute renal failure. However, the source(s) of those free radicals which trigger lipid peroxidation in proximal tubular cells remain unknown. In conclusion, the terminal mitochondrial respiratory chain is the dominant source of free radical.

**Fibrosis:** Oxygen, paraquat, nitrofurantoins, and bleomycin, produces pulmonary fibrosis. Radical-generating agents such as iron and copper are also associated with liver fibrosis (cirrhosis) and fibrotic changes in other organs such as the heart. The induction of vitreous scarring by interocular iron or copper is also well known, as is the association of homocystinuria with fibrotic lesions of the arteries. Adult Respiratory Distress Syndrome (ARDS) occurs due to production of active oxygen species by inflammatory cells (Sarma et al, 2010).
**Types of free radicals**

- Hydroperoxyl radical
- Superoxide radical
- Hydrogen peroxide
- Triplet oxygen
- Active oxygen

**Hydroperoxyl radical:** The hydroperoxyl radical, also known as the perhydroxyl radical, is the protonated form of superoxide with the chemical formula HO\(_2\). Hydroperoxyl is formed through the transfer of a proton to an oxygen atom. HO\(_2\) can act as an oxidant in a number of biologically important reactions, such as the abstraction of hydrogen atoms from tocopherol and polyunsaturated fatty acids in the lipid bilayer. As such, it may be an important initiator of lipid peroxidation.

**Superoxide:** Superoxide can act either as oxidant or reductant, it can oxidize sulphur, ascorbic acid or NADPH and it can reduce Cytochrome C and metal ions. A dismutation reaction leading to the formation of hydrogen peroxide and oxygen can occur spontaneously or is catalyzed by enzyme superoxide dismutase. In its protonated form (pKa 4.8), superoxide forms and perhydroxyl radical, which is a powerful oxidant (Gebicki and Bielski, 1981) but its biological relevance is probably minor because of its low concentration at physiological pH.

**Hydrogen peroxide:** The univalent reduction of superoxide produces hydrogen peroxide, which is not a free radical because all its electrons are paired. It readily permeates through the membranes and is therefore not compartmentalized in the cell. The main damages caused by this are breaking up of DNA, resulting in single strand breaks and formation of DNA protein crosslink. Numerous enzymes (peroxidases) use hydrogen peroxide as a substrate in oxidation reactions involving the synthesis of complex organic molecule. This is an oxidizing agent but not specially reactive and its main significance lies in it being a source of hydroxyl radical in the presence of reactive transition metal ions.

**Singlet oxygen:** It is not a free radical but it can be formed in some radical reactions and can trigger off others. This arises from hydrogen peroxide molecules. Singlet oxygen on decomposition generates superoxide and hydroxyl radicals.
**Triplet oxygen:** Triplet oxygen can react with elements and ions to form oxides, but usually not with organic compounds, which are in singlet state. However, it reacts easily with free radical molecules produced by the action of other active radicals, radiations, ultra violet light, and heat or by complex formation with oxygen and transition metal to produce active peroxide radicals and trigger auto-oxidation of unsaturated fatty acids and others (Shiv Kumar, 2011).

**Antioxidants**

Antioxidants are an inhibitor of the process of oxidation, even at relatively small concentration and thus have diverse physiological role in the body. Antioxidant constituents of the plant material act as radical scavengers, and helps in converting the radicals to less reactive species. A variety of free radical scavenging antioxidants is found in dietary sources like fruits, vegetables and tea, etc (Mandal et al, 2009). Antioxidants defense both enzymatic and non enzymatic reactions protect the body against oxidative damage. Non enzymatic antioxidants are frequently added to the food to prevent lipid oxidation. Several lipid antioxidants can exert pro-oxidant effect towards other molecule under certain circumstances thus the antioxidants for food and therapeutic use must be characterized carefully.

**Classification of antioxidants**

A) Natural antioxidants
B) Synthetic antioxidants

**A) Natural antioxidants:** Naturally occurring antioxidants of high or low molecular weight can differ in their composition, in their physical and chemical properties, in their mechanism and in their site of action. They can be divided into following categories:

1. **Enzymes:** Enzyme such as superoxide dismutase (SOD), catalase and glutathione peroxidase attenuate the generation of reactive oxygen species by removing potential oxidants or by transferring ROS/RNS (reactive nitrogen species) into relatively stable compounds. SOD which was discovered in late 60s, catalyses the transformation of the superoxide radical into hydrogen peroxide, which can then be transformed by enzyme catalase into water and molecular oxygen. While superoxide anion in itself is not particularly reactive, it can reduce transition metal ions, such as iron and gets converted to most reactive radicals - the hydroxyl radical. Thus,
elimination of superoxide radical can attenuate the formation of hydroxyl radical. Glutathione peroxidase (GPx) reduces lipid peroxides, formed by the oxidation of polyunsaturated fatty acids, to a stable, non toxic molecule - hydroxyl fatty acid. Together with phospholipase, GPx can also convert phospholipids hydro peroxide into phospholipids hydroxide.

ii) Low molecular weight antioxidants: These are subdivided into lipid-soluble antioxidants (tocopherol, carotenoids, quinones, bilirubin and some polyphenols) and water soluble antioxidants (ascorbic acid, uric acid and polyphenols). These delay or inhibit cellular damage mainly through free radical scavenging property.

Lipids soluble antioxidants:

These antioxidants tend to accumulate in lipid plasma lipoprotein (eg.LDL); upon supplementation. This group of antioxidants is supposed to act as highly efficient scavengers, such as against lipid peroxyl radical, which are formed within the lipoprotein as a consequence of free radical chain reaction of lipid peroxidation.

Water soluble antioxidants:

These antioxidants cannot enter the lipid moiety of low density lipoprotein (LDL); these will be less efficient as these are principally unable to encounter most of these lyophilic radicals; however, such a compound may act in a synergistic manner with lipophilic antioxidants by regenerating them.

B) Synthetic antioxidants These are most effective antioxidants and are synthetic chemicals, approved by Food and Drug Administration for addition to foods, e.g BHA (Butylated Hydroxy Anisole), BHT (Butylated Hydroxy Toluene), TBHQ (Tertiary Butylated Hydroxy Quinone) etc.

Mode of action of antioxidants

In general, the antioxidants act by the following routes:

- Chain breaking reaction eg. α-tocopherol, which act in lipid phase to trap free radical.
- By reducing concentration of reactive oxygen species eg. Glutathione.
- By scavenging initiating radicals ego superoxide dismutase which acts in the lipid phase to trap superoxide free radicals.
• By chelating transition metal catalyst: a group of compound which act by sequestration of transition metals that are well established prooxidants. In this way transferrin, lactoferrin and ferritin function to keep iron induced oxidant stress in check and ceruloplasmin and albumin as copper sequestrants (Shiv Kumar, 2011).

**Antioxidant Capacity of honey:**

Lots of methods for determining the antioxidant activity in honey have been used, e.g., determination of active oxygen species (viz. the superoxide anion, peroxyl and hydroxyl radicals), their radical scavenging ability (Gheldolf and Engeseth, 2002), the 1,1-diphenyl-2-picrylhydrasyl (DPPH) antioxidant content, enzymatic or nonenzymatic measurements of lipid peroxidation inhibition (Chen et al, 2000), the ferric reducing/antioxidant power assay (Aljadi and Kamaruddin, 2004) and the TEAC (Trolox equivalent antioxidant capacity) assay (Huang et al, 2005).

**a) Ferric Reducing Antioxidant Power (FRAP)**

The principle of this method is based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex ($Fe^{3+}$-TPTZ) to its ferrous colored form ($Fe^{2+}$-TPTZ) in the presence of antioxidants (Jose et al, 2009). The FRAP assay “Ferric reducing ability of plasma” was described by Benzie and Strain (1996), and it has been used by several authors for the study of the antioxidants capacity of natural sources such as honey (Aljadi and Kamaruddin, 2004 and Prior et al, 2005). As the FRAP assay measures the reducing capacity based upon reduction of ferric ion, antioxidants that act by radical quenching (H transfer), particularly carotenoids (present in the honey bee), will not be determined. Another point to take into consideration is the concomitant production of Fe (II), which is a well-known pro-oxidant and may result in the generation of additional radicals in the reaction medium, as $OH^*$ from hydrogen peroxide. Finally, compounds that absorb at the wavelength of the determination may interfere, causing overestimation of the FRAP value (Jose et al, 2009). Concerning limitations, any compound (even without antioxidant properties) with redox potential lower than that of the redox pair $Fe(III)/Fe(II)$, can theoretically reduce $Fe(III)$ to $Fe(II)$, contributing to the FRAP value and inducing falsely high results. On the other hand, not all antioxidants reduce Fe (III) at a rate fast enough to allow its measurement within the observation time (typically 4 min). Indeed, many polyphenols
react more slowly and require longer reaction times (≥30 min) for total quantification and, depending on the analysis time, the order of their reactivity is changed. However, some polyphenolic compounds (caffeic acid, ferulic acid, quercetin, and tannic acid) may have slower reactions, requiring more time (30 minutes) until completing the complex reduction. When used to determine the antioxidant potential of polyphenols in water and methanol (solvent used for their extraction form in honey bee), the change in absorbance continued after 4 min. Therefore the FRAP values for these compounds cannot be accurately determined in 4 min, and for this reason, 10 minutes are strongly recommend as a useful reaction time (Jose et al, 2009).

b) Diphenyl-1-picrylhydrazyl Radical Scavenging Activity (DPPH RSA) Assay

In the DPPH assay, antioxidants reduce the free radical 2, 2-diphenyl-1-picrylhydrazyl. In the presence of an antioxidant, the purple colour of DPPH⁻ fades and the change of absorbance can be followed spectrophotometrically at 515 nm (Schlesier et al, 2002). The DPPH analysis is a quick and simple test; it guarantees reliable results and needs only a UV-visible spectrophotometer to perform, which probably explains its widespread use in antioxidant screening. However, interpretation is complicated when the test compounds have spectra that overlap DPPH at 515 nm. Carotenoids, present in honey interfere. The assay is not a competitive reaction because DPPH is both radical probe and oxidant. DPPH is stable nitrogen radical that bears no similarity to the highly reactive and transient peroxyl radicals involved in lipid peroxidation. One more important disadvantage is many antioxidants that react quickly with the radical peroxide are almost or completely inert to DPPH. Despite the above mentioned limitations, DPPH⁻ is stable, commercially available, and does not have to be generated before assay like ABTS⁺⁺. For these reason it could be considered an easy and useful spectrophotometric method with regard to screening/measuring the antioxidant capacity in honey (Jose et al, 2009).

c) 2,2' - azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS)

This assay assesses the total radical scavenging capacity based on the ability of a compound to scavenge the stable ABTS radical cation (ABTS⁺⁺). The fact that ABTS⁺⁺ is not found in biological systems and is not similar to radicals found in those systems is also a problem and thus represents a “nonphysiological” radical source. In spite of this, the ABTS assay is considered an easy and accurate method for use in the
antioxidant capacity studies in honey because it allows determine the radical scavenging ability present in the honey by the hydrogen-donation reaction. This spectrophotometric assay is technically simple, which accounts for its application for screening and routine determinations. The ABTS•⁺ scavenging can be evaluated over a wide pH range, which is useful to study the effect of pH on antioxidant mechanisms in honey. Furthermore, the ABTS•⁺ radical is soluble in water and organic solvents. However, as the results obtained for samples are related to an antioxidant standard compound that shows different kinetic behaviour, the results provided by this assay are dependent on time of analysis. This is one of the technique that allow determination of the action mechanism with which the honey presents its antioxidant capacity, but it is not enough in the antioxidant capacity studies of a compound (Jose et al, 2009).

**In vitro antioxidant capacity of honey**

The antioxidant activity of Portuguese honeys was evaluated by (Isabel et al, 2009) considering the different contribution of entire samples and phenolic extracts. It was concluded that the samples submitted to phenolic extraction clustered proximately being well separated from the entire honey. The proximity of all the extracted samples indicate that a methanolic extraction after separation in Amberlite led to more reliable and accurate antioxidant activity results in a non-honey type dependent manner. The use of the whole extract instead of individual antioxidants allows advantage to be taken of additive and synergistic effects of different phenolic compounds present in the samples. This effect is more easily seen in the phenolic extracts than in the entire honey, once the extracted compounds should be structurally related and therefore, responsible for similar biological properties.

Elena et al (2008) analysed twenty-seven Italian honey samples of different floral origin for total phenolic and flavonoid contents by a spectrophotometric method and for antioxidant power and radical-scavenging activity by the ferricreducing/antioxidant power (FRAP) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) assays respectively. In addition, the phenolic and flavonoid profiles were analysed using high-performance liquid chromatography with UV detection (HPLC-UV). The results of this study showed that honey contains copious amounts of phenolics and flavonoids. HPLC-UV analysis showed a similar qualitative polyphenolic profile for
all honey samples analysed. The main difference among samples was in the contribution of individual analytes, which was affected by floral origin. Total phenolic and flavonoid contents varied from 60.50 to 276.04 mg gallic acid equivalent kg$^{-1}$ and from 41.88 to 211.68 mg quercetin equivalent kg$^{-1}$ respectively. The antioxidant capacity was high and differed widely among samples. The FRAP value varied from 1.265 to 4.396 mmol Fe$^{2+}$ kg$^{-1}$, while the radical-scavenging activity expressed as DPPH-IC$_{50}$ varied from 7.08 to 64.09 mg mL$^{-1}$. Correlations between the parameters analysed were found to be statistically significant (P < 0.05).

Mohamed et al (2002) evaluated the antioxidant activities and total phenolic contents of five different types of Yemeni honey. Total phenolic contents of diluted honey samples varied from 56.32 to 246.21 mg/100g honey as catechin equivalent by the Folin-Ciocalteu method. Four of five Yemeni honey samples contained significantly higher total phenolic content as compared with the imported honeys. Percentage antioxidant activities of diluted honey samples were assayed in vitro by the inhibition of liver homogenate oxidation mediated by ferrous sulphate/ascorbate system. The antioxidant activity of diluted honey samples increased with increasing the levels of honey samples. The total antioxidant activities of diluted samples varied from 6.48% (prooxidant activity) to 65.44% inhibition.

The antioxidant properties of 15 honey samples from different floral sources and various Slovak regions were investigated by Michal et al (2008). Cation radical of ABTS, and hydroxyl radicals generated by the photochemical decomposition of hydrogen peroxide were used as oxidants. The antioxidant activities found with ABTS, expressed as trolox equivalent antioxidant capacity (TEAC), ranged from 0.15 to 1.14 mmol kg$^{-1}$, and those determined with DPPH, from 0.04 to 0.32 mmol kg$^{-1}$. TEAC values correlated well with results found by elimination of DPPH, and both values revealed a linear relationship with the concentration of phenolics obtained with the Folin–Ciocalteu phenol test (expressed as gallic acid equivalents, GAE).

Antioxidant analysis of the different honey fractions was carried by Gheldof et al (2002). The results suggested that the water-soluble fraction contained most of the antioxidant components. Specific water-soluble antioxidant components were quantified, including protein; gluconic acid; ascorbic acid;
hydroxymethylfuraldehyde; and the combined activities of the enzymes glucose oxidase, catalase and peroxidase. Of these components, a significant correlation could be established only between protein content and ORAC activity. In general, the antioxidant capacity of honey appeared to be a result of the combined activity of a wide range of compounds including phenolics, peptides, organic acids, enzymes, maillard reaction products, and possibly other minor components. The phenolic compounds contributed significantly to the antioxidant capacity of honey but were not solely responsible for it.

Rosa et al (2008) examined sixty-seven Spanish honeys from different floral origins, nectars and honeydews for potential antimicrobial and antioxidant activities. Antioxidant capacities were determined in terms of their antiradical capacity using the stable free radical DPPH•. The bacterial species Micrococcus luteus and Staphylococcus aureus were used as the resistant microorganism in the antibacterial assays. The results showed that honeydew honeys presented higher antioxidant capacities than nectar honeys. On the other hand, honey capacity to inhibit Micrococcus luteus and Staphylococcus aureus was firstly evaluated by an agar diffusion method. Secondly, the active samples were tested with a spectrophotometric method to quantify their “non-peroxide” antimicrobial capacity to inhibit Staphylococcus aureus.

Forty honey samples which came from various locations of the Czech Republic and varied in their origins, were evaluated spectrophotometrically for their total polyphenol content, total flavonoids and 3’, 4’-dihydroxyflavones and flavonols, and major antioxidants were identified by High Pressure Liquid Chromatography – Diode Array Detection and Gas Chromatography-Mass Spectrometry. The kind of honey, location, and date of the honey harvest were shown to have a significant effect on the contents of phenolic antioxidants (average content 11.02 mg gallic acid equivalents/100g), total flavonoids (0.66 mg quercetin equivalent/100 g), and 3’,4’-dihydroxyflavones and flavonols (4.32 μg quercetin equivalents/100 g). In the Czech honey, ferulic acid (0.11 mg/100 g) and chrysin (0.06 mg/100 g) and other minority phenolics and flavonoids were identified and quantified as honey phenolic antioxidants contained (Lachman et al., 2010).
**In vivo antioxidant capacity of honey**-

More recently, a small number of studies have investigated the *in vivo* antioxidant capacity of honey (Ghelfod et al, 2003, Besserroles, 2002 and Schramm et al, 2003). Ghelfod et al (2003) found that the serum antioxidant capacity, measured using oxygen radical absorbance capacity (ORAC), was increased by 7% in individuals who had consumed a 500 ml solution of Buckwheat honey (160 g/l), but not in those who consumed 500 ml of black tea or black tea plus 160 g/l of syrup of mixed sugars (45% fructose, 35% glucose, 20% water). Similarly, in another study, plasma antioxidant capacity increased by 12–25% six hours after the ingestion of a single dose of Buckwheat honey (1.5 g/kg body weight), and the plasma total-phenolic content was increased by 4–8% (Schramm et al, 2003). No significant effects on either parameter were reported in subjects who consumed an equivalent amount of corn syrup (Schramm et al, 2003). The plasma reducing capacity was also shown to be improved by approximately 10% following ingestion of honey (Schramm et al, 2003). In a 2-week rat feeding study in which animals were fed diets containing 65 g of carbohydrate (starch, honey or a mixture of fructose and glucose as in honey) per 100 g of diet, honey-fed rats had higher plasma α-tocopherol levels, lower plasma nitrogen oxide levels and a lower susceptibility of lipid peroxidation in the heart (Besserroles et al, 2002).

**Phytochemicals in honey**

Phytochemicals are one wide class of nutraceuticals found in plants which are extensively researched by scientists for their health-promoting potential. Honey has a wide range of phytochemicals including polyphenols which act as antioxidants. Polyphenols and phenolic acids found in the honey vary according to the geographical and climatic conditions. Some of them were reported as a specific marker for the botanical origin of the honey. Considerable differences in both composition and content of phenolic compounds have been found in different unifloral honeys (Khalil et al, 2010). The antioxidant capacity of honey has been attributed to several factors including α-tocopherol, polyphenolics, organic acids, ascorbic acid, β-carotene and enzymes (Crane, 1975 and Ghelfod et al, 2003). In particular, research has suggested that the antioxidant capacity of honey is largely due to its total phenolic content (Ghelfod and Engeseth, 2002 and Ghelfod et al, 2002), and several studies have demonstrated that many honeys do have a rich phenolic profile consisting of benzoic
Review of Literature


Many authors have studied the phenolic and flavonoid contents of honey to determine if a correlation exists with floral origins (Tomas-Barberan et al, 2001 and Meda et al, 2005) and also to determine the presence of antimicrobial activity (Lachman et al, 2010). The content of phenolic antioxidants showed a good correlation with the characteristic antioxidant activities ($R^2 = 0.96$ for propolis and 0.90 for honey) (Buratti et al, 2007). Flavonoids pinobanksin, pinocembrin, quercetin, chrysin, galangin, luteolin and kaempferol were reported to be present in honey (Gheldof et al, 2002), while pinocembrin, pinobanksin and chrysin are characteristic flavonoids of propolis (Gardana et al, 2007); these flavonoids were determined in most of the previously analysed European honey samples (Yao et al, 2004). The screening of honey phenolic extracts by HPLC resulted in the identification of $p$-coumaric acid, chrysin, kaempferol, and apigenin in all samples tested. Honey with pine, birch, and stinging nettle extracts was richer in apigenin than other natural honey samples (Baltrušaityte et al, 2007).

Phenolic content expressed as gallic acid equivalent ranged from 44.8 mg/kg in acacia honey to 241.4 mg/kg in fir honey (average 83.7 mg/kg) (Bertoncelj et al, 2007). The antioxidant activity was the lowest in the brightest acacia and lime honey kinds and the highest in darker kinds of honey, namely fir, spruce, and forest honey kinds. Flavonoid contents in ether and water fractions were 2.57 mg and 1.64 mg catechin equivalents in 100 g honey, respectively (Blasa et al, 2007). The comparison of the contents of flavonoids in Italian Acacia and Millefiori kinds of honey recently revealed that Millefiori samples showed the highest contents of flavonoids and antioxidant activity and also demonstrated that these parameters are dependent upon the honey origin (Blasa et al, 2007). In Burkina Fasan honey, total phenolic content (mg gallic acid equivalents GAE/100 g of honey) varied from 32.59 mg to 114.75 mg with a mean of 74.38 ± 20.54 mg using the standard curve of gallic acid (Meda et al, 2005). The total phenolic content varied from 32.59 mg in multifloral honey to 93.66 mg in honeydew honey. Using the standard curve generated by quercetin, the total flavonoid content of honey samples (mg Quercetin Equivalent/100 g) varied from 0.17 mg to 8.35 mg with a mean value of 2.57 ± 2.09 mg, with the highest and the
lowest levels observed in multifloral kinds of honey. Only a low correlation ($R^2 = 0.11$) was shown between total phenolic and total flavonoid contents. The comparison of the phenolic contents of several Chilean kinds of honey showed great variations in flavonoid concentration among the samples analysed (Munoz et al, 2007). The major flavonoids detected were pinobanksin, chrysin, hesperetin, luteolin, 3-methylquercetin, isorhamnetin, pinocembrin, galangin, 3-methylgalangin, tectochrysin, 8-methoxykaempferol, apigenin, quercetin, kaempferol, pinobanksin-3-acetate, ellagic acid, and esters of caffeic acid.

**Applications of honey in the food industry**

Due to its various favourable properties honey is used as an additive to a variety of food and beverages. The application of honey as a food additive is based on its manifold properties (Table 3). The antibacterial effect of honey counteracts microbial spoilage of food, e.g. of meat (Nagai et al, 2006). The antioxidant effect of honey prevents oxidation of food during storage.

Honey acts against lipid oxidation of meat Mckibben and Engeseth (2002) and is thus an efficient meat additive for preventing oxidation spoilage, e.g. to poultry (Antony et al, 2000) or to meat and muscle of unspecified origin (Nagai et al, 2006). Effects of honey against enzymatic browning of fruits and vegetables (Chen et al, 2000), soft drinks (Lee, 1996) light raisin (Mclellan et al, 1995) apple slices (Ozmilanski and Lee, 1990) have been reported. Other physical and sensory properties make honey a good candidate for an additive to a wide variety of food: good sensory and rheological properties, superior microwave reactivity than synthetic sugars etc.

Honey enhances the growth of dairy starter cultures in milk and milk products. Especially species with weak growth rates in milk such as bifidobacteria are usually fortified by growth enhancers or by honey. The growth rate of two bifidobacteria Bf-1 and Bf-6 in milk can be stimulated by the addition of honey to milk (Ustunol and Gandhi, 2001). The effect of honey was more pronounced than the one caused by common growth enhancers based on other oligosaccharides. Thus, honey can be used as a prebiotic additive to probiotic milk products. Honey added to non fat dry milk has a favourable influence on some other “good bacteria”. The milk was incubated with *Streptococcus thermophilus*, *Lactobacillus acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, or *Bifidobacterium bifidum*. Honey
### Table 3: Application of honey as a food additive

<table>
<thead>
<tr>
<th>Use</th>
<th>Explanation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweetener for sport beverages, non-alcoholic fruit beverages, ice tea, yoghurt drinks, chocolate milk beverages, fermented beverages, vinegar, vegetable juices, mead production</td>
<td>Supplies different natural flavours and colours, honey sugars are fermentable and give alcoholic drinks unique flavours, prevents browning due to antioxidant properties</td>
</tr>
<tr>
<td>Additive to poultry and other meat, to fruit and vegetable processing</td>
<td>Antioxidative and preservative (antibacterial) properties reduce browning, improves sensory properties</td>
</tr>
<tr>
<td>Additive to microwave foods: cakes, muffins, cookies, glazes</td>
<td>Superior microwave reactivity and water activity managements and synthetic sugars</td>
</tr>
<tr>
<td>Additive to flour bagels, cereals, chicken marinades, French fries, bread, pasta, extruded snacks, corn chips, potato chips</td>
<td>Improves sensory properties, adds/retains moisture due to hygroscopic properties, improves browning due to reducing sugar</td>
</tr>
<tr>
<td>Additive to frozen ice cream and dough</td>
<td>Better stability and sensory properties</td>
</tr>
<tr>
<td>Additive to fruit spreads, peanut butter, nut spread</td>
<td>Better storability and sensory properties</td>
</tr>
<tr>
<td>Additive to salad and sauces</td>
<td>Neutralizes sour and burn intensity</td>
</tr>
<tr>
<td>Additive to fried or roasted beef, poultry</td>
<td>Reduces the formation of heterocyclic aromatic amines and their mutagenic effects</td>
</tr>
<tr>
<td>Dried honey</td>
<td>Convenient as consistent in texture, flavor and colour, allowing blending with other dry ingredients</td>
</tr>
</tbody>
</table>

**Source – Bogdanov, S (2010)**
supported the growth of all strains. The authors conclude that various oligosaccharides found in honey may be responsible for the enhanced lactic acid production by bifidobacteria (Chick et al, 2001). Due to its antioxidant activity the addition of honey to patties seems to prevent formation of heterocyclic aromatic amine and overall mutagenicity in fried ground-beef patties (Shin et al, 2003).

**Probiotics and Prebiotics –**

**Probiotics:** Probiotics was derived from greek words which means “for life”. The term “probiotics” was first introduced in 1953 by Kollath (Hamilton-Miller, 2003). An attempt on the definition of probiotics was made in 1989 by Roy Fuller who defined it like so; A live microbial supplement which beneficially affect the host animal by improving its intestinal microbial balance (Fuller, 1989). The definition by Fuller emphasized the requirement of viability for probiotics and introduced the aspect of a beneficial effect on the host. It can also be defined as “a preparation or product containing viable, defined microorganisms in sufficient numbers, which alter the microflora of the host intestine and by that exert beneficial health effects on the host (Schrezenmier and Vrese, 2001).

**Prebiotics:** The large intestine is by far the most heavily colonized region of the digestive tract, with up to $10^{12}$ bacteria for every gram of gut content. Through the process of fermentation, colonic bacteria are able to produce a wide range of compounds that have both positive and negative effects on gut physiology as well as other systemic influences (Gibson and Roberfroid, 1995). It is therefore important to manipulate the content of the gut flora with the view to increasing the numbers and activities of the presumed probiotics and reducing those of the pathogens. This can be brought about by the supplementation of human diet with some food ingredients that have been termed prebiotics. A prebiotic is a non digestible food ingredient that beneficially affects the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon and thus improves host health (Gibson and Roberfroid, 1995). Therefore intake of prebiotics can significantly modulate the colonic microflora by increasing the number of specific bacteria and thus changing the composition of the gut bacteria to favour the probiotics (International Food Information Council, 2006). It must be neither hydrolyzed nor absorbed in the upper part of the gastrointestinal tract. It must be a selective substrate for one or a limited number of beneficial bacteria commensal to the colon, which are
stimulated to grow and/or are metabolically activated. It must consequently, be able to alter the colonic flora in favour of a healthier composition. It must induce luminal or systemic effects that are beneficial to the host health. Based on these criteria listed, only a few groups of food ingredients qualify to be used as prebiotics. A good number of food materials because of their chemical structure are not absorbed in the upper part of the GIT or hydrolyzed by the digestive enzymes in humans. Such foods have been called “colonic foods” (Gibson and Roberfroid, 1995) i.e., foods entering the large intestine which also serve as food for the endogenous microorganisms. Amongst these colonic foods are non-digestible carbohydrates, some peptides and proteins. The use of peptides and proteins as prebiotics will have a major problem; their anaerobic decomposition is likely to produce potentially harmful compounds such as ammonia and amines. Studies on non-digestible oligosaccharides have shown that the fructooligosaccharides and galactooligosaccharides are those that have been found to selectively stimulate the growth and/or metabolic activity of the potentially beneficial bacteria (probiotics) in the colon (Roberfroid et al, 1998 and Ito et al, 1990).

**Prebiotic effect of honey -**

Recently, a small number of researchers have demonstrated that honey can stimulate the growth of bifidobacteria in vitro (Chick et al, 2001, Ustunol and Gandhi, 2001 and Kajiwara et al, 2002). In a large study funded by the National United States Honey Board, 3–5% (w/v) honey significantly enhanced the growth and survival of bifidobacteria BF-1, BF-6 and *B. longum in vitro* when added to inoculated samples of 12% reconstituted non-fat dry milk but no effect on bifidobacteria growth was observed with the equivalent concentrations of either fructose, glucose or sucrose (Ustunol and Gandhi, 2001). Similarly, 5% honey stimulated the growth of the human intestinal bifidobacterium spp., *B.longum, B. adolescentis, B. breve, B. bifidum and B infantis* when cultured in vitro in reinforced clostridial medium (Kajiwara et al, 2002). The mean doubling times of these bacteria decreased from 147–690 hours to 9.9–14.3 hours after 48 hours of anaerobic incubation (Kajiwara et al, 2002). In both these studies the effects of honey were equal to or greater than that observed with fructooligosaccharides (FOS), glucooligosaccharides (GOS) and inulin (Ustunol and Gandhi, 2001 and Kajiwara et al, 2002). However, in both of these studies, no control tests were performed to determine what level of bacterial growth would occur due to the glucose and fructose present in the honey. Bergey's Manual of Systematic
Bacteriology (1984) states that bifidobacteria will grow on a medium of glucose and/or fructose, thus it is possible that the bacterial growth reported by Ustunol and Gandhi (2001) and Kajiwara et al (2002) was due to the sugar components of the honey.

In line with other prebiotic research, it has also been suggested that the prebiotic effect of honey is due to its oligosaccharide content. As mentioned earlier, honey consists primarily of the monosaccharides glucose and fructose. However, a detailed examination of 27 Spanish honeys revealed that honey can also contain 10–25% minor carbohydrates, including disaccharides and tri-saccharides (Sanz et al, 2004). Astwood et al (1998) has demonstrated the presence of four tetra-saccharides, one penta-saccharide and one hexasaccharide in New Zealand honeydew honeys and it is highly likely that there are also other as yet unknown oligosaccharides present in honey. To conclusively demonstrate that the prebiotic effects of honey are due to oligosaccharides, a group of British and Spanish researchers recently investigated the growth of faecal bifidobacteria and lactobacilli when in the presence of monosaccharide-devoid honey in an in vitro fermentation system (Sanz et al, 2005). After removing the glucose and fructose from a honeydew sample via nanofiltration, yeast treatment and adsorption onto active charcoal, the remaining oligosaccharide mixture was shown to increase the bacterial populations after 12 hours of fermentation, with a prebiotic index (PI) in the range of 3.38–4.24 (the PI is calculated to give a comparable index between the growth of beneficial faecal bacteria (e.g. bifidobacteria, lactobacilli and eubacteria) and the less desirable ones (e.g. clostridia and bacteroides)) related to changes in the total number of bacteria (Palframan et al, 2003). Unlike previous studies that reported that honey appeared to have a prebiotic effect that was equal to or greater than that of FOS, this study demonstrated that the prebiotic potential of neither the total honey nor the oligosaccharide mixture was as potent as the FOS (PI = 6.89; GOS and inulin were not investigated in this study). However, this same study did demonstrate that lactic acid and acetic acid production was at least as high with honey and honey oligosaccharides as with FOS, suggesting that growth of bifidobacteria was enhanced to an equal extent in these samples.

Similarly, Shamala et al (2000) showed that Lactobacillus plantarum counts increased 10–100 fold when grown in vitro in the presence of 1% honey compared
with 1% sucrose or 1% lactose + 1% glucose. In contrast, Curda and Plockova (1995) demonstrated that the growth of *Lactococcus lactis* bacteria in skim milk was inhibited by the addition of 5–10% honey. The effects of honey on the growth of lactic acid bacteria therefore require further investigation. With the exception of the two studies mentioned earlier (Astwood et al, 1998 and Sanz et al, 2004), there appears to be very little information available as to the oligosaccharide content of honey.

**Health effects of honey:**

**Anti-inflammatory effects**

Anti-inflammatory effects of honey in humans were studied by Al Waili and Boni (2003) after ingestion of 70 g honey. The mean plasma concentration of thromboxane B(2) was reduced by 7%, 34%, and 35%, that of PGE (2) by 14%, 10%, and 19% at 1, 2, and 3 hours, respectively, after honey ingestion. The level of PGF (2α) was decreased by 31% at 2 hours and by 14% at 3 hours after honey ingestion. At day 15, plasma concentrations of thromboxane B(2), Prostaglandin E(2) and Prostaglandin F(2α) were decreased by 48%, 63% and 50%, respectively. Ingestion of honey had a positive effect in an experimental model of inflammatory bowel disease in rats. Honey administration is as effective as prednisolone treatment in an inflammatory model of colitis. The postulated mechanism of action is by preventing the formation of free radicals released from the inflammed tissues. The reduction of inflammation could be due to the antibacterial effect of honey or to a direct anti-inflammatory effect. A support of the latter hypothesis was shown in animal studies, where anti-inflammatory effects of honey were observed in wounds with no bacterial infection (Postmes, 2001).

**Immunoactivating and immunosuppressive properties**

The effect of honey on the antibody production against thymus-dependent antigen sheep red blood cells and thymus-independent antigen (*Escherichia coli*) in mice was studied. According to this study oral honey stimulates antibody production during primary and secondary immune responses against thymus-dependent and thymus-independent antigens (Al Waili and Haq, 2004). In a study by Al-Waili with humans receiving a diet supplemented with a daily honey consumption for two weeks
of 1.2 g/kg body weight ingestion of honey has the following effects: Increase of serum iron by 20% and decrease of plasma ferritin by 11%, an 50 % increase of monocytes and slight increases of lymphocyte and eosinophil percentages, reduction in serum of immunoglobulin E (34%) aspartate transaminase (22%) and alanine transaminase (18%), lactic acid dehydrogenase (41%), fasting sugar (5%) and creatine kinase and finally an increase in blood of copper (33%) and slight elevations of zinc and magnesium, hemoglobin and packed cell volume (AL-Quassemi and Robinson, 2003).

**Gastroenterological effects**

Honey is a potent inhibitor of the agent that causes peptic ulcers and gastritis, *Helicobacter pylori*. *In vitro* studies of *Helicobacter pylori* isolates that cause gastritis have shown it is inhibited by a 20% solution of honey. Even isolates that exhibited a resistance to other antimicrobial agents were susceptible. In a clinical study, the administration of a bland diet and 30 ml of honey three times a day was found to be an effective remedy in 66% of patients and offered relief to a further 17%, while anaemia was corrected in more than 50% of the patients. A clinical study of honey treatment in infantile gastroenteritis was reported by Haffejee and Moosa (1985). They found that by replacing the glucose (111 mmol/l) in the standard electrolyte-containing oral rehydration solution recommended by the World Health Organization/United Nation International Children’s Emergency Fund (1976), as well as the solution of electrolyte composition 48 mmol/l sodium, 28 mmol/l potassium, 76 mmol/l chloride ions, with 50 ml/l honey (Chaterjee et al, 1978), the mean recovery times of patients (aged 8 days to 11 years) were significantly reduced. Honey was found to shorten the duration of diarrhoea in patients with bacterial gastroenteritis caused by organisms such as *Salmonella, Shigella* and *E. coli*. They recommended that honey was a safe substitute for glucose as long as it provided 111 mmol/l each of glucose and fructose. The high sugar content of honey means that it could be used to promote sodium and water absorption from the bowel.

**Cardiovascular effects**

It has been found that honey ameliorates cardiovascular risk factors in healthy individuals and in patients with elevated risk factors. Yaghoobi et al (2008) investigated the effect of natural honey on total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), triacylglycerole, C-reactive protein (CRP), fasting blood glucose (FBG) and body weight in
overweight individuals. There were 55 patients, overweight or obese, who were randomly recruited in the study and assigned to two groups: control group (17 subjects) and experimental group (38 subjects). Patients in the control group received 70 g of sucrose daily for a maximum of 30 days and patients in the experimental group received 70 g of natural honey for the same period. In this experiment the body weight, body mass index, body fat weight, total cholesterol, LDL-C, HDL-C, triacylglycerole, FBG and CRP were measured before treatment and at day 31 after the commencement of treatment. Results showed that honey caused a mild reduction in body weight (1.3%) and body fat (1.1%). Honey reduced total cholesterol (3%), LDL-C (5.8), triacylglycerole (11%), FBG (4.2%) and CRP (3.2%), and increased HDL-C (3.3%) in subjects with normal values. Meanwhile, in patients with elevated variables, honey caused reduction in total cholesterol by 3.3%, LDL-C by 4.3%, triacylglycerole by 19% and CRP by 3.3% (P ≤ 0.05). The authors concluded that consumption of natural honey reduces cardiovascular risk factors, particularly in subjects with elevated risk factors, and it does not increase body weight in overweight or obese subjects.

The effects of ingestion of 75 g of natural honey compared to the same amount of artificial honey (fructose plus glucose) or glucose on plasma glucose, plasma insulin, cholesterol, triglycerides (TG), blood lipids, C-reactive proteins and homocysteine, most of them being risk factors for cardiovascular diseases, were studied in humans (Al Waili, 2004). Elevation of insulin and C-reactive protein was significantly higher after glucose intake than after honey consumption. Glucose reduced cholesterol and LDL-C. Artificial honey slightly decreased cholesterol and LDL-C and elevated TG. Honey reduced cholesterol, LDL-C and TG and slightly elevated HDL-C. In patients with hypertriglyceridaemia, artificial honey increased TG, while honey decreased TG. In patients with hyperlipidaemia, artificial honey increased LDL-C, while honey decreased LDL-C. In diabetic patients, honey caused a significantly lower rise of plasma glucose than dextrose.

In a study with 30 persons and 30 controls showed that no significant decrease of cholesterol HDL and TG was encountered. While there were no effects in men, in women HDL values were increased in the controls having ingested sucrose, while in
the honey group no increase was encountered, pointing out that honey has a positive effect in women (Munstedt et al, 2009).

Bahrami et al (2009) tested the effect of honey intake on the blood risk factors of diabetes 2 patients (controls with no intake). Body weight, total cholesterol, low-density lipoprotein-cholesterol and triglyceride decreased, while and high-density lipoprotein-cholesterol ratio increased significantly.

Ahmad et al (2009) tested the effect of honey on bovine thrombin -induced oxidative burst in human blood phagocytes. Honey treatment of phagocytes activated by bovine thrombin showed effective suppression of oxidative respiratory burst. It can be assumed that this suppressive activity of honey could be beneficial in the interruption of the pathological progress of cardiovascular disease and may play a cardioprotective role.

Honey can contain nitric oxide (NO) metabolites which are known indicators for cardiovascular disease risk. Increased levels of nitric oxides in honey might have a protecting function in cardiovascular diseases. Total nitrite concentration in different biological fluids from humans, including saliva, plasma, and urine was measured after ingestion of 80g of honey. Salivary, plasma and urinary NO metabolite concentrations showed a tendency to increase. Different honey types contained various concentrations of NO metabolites, darker or fresh honeys containing more NO metabolites than light or stored honey. After heating, NO metabolites decreased in all honey types (Al Waili, 2004 and Al Waili and Boni, 2003). Busserolles et al (2002) observed that compared to fructose-fed rats, honey-fed rats had a higher plasma α-tocopherol level, and a higher α-tocopherol /triacylglycerol ratio, as well as lower plasma nitrate levels and lower susceptibility of the heart to lipid peroxidation.