Review of Literature

2:1 Fruit growth and ripening:

As a subject of analysis, fruit development has the same advantages as the other systems of comparable sophistication. Right from the early stage of fruit set to the ripening of fruits, it undergoes through periods of slow growth, accelerated growth, declining growth and senescence. To enable commercial handling of fruits, they are usually harvested while still firm and are allowed to ripen later before consumption. The determination of number of days between full bloom and maturity gives an approximate guide to harvest date, but the length of this period depends on seasonal climatic conditions. The maturity indices are said to vary among cultivars, climactic conditions, crop load, production area, seasons etc. Moreover, maturity index of fruit is either based upon its physiological or commercial maturity. The physiological maturity is defined as the stage when growth ceases and ripening begins, while commercial maturity is based on components of the fruit which changes during fruit development (Reid, 1992). Maturity indices can also be based on a mixture of physico-chemical characteristics that are judged either objectively or subjectively. As several physico-chemical factors can be integrated to give an assessment of harvest date but no single factor is said to be reliable. On the other hand experienced growers rely mainly on the subjective assessments based on their previous observations (Kays, 1991). Hence, the assessment of maturity still remains problematic.

The nutritional and climatic condition during the life of the plant usually affects the post harvest behavior of the fruits. Freshly harvested fruit is a group of living cells, still undergoing metabolic reactions. It lives an independent life, using the nutrients accumulated during growth and no longer depends upon absorption of water and minerals by the roots or photosynthetic activity of the leaves or conduction of vascular tissue. In contrast to most of the other tissues, where there is an inflow and outflow of metabolites it influences the metabolism. Thus, fruits can be considered as an isolated system (Stanley, 1998).

The ripening of fruit does not start until it has developed completely. It is also difficult to stimulate ripening in fruits before a certain state of readiness. Physiologically, ripening of a fruit is a stage of senescence reached after growth has ceased on the plant, leading ultimately to the death of the fruit. Fruit ripens by a sequence of physical and biochemical events and this irreversible process has received attention of many plant
physiologists, biochemists and horticulturists. The fruits have been extensively worked out to understand the ripening changes and biochemical regulations, to improve the methods of post harvest handling of the fruits. Although a number of studies have been carried out regarding the behavior of different fruits during ripening, the exact mechanism of these changes are not very clearly understood.

Fruit ripening involves many physiological and biochemical changes in the tissues of fruit which under ideal conditions, are integrated and lead to the production of acceptable ripe fruit (Thompson, 1996). These changes in quality make the fruit attractive to consumers and in nature these changes are thought to favor seed dispersal. Ripening frequently involves changes in fruit colour often involving the destruction of chlorophylls and the synthesis of new pigments (Rhodes, 1980; Seymour and Tucker, 1993), the production of the volatiles responsible for aroma (Davies and Hobson, 1981), the conversion of starch into sugars (Biale and Young, 1981), an increase in carbon dioxide and ethylene production in climacteric fruits (McGlasson et al., 1978), a decline in acidity and textural changes associated with fruit softening (Huber, 1983). Changes in these parameters are thought to be regulated and coordinated by the expression of specific proteins (Brady, 1992). Overall, ripening processes are probably under the control of plant growth regulators (McGlasson et al., 1978). Finally ripening is said to be a genetically programmed phase involving a series of changes occurring during the early stages of senescence of fruits in which the structure and composition of the unripe fruit is altered so that it becomes edible (Rhodes, 1980). Thus, these changes lead to a complex network of anabolic and catabolic reactions occurring during the ripening of fruits.

A perusal of published literature manifests that the subject of fruit growth and ripening has been reviewed a number of times. The work of several workers like Biale (1954); Hansen (1966); Hulme (1971 & 1972); Coombe (1976); Rhodes (1980); Brady (1987); Seymour et al. (1993); Raghavan (2000); Giovannoni (2001); Salunke and Kadam (2005) and Prasanna et al. (2007) could be found in the literature regarding the growth and ripening of fruits.
2:2 Histological studies:

The importance of fruit anatomy is well understood while analyzing the statements of various authors. Cuter (1971) is of the opinion that fruit anatomy is not often used as a taxonomic character. Carlquist (1961) commented that the anatomical studies on the vegetative organs and to some extent floral parts have developed in greater measure but reflections on fruit anatomy are less. Eames and Mac Daniels (1947) also stressed the necessity of considering anatomical characters of mature fruit to determine its morphological nature. Structure and development are the two closely related aspects of any living matter. Many structures can be better understood if their development is known thoroughly or in other words it is frequently necessary to investigate the development of an organ, organism or character in order to allow its accurate interpretation (Bhatnagar, 1998).

When an ovary develops into a fruit, the ovary wall becomes the pericarp. The term fruit wall is applied to the pericarp of fruit derived from superior ovaries and to the combination of pericarp and non-carpellary parts in fruits originating from inferior ovaries. During maturation the pericarp frequently shows an increase in the number of cells. The pericarp may be differentiated into three parts, more or less distinct morphologically: the exocarp or epicarp, the mesocarp, and the endocarp; that is, the outer, median and inner layers respectively (Esau, 1953).

The fleshy tissue of fruits may develop by cell enlargement without any cell division or by cell division followed by cell enlargement (Sinnott, 1939). If the entire ground tissue develops into a fleshy tissue, the fruit is a berry. All the fleshy tissue may originate from the ovary walls as in the tomato, where the main body of the mature fruit consists of placenta. The placental tissue constitutes the pulp of the mature fruit. The exocarp functions as the protective layer of the fruit. In many cases, the sub-epidermal layers are included in exocarp formation; a specially differentiated hypodermis may take part in the formation of the outer rind of the fruit and even several sub-epidermal layers may be involved in shell development. The mesocarp lies between exo and endocarp and often develops as a fleshy parenchymatous tissue, called sarcocarp. The endocarp of the drupe may develop as a hard stony and lignified layer which protects the seed (Roth, 1977).
Fruit development is frequently initiated by cell division which is later followed by cell enlargement. The period of cell division may be short and eventually restricted to pre-anthesis time, while cell enlargement is always of greatest importance. Two principal growth phenomena are involved in fruit development: longitudinal growth and growth in thickness which causes growth in circumference of the peripheral pericarp parts. Growth in thickness, on the other hand, can be accomplished by cell division periclinally to the fruit surface or by radial cell enlargement which is also related to the radial symmetry of the fruit (Roth, 1977). However, a pronounced change in shape or structure takes place, as the ovary enlarges to transform into the mature fruit, this is usually accomplished by localized cell division. After a specific cell size is reached, division ceases. There is apparently an upper limit of cell size for cell division which also includes the state of the cell. When the cell has become vacuolated, cell division is usually suspended and cell enlargement begins. But also the amount of cell enlargement is limited by deposition of secondary wall material, which may become so strong that further enlargement is impossible (Sinnott, 1939).

It is well known that fruits are rich in ergastic materials, such as sugars, starch, cellulose, oil, fats and proteins which are usually stored in parenchymatous cells or in special idioblasts and that these substances may appear and disappear at different times during the life history of the fruit. In unripe fruits, starch is often present in high quantities but disappears gradually during maturation, when it is transformed to sugar. Organic acids are another feature of fruits and their presence adds the special taste and flavour to them. Acids are usually abundant in unripe fruits, but their content is considerably reduced during ripening. Tannins are particularly abundant in unripe fruits. The free tannin content diminishes during fruit maturation and ripening fruits gradually lose their astringent character, probably by conversion of soluble tannin into insoluble compounds (Roth, 1977).

Although all other facets or ripening have been extensively investigated, the structural/histological changes have received very little attention. A comprehensive account of anatomical structure of plant tissues including fruits and vegetables have been published by Esau (1953). The survey of literature shows that some of the earlier students of Department of Biosciences have contributed significantly and enriched knowledge on anatomy and morphology of fruits in relation to development. Such as the
histo-morphological and histochemical changes during growth and development of the fruits of Solanaceae, Cucurbitaceae, Caesalpiniaceae, Mimosaceae, Umbelliferae, Asclepiadaceae, Periplocaceae, Apocynaceae, Papilionaceae, Acanthaceae, have been worked out (c.f. Sebastian, 1994). Morpho-histological studies on different types of fruits such as capsules, samara, schizocarps, berries, drupes and achenes have been worked out by Sebastian (1994). Besides, Bhatnagar (1998) has studied the Morpho-histogenic and histo-chemical studies in the fruits of Combretaceae, Lythraceae and Myrtaceae, while recently certain physiological and histological studies have also been carried out in some Cucurbits by Shah (2006).

The screening of literature further reveals that many research articles have been published dealing with the histological studies on fruits. Seidemann (1993) studied the anatomy and food uses of Aronia fruits. Comparative anatomy of the pericarp in the fruit of plum, apricot and peach is studied by Rotaru (1994). Morphology of fruits, seeds and seedlings of Platonia insignis Mart. (Clusiaceae) with anatomical aspects of fruit and seed development is reported by Mourao and Beltrati (1995). The structure of mature fruits of some species of Potamogeton (Potamogetonaceae) is investigated by Karcz and Toma (1996). An ultrastructural and anatomical factors associated with resistance to cuticle cracking in tomato (Lycopersicon esculentum Mill.) is studied by Emmons and Scott (1998). Fruit anatomy and development in Malpighia emarginata is reported by Laskowski and Bautista (2000).

Peculiarities of the Malus domestica (Rosaceae) fruit anatomy depending on the altitude of cultivation in mountains is described by Kumakhova (2003). Anatomical investigation of lenticel development and subsequent discolouration of 'Tommy Atkins' and 'Keitt' mango (Mangifera indica L.) fruit is carried out by Bezuidenhout et al. (2005). Development of fruit and seed covers in Calligonum junceum is investigated by Ashurmetov et al. (2006). Histo-architecture of the pericarp and seed liberation in the schizocarpic fruit of Sida rhombifolia L. is studied by Rao and Dave (2007). Coan et al. (2008) made comparative study of ovule and fruit development in the species of Hypolytrum and Rhynchospora. Ultrastructure of pericarp in the developing walnut (Juglans regia L.) fruit is studied by Wu et al. (2009).
2:3 Compositional studies:

2:3.1 Pigments:

Colour change in fruits during ripening often serves as visual maturity index. Fruit color or visual appearance is determined by the various pigments present in the skin and flesh (Rood, 1957). As fruit matures and ripen, green colour decline and develops yellow, red or other colours due to the presence of accessory pigments, which are characteristic of the various cultivars (Romani and Jennings, 1971). Colour changes can be either dependent or independent of ethylene action according to the pigments involved and the fruit species (Lelievre et al., 1997).

Chlorophyll is the major pigment that dominates in unripe stages of fruits and vegetables. Chlorophyll ‘a’ and ‘b’ are most common pigments in all photosynthetic and storage tissues. The surface pigment changes have been recorded during ripening/ senescence of fruits, but in vivo degradation of chlorophylls and its derivatives is least understood (Durand and Martin, 1974). Chlorophyllase activity is often correlated with chlorophyll degradation and also with its synthesis (Aljuburi et al., 1979). Enzymatic cleavage of chlorophylls into small colourless fragments and distribution of chlorophyll ‘a’ and ‘b’ in various chlorophyll protein complexes is also suggested (Thornber, 1975).

The colour change in fruits from green to yellow is mainly due to chlorophyll degradation, which subsequently reveals the presence of yellow carotenoids pigments (Seymour et al., 1993). Carotenoids are C₄₀ iso-propanoid compounds that participate in a number of physiological processes in plants and other organisms (Fraser et al., 2001). These compounds are essential in photosynthesis where they function as energy carriers and photo-oxidation protectors (VanDen Berg et al., 2000). They are also important for pollination mediated by insects in flowers, while in fruits they serve as indicators of maturity that make fruits attractive for human consumption (Arias et al., 2000).

One of the most significant values of carotenoids is its essential role in human nutrition and prevention of many diseases (Ye et al., 2000). Carotenoids are free radical scavengers and also natural antioxidants. Unfortunately, humans do not synthesize carotenoids. Therefore, they consume fruits and vegetables to meet this nutritional requirement. The most studied carotenoids include β-carotene and lycopene, although other carotenoids such as lutein and zeaxanthin have also received considerable attention because of their antioxidant properties (Volker et al., 2002).
Lycopene and β-carotene both inhibit reactive oxygen species-mediated reactions, which have been associated with many diseases (Giovanelli et al., 1999). Lycopene is also involved in the prevention of heart attacks and different types of cancer (Shi et al., 1999). It is interesting to note that in some fruits, lycopene and β-carotene accumulate late in fruit maturation and continue accumulating even after ripening (Giovanelli et al., 1999). The composition of carotenoids differs from one species to another. They are relatively simple in tomato with lycopene and β-carotene (Bartley, 1974), but highly complex with 115 different carotenoids in mature citrus fruits (Stewart, 1977) and with less degree of complexity in capsicum (Camara et al., 1983) and mango (Nagoway et al., 1982).

The pigment anthocyanins are the members of a class of nearly universal, water-soluble, terrestrial plant pigments that can be classified chemically as both flavonoid and phenolic. They contribute colors to flowers, leaf and fruits ranging from shades of red through crimson and blue to purple, including yellow and colorless. According to Francis (1977), the blue to red colour imparted by the anthocyanins depends largely upon the pH of the medium. Anthocyanin-related pigments serve as a UV screen and are produced in response to exposure of the plant to UV radiation, protecting the plants DNA from damage by sunlight. Environmental factors affecting anthocyanin production includes light, temperature, water, carbohydrate and the concentrations of the elements nitrogen, phosphorous and boron.

There are more than 300 different types of anthocyanins that fall into six major groups: delphinidin; cyanidin; petunidin; pelargonidin; peonidin; and malvidin. Kuhnau (1976) describes anthocyanins as water-soluble plant pigments of the 2-phenylbenzophyryylum (flavylium) structure. Anthocyanins normally are bound to sugar molecules (as glycosides) and it is known that without bound sugars (the aglycone) it is extremely unstable. Anthocyanins have long been the subject of investigation by botanists and plant physiologists because of their roles as pollination attractants and phyto-protective agents. Food scientists and horticulturists continue to study these compounds because of their obvious importance to the color quality of fresh and processed fruits and vegetables. Also interest in anthocyanin pigments has intensified because of their possible health benefits as dietary antioxidants.
Thus, the pigment changes appear to be controlled separately from other processes of ripening such as softening, sugar accumulation, respiration, etc. However, there is striking similarity in the hormonal control of pigment changes occurring within plastids (chlorophylls, carotenoids) and in the vacuole (anthocyanins). Also, pigments changes in maturing fruits are similar to patterns of leaf senescence (Goldschmidt, 1984).

2:3.2 Carbohydrates:

Carbohydrates are the most abundant biochemical constituents in plants, representing 50-80% of the total dry weight. They function as forms of stored energy reserves and make up much of the structural framework of the cells. In addition, simple carbohydrates such as the sugars - sucrose and fructose impart important quality attributes to many harvested products (Wills, 1996).

Carbohydrates is a group that is defined as polyhydroxy aldehydes or ketones or substances that yield either of these compounds upon hydrolysis as carbohydrates are an energy source for the respiratory processes during fruit storage and ripening (Kozlowski, 1992). In addition, sugars that have a free or potentially free aldehyde group are classified as reducing sugars based on their ability to act as a reducing agent (accept electrons) in an alkaline solution. Most of the common sugars are reducing sugars (e.g., glucose, fructose, galactose, mannose, ribose and xylose). Sucrose and raffinose are the most common non-reducing sugars. Glucose and fructose are the predominant simple sugars found, especially in fruits; however, mannose, galactose, arabinose, xylose and various others are found in a number of harvested products. Sucrose is a disaccharide which yields glucose and fructose upon hydrolysis (Stanley, 1998).

Cellulose is a straight chain polymer of glucose and represents one of the most abundant compounds. Cellulose is not, however, present in large quantities in many storage organs where storage carbohydrates or lipids are found. Cellulose molecules are extremely stable and can be broken down only with strong acids or by enzymes, called cellulase, capable of hydrolyzing the molecules. There is often little change in the cellulose structure in fruits during ripening and the activity of enzymes do not correlate with softening changes that occur during ripening (Hobson, 1993).
Starch is a mixture of branched and straight chained glucose polymers that represents the major storage carbohydrate in fruits. It is stored in the form of starch grains found in specialized storage plastids (amyloplast). Starch is comprised of two compounds; a straight chain molecule – amylase and amylopectin, a branched chain molecule. The conversion of starch to sugars in the fruit is an important component of the ripening process, giving the fruit its distinctive flavor as well as precursors for many of the aromatic flavor compounds. Starch can be converted back to glucose by at least three different enzymes; α-amylase, β-amylase and starch phosphorylase (Lehninger, 2005).

The bulk of the primary cell wall in plants is comprised of dense gel like noncellulosic polysaccharides called pectic substances. Pectin is found extensively in the middle lamella where it functions as the binding agent between neighboring walls. There are three primary forms of pectic substances; pectic acids, pectins and protopectins. Each is comprised largely of α-(1-4) linked D-galacturonic acid subunits. The activity of pectic enzymes has been shown to be closely correlated with increase in soluble pectins. This increase is due to hydrolytic cleavage of the long pectic chains increasing their solubility. The principal pectic enzymes are pectinesterase, endopolygalacturonase and exopolygalacturonase. The hemicelluloses represents a heterogenous group of polysaccharide compounds that are closely associated with cellulose; hence the name (half) cellulose. They are one of the major components of cell walls and are quite stable once formed (Stanley, 1998).

The review of literature revels that the fruit ripening in relation to change in carbohydrates has been studied by a number of researchers. Carbohydrate metabolism during fruit development in sweet pepper is observed by Nielsen et al. (1991). Cell wall polyuronides and carbohydrates in papaya fruit during ripening is reported by Lazan et al. (1995). Wegrzyn and MacRae (1995) reported the role of amylase in the degradation of starch in kiwi fruit. Changes in carbohydrates content during cellular development of tomato fruit is reported by Davies and Cocking (1995). Sucrose to starch metabolism in tomato fruit is described by Schaffer and Petreikov (1997). Beruter and Feusi (1997) reported carbohydrate partitioning in growing apple fruit. Prabha and Bhagyalakshmi (1998) described carbohydrate metabolism in ripening banana fruit. Carbohydrate metabolism during early fruit development of sweet melon is noted by
Gao et al. (1999). Holland et al. (2002) observed carbohydrates in the mandarin fruits when harvested at different maturity stages. Zhang et al. (2005) reported production, physiochemical properties and digestibility of banana starch. Metabolism of starch in developing strawberry fruits was reported by Souleyre et al. (2004). Degradation of starch in mango fruit has recently been reported by Simao et al. (2008).

2:3.3 Protein and amino acids:

Proteins are extremely important components of living cells in that they regulate metabolism, act as structural molecules and in some represent storage forms of carbon and nitrogen. Proteins are composed of chains of amino acids each joined to the next in a sequence by peptide bond. Thus, they are often referred as polypeptides. There are approximately 22 amino acids that are commonly found; however, over 100 non-protein amino acids have also been identified. Amino acids are small in size and soluble in water (Stanley, 1998).

During the growth of the fruit various metabolic processes continue, requiring specific proteins in appropriate quantities and at precise times. Proteins can be grouped into three general classes based on their function – (i) structural proteins (membrane and cell wall proteins), (ii) storage proteins and (iii) enzymes. Enzymatic proteins are extremely important in that they regulate virtually all of the biochemical reactions within the cells of the fruit. Primarily through enzyme synthesis, activation and degradation, control is exerted over the rate of specific processes, thus allowing the fruit to adjust its metabolism to change the environment in which it is held to genetically control metabolic shifts (e.g. ripening). Many proteins within the cells are in a continuous state of synthesis and degradation. After synthesis, they begin to progress towards eventual degradation and recycling of their component parts. Protein degradation studies have been directed in seeds during germination, while other plant parts such as leaves, fruits and roots are poorly understood (Stanley, 1998).

The relative change in the protein and amino acid content and composition in fruit is those that undergo significant changes in homeostasis during fruit ripening. Therefore, while proteins are broken down and the component amino acids are recycled, a small but extremely important number of specific proteins are synthesized. During the onset of ripening it has been shown that the actual concentration of protein increases.
Proteins and amino acids are minor constituents of fruit and have no role in determining eating quality. Changes in nitrogenous constituents however indicate variations in metabolic activity during different growth phases. During the climacteric phase of many fruits, there is a decrease in free amino acids which often reflects an increase in protein synthesis. During senescence, the level of free amino acids increases reflecting a breakdown of enzymes and decreased metabolic activity (Stanley, 1998).

Numerous studies have been reported on the metabolism and physiology of many fruits, including their free sugar, organic acid, and amino acid compositions. The relationships between these compounds and fruit ripening have been extensively studied in different fruits such as peach (Chapman & Horvat, 1990), persimmon (Ayaz and Kadioglu, 1998; Senter et al., 1991), apples (Ackerman et al., 1992), strawberry (Perez et al., 1992), blueberry (Ayaz et al., 2001).

The published literature in relation to fruit growth and ripening includes. Frankel et al. (1968) has investigated the synthesis of proteins in relation to ripening of pome fruits. Changes in yellow pigments, protein and total carbohydrates content during ripening of ber fruits is studied by Bal et al. (1978). The protein and amino acid composition of ten tropical fruits has been investigated by using gas-liquid chromatography by Hall et al. (1980). The effect of calcium on cell wall structure, protein phosphorylation and protein profile in senescing apples was described by Glenn et al. (1988). Influence of cultivar and fruit ripening on fruit protein content, composition, and antioxidant activity of olive (Olea europaea) is reported by Zamora et al. (2001). Changes in amount of sugar, organic acids and amino acids in medlar fruit during development and maturation is reported by Glew et al. (2003). Araujo et al. (2004) studied the biological activity of proteins from the pulps of some tropical fruits.

2.3.4 Phenolics:

Phenolic compounds increase under environmental stress, playing a vital role in plant survival. They provide cell-wall support materials (lignin), function as inhibitory compounds in competition among plants (allelopathy), and act as signal molecules in plant defense mechanisms against invasive stress of microorganisms, excessive UV light, drought and wounding.
Polyphenolic compounds commonly serve as a protective mechanism in plants, warding off predator and microbiological attack. Many factors affect polyphenolic concentrations, including cultivar differences, growing conditions, maturity and postharvest handling of fruit (Lakshminarayana et al., 1970; Selvaraj and Kumar, 1989; Häkkinen and Törrönen, 2000). Abiotic stresses such as excessive UV, heat and chilling temperatures, wounding and drought that are introduced before or after fruit harvest may affect the biosynthetic pathways of secondary metabolites (Cisneros-Zevallos, 2003), as fruit attempt to protect themselves. Under normal ripening conditions, polyphenol content is highest during fruit growth and decreases with ripening (Lakshminarayana et al., 1970, Selvaraj and Kumar, 1989). These observations indicate a correlation between fruit ripening and loss of secondary metabolic substrates. However, polyphenolic content may increase under conditions of abiotic stress as phenolic compounds have been reported to increase following chilling injury of spinach leaves (Howard et al., 2002) and wound healing of lettuce leaves (Kang and Saltveit, 2002).

Polyphenolic compounds such as benzoic acids, flavonoids, cinnamic acids and tannins possess an aromatic ring bearing hydroxyl substituents that will readily take part in hydrogen bonding unless hindered, allowing them to donate hydrogen ions to 15 free radicals. These hydrogen donations classify them as antioxidants in many in vivo and in vitro systems because of their ability to scavenge free radicals. Their function is to delay or prevent the oxidation of important biological components. The efficiency of a given polyphenolic compound in acting as an antioxidant, is dependent on the number and/or position of hydroxyl groups available for donation (DeWhalley et al., 1990).

Polyphenolic compounds in fruit and fruit products (such as wine, grapes, prune juice, strawberries, cranberries and apples) may, therefore, be linked to prevention of degenerative diseases due to their antioxidant activity. Epidemiological studies indicate that fruit phenolic phytochemicals are capable of reducing the risks of cardiovascular disease, stroke, and atherosclerosis (Kelly et al., 2001), through the prevention of cellular oxidative damage. Their antioxidant activities also have the potential to inhibit oxidative damage to cellular DNA, preventing mutagenesis and tumorigenesis (Kelly et al., 2001). Acting as antioxidants on LDL free radicals, they can lower the amount of oxidized LDL cholesterol, which in turn can reduce the risk of coronary atherosclerosis (DeWhalley et al., 1990; Hertog et al., 1992).
The chemical composition of fruit and thus their health benefits to consumers are largely dependent on the maturity of the fruit and the degree of processing applied prior to consumption. Generally, fruit will reach their highest levels of sugars, ascorbic acid, soluble solids, fats and pectin, and their lowest levels of acidity and phenolic acids as they ripen (Bulk et al., 1997). Recent awareness of the role of antioxidants play in the promotion of health, due to their ability to act as chemoprotective agents (Teissedre and Waterhouse, 2000), has contributed to the rise in consumer demand for fresh fruits. Epidemiological studies have linked numerous food polyphenolic compounds with the reduction in the risk of various diseases, attributed to their natural antioxidants ability to act as free-radical scavengers. Sufficient evidence exists between in vivo and in vitro studies to support the role of antioxidant polyphenolic compounds in the prevention of disease and thus promotion of health.

2:3.5 Textural and firmness changes:

Texture is an attribute, difficult to define and measure. Fruits and vegetables are often characterized by different textural types that alter with advances of ripening and senescence. The texture profile varies according to maturity and is greatly influenced by the structure and composition of the cell walls (Bourne, 1982). In large parenchymatous tissues of fruit cells, the degree of contact between cells is also an added factor.

The chemical composition and nature of cell walls has been well studied and established by several workers (Ahmed and Lambavitch, 1980; Knee, 1973). The main components of cell wall carbohydrates are pectin, cellulose and other polygalacturonase (Albersheim, 1974). Pectins make up by one third of the dry matter of primary cell wall of fruits and vegetables and are mainly present in the middle lamella. The chemistry of these substances has been extensively studied (Pilnik and Voragen, 1970). Along with pectins, hemicelluloses contribute to the bulk of non-cellulosic dry material of primary cell wall (Northcote, 1972). Cellulose (β, 1-4 glucon) is present in primary cell wall in linear association of the polymer molecules called fibrils. They greatly influence the cell wall properties (Walter et al., 1977). These fibrils usually form a loose network (Itoh, 1975) but parallel arrangement is not uncommon (Northcote, 1972). In addition to these complex carbohydrates, 0.5 – 2 % of the dry weight of fruits and vegetable cell walls is constituted by proteins (Lamport, 1977). Lignin is absent or present in very low concentration in the cells of fruits and vegetables at their edible stages. Lignin is a complex polymer derived from phenolic compounds appears in senescent vegetables in their secondary cell walls (Nelmes and Preston, 1968).
Texture is also influenced by varieties, maturity stages and several pre and post harvest factors. Various methods are used to measure these properties. They are based on the principles of puncture, deformation and extrusion (Ben-Yehoshua et al., 1983). Comprehensive review has been published by Bourne (1982) is available on the principles, methods and instrumentation of textural evaluation of horticultural corps. Most fruits soften during ripening and this is a major quality attribute that influences consumer acceptance for fruits and the shelf life of fruits. Fruit softening is regulated by three mechanisms, viz. (i) loss of turgor, (ii) degradation of protopectin and (iii) the enzymatic breakdown of the fruit cell walls (Seymour et al., 1993).

Two broad mechanisms for softening of fruit cell walls have been proposed. One proposal involves a leakage of organic acids into the apoplast or a decrease in the pH of the apoplast due to the action of a plasma membrane bound H+ and ATPase, which results in the removal of Ca++ bridges between pectin chains (Brady, 1992). According to the second proposal, softening is the result of enzymatic degradation of protopectin and cell wall hydrolysis during fruit ripening and senescence (Perring and Pearson, 1988; Weichmann, 1986). Other changes in cell wall composition that accompany fruit softening include decrease in wall bound pectic polysaccharides and corresponding increases in soluble pectic components (Pilnik and Voragen, 1970) and changes in neutral sugar components such as arabinose, galactose and xylose (Gross and Sams, 1984).

Fruits soften, while vegetables toughen during later stages of storage. Degradation of pectic and cellular material results in textural weakening of senescent tissues (Kim, 1984). The semi-permeability of cell membranes leads to the loss of turgor and softening (Massey, 1968). A reduction in cell wall pectin due to increased solubility has been reported in a whole range of ripening fruits (Pilnik and Voragen, 1970). Changes in polyuronides have been intensively investigated by Labavitch (1981). In addition a net loss of non cellulosic neutral sugar residues has been recorded during softening (Ahmed and Labavitch, 1980; Gross and Sans, 1984; Knee et al., 1977).
In order to understand the changes occurring in cell walls during softening, knowledge of the primary cell wall, middle lamella and factors such as enzymatic or non-enzymatic is required as they could influence the cell wall architecture. However, there is little detailed knowledge of the cell wall structure of many mature fruits (Brady, 1987). The change in texture of fruits during ripening has been linked with several enzymatic changes including the loss of neutral sugar side chains, changes in the synthesis of cell wall components products and the breakdown of calcium pectate (Hobson, 1993). However, some of the products of cell wall metabolism have also shown to promote ripening.

It has been established that certain proteins, at least some of them metabolically active, are bound to fruit cell walls, while those rich in hydroxyproline residues play an intrinsic role in wall structure. There is evidence that endopolygalacturonase (PG), one of the enzymes responsible for the degradation of pectin, is not evenly distributed in cell walls. As PG is immobile, the enzyme would be required to accumulate if extensive hydration of cell walls accompanies ripening, so that pectin becomes solubilized and that hemicelluloses are also degraded at this time (Hobson, 1993). Therefore, cellulose microfibrils are left without support and starts to breakdown leading to the cell separation or changes in wall elasticity. These changes are thought to be caused by a small group of hydrolytic enzymes (Huber, 1983).

Enzymes thought to be involved in fruit cell wall metabolism during ripening include polygalacturonase (PG), pectin methyl esterase (PME), cellulose and β-galactosidase (Awad and Young, 1979; Seymour and Tucker, 1993). PME removes methyl groups from the pectin chains which may then be later attacked by PG (Tucker and Grieren, 1987). The relative importance of these cell wall hydrolyses may vary among species (Award and Young, 1979).

2.3.6 Oxidative changes:

Fruit ripening has been described as an oxidative phenomenon, which requires a turnover of active oxygen species, such as H₂O₂ and superoxide anion (Hamilton, 1974). For this to be the case there must be a balance between the production of active oxygen species and their removal by antioxidant systems. It is likely, therefore, that the antioxidant system will play a crucial role in the ripening process (Foyer and
Halliwell, 1976). The term antioxidant can be considered to describe any compound capable of quenching active oxygen species without itself undergoing conversion to a destructive radical (Nishikimi and Yagi, 1996). Antioxidant enzymes are considered as those that either catalyze such reactions and/ or are involved in the direct processing of active oxygen species. Hence, antioxidants and antioxidant enzymes function to interrupt the cascades of uncontrolled oxidation (Halliwell et al., 1995).

The term active oxygen species is generic, embracing not only free radicals such as superoxide and hydroxyl radicals but also H$_2$O$_2$ and singlet oxygen. It is generally assumed that the hydroxyl radical and singlet oxygen are so reactive that their production must be minimized (Jakob and Heber, 1996). Hydroxyl radicals (and their derivatives) are among the most reactive species known to chemistry (Cadenas, 1989), and are able to react indiscriminately to cause lipid peroxidation, the denaturation of proteins, and the mutation of DNA. Lipid peroxidation is commonly used as an indicator of oxidative stress, although it can be caused by other reactive species (Bowler et al., 1992). In addition, singlet oxygen, which is formed when excitation energy is transferred to oxygen, also produces deleterious effects (Cadenas, 1989).

The antioxidant enzymes are involved in virtually all major areas of aerobic biochemistry (e.g. respiratory and photosynthetic electron transport; oxidation of glycolate, xanthine, and glucose) and are produced in copious quantities by several enzyme systems (e.g. plasmalemma-bound NADPH-dependent superoxide synthase and superoxide dismutase) (Bowler et al., 1992). The chief toxicity of O$_2$ and H$_2$O$_2$ is thought to reside in their ability to initiate cascade reactions that result in the production of the hydroxyl radical and other destructive species such as lipid peroxides. These dangerous cascades are prevented by efficient operation of the cells antioxidant defense. In some circumstances, the destructive power and signaling potential of active oxygen species are utilized as an effective means of defense (Chen et al., 1993, Foyer et al., 1997).

The antioxidant enzyme includes catalase, superoxide dismutase, some peroxidases and the enzymes involved in the ascorbate-glutathione cycle (Scandalios et al., 1980; Dalton, 1995; Asada, 1996). These enzymatic components, together with the low-molecular-weight antioxidants ascorbate and glutathione, ultimately scavenge H$_2$O$_2$ at the expense of NADPH or NADH (Foyer and
Halliwell, 1976; Jimenez et al., 2002). Ascorbate is an antioxidant enzyme involved in removing active oxygen species. It is also utilized in cell metabolism and may be involved in the control of growth (Navas and Gomez-Diaz, 1995), cell division (Liso et al., 1984; Kerk and Feldman, 1995) and cell wall expansion (Takahama and Oniki, 1994). Efficient destruction of O₂ and H₂O₂ requires the action of several antioxidant enzymes acting in synchrony. Superoxide produced in the different compartments of plant cells is rapidly converted to H₂O₂ by the action of superoxide dismutase (Bowler et al., 1992). In plant cells, the most important reducing substrate for H₂O₂ detoxification is ascorbate (Mehlhorn et al., 1996).

Superoxide radicals, hydrogen peroxide, and singlet oxygen are formed from many cellular reactions (Bowler et al., 1992). In general, superoxide can arise when electrons are misdirected and donated to oxygen. Mitochondrial electron transport, for example, is a well-documented source of superoxide radicals, as is the electron transport chain of the photosynthetic apparatus within the chloroplast. An additional problem for the chloroplast is the transfer of excitation energy from chlorophyll to oxygen, which can generate singlet oxygen (Bowler et al., 1992). Hydrogen peroxide is disposed by catalases and peroxidases. In plants, catalase is found predominantly in peroxisomes (and also in glyoxysomes) where it functions chiefly to remove the H₂O₂ formed during photorespiration (or during β-oxidation of fatty acids in glyoxysomes) (Bowler et al., 1992). In spite of its restricted location it may play a significant role in defense against oxidative stress since H₂O₂ can readily diffuse across membranes. Some of these enzymes have broad substrate specificity while others can only function with one.

Catalase is an enzyme related to the cellular control. Catalase catalyses the dismutation of hydrogen peroxide into water and oxygen (Redinbaugh et al., 1988). Peroxidase enzymes participate in hormone catabolism, phenol oxidation, polysaccharides and cell wall proteins intercrossing, lignin polymerization, fruit ripening and defense against pathogens. During fruit ripening and particularly during climacteric rise, the activity of peroxidase increases along with the polygalacturonase and cellulase enzymes (Robinson, 1991).
The peroxidases with broad specificity are often found in cell wall where they utilize \( \text{H}_2\text{O}_2 \) to generate phenoxy compounds that then polymerize to produce components such as lignin (Greppin et al., 1986). In addition to their role in the biosynthesis of cellular components, reactive oxygen species are thought to act as secondary messengers in cells. Plant peroxidase activity seems to be under the strict control depending on the development stage and the environmental stimulus (Gadea et al., 1999). Ali et al. (2005) reported that peroxidase activity increases when higher temperatures were applied. On the other hand, the activation of peroxidase is correlated to the defense responses of fruit in presence of pathogens (Maksimov et al., 2003).

The oxidation of phenolic compounds, in presence of oxygen by enzyme polyphenoloxidase results in the browning of the tissue (Haslam, 1998). Phenolic compounds are oxidized to Quinones. These quinines may polymerize into coloured, usually brown, products (Amiot et al., 1997). Rate of enzymic browning depends on whether the responsible enzyme is present, active and physically available. Enzymes other than polyphenoloxidase such as laccase, peroxidase and \( \beta \)-galacturonase are also reported to be involved in browning in a minor way (Mayer and Harel, 1979). It has been suggested that high levels of oxidative changes, together with high levels of phenolic substrate early in fruit development may act as a defense mechanism by protecting seeds from infection or predation prior to maturity (Mayer and Harel, 1979; Knee et al., 1994). Maturity and ripeness are reported to be important predictors of browning in fruit that are dried, while the unripe fruit is more likely to show browning when dried (McBean, 1985). In plants a large number of O-diphenolic compounds are present, which are more or less oxidisable with oxidative enzymes which includes 3, 4-dihydroxyphenylalanine, the chlorogenic acids, adrenaline, phenylalanine, caffeic and gallic acids and flavonoids (Mayer and Harel, 1979).

### 2:3.7 Ethylene and Respiration changes:

Fruit can be classified into two main categories, ‘climacteric’ and ‘non-climacteric’, according to changes in respiratory behavior and ethylene responses during ripening (Kidd and West, 1925). In non-climacteric fruits (e.g. cherry, blueberry, grape, etc.) changes associated with ripening (color, flavor, texture, aroma, etc.) occurs relatively slowly and respiration rate declines throughout fruit development (including ripening). In contrast, respiration of climacteric fruits (e.g. raspberry, apple,
par, avocado, banana etc.) declines until it reaches physiological maturity; increases substantially during ripening and then decreases as maturation precedes leading eventually to senescence. Physiological maturity is thus a stage of development just prior to ripening, where fruits will continue to demonstrate changes even if harvested. It is important to recognize physiological maturity for climacteric fruits as harvesting during this stage results in maximum storage life and the ability to ripen normally even when presented with appropriate environmental conditions. Also increased respiration provides the energy (ATP) required to fuel ripening metabolism (Salunkhe and Desai, 1984).

Respiration is an essentially irreversible active metabolic process of fruits and vegetables. This is associated with physiological, histo-chemical and biochemical changes (Sacher, 1973; Limberman, 1979). Respiration is considered as a programmed continuation of both catabolic and anabolic processes. The various physico-biochemical changes involved in respiration have also been well reviewed (e.g. Biale and Young, 1971; 1981; Rhodes, 1980). The respiratory climacteric is accompanied by an increase in autocatalytic, endogenous ethylene production (Sekse, 1988). Application of exogenous ethylene or propylene to fruit of the climacteric class at a pre-climacteric stage advances the onset of endogenous ethylene production and ripening (McMurchie et al., 1972). In non-climacteric fruit, the respiration rate gradually decreases during maturation and senescence and ethylene production remains low (Biale and Young, 1981).

The function of increased respiration during the ripening of climacteric fruit has been the subject of much debate. A popular view is that an increase in respiration is necessary to provide adenosine triphosphate (ATP) and substrates for various anabolic processes associated with ripening (Blanke, 1991). During the process of respiration in fruits, it involves the oxidation of glucose to carbon dioxide and water which produces energy that is stored as ATP. This is a very efficient system with about 90 % of the energy generated being conserved within the fruit and the remainder being lost as heat (Blanke, 1991).
Ripening of climacteric fruits is controlled by ethylene. Fruit ethylene production is very low prior to the climacteric. Soon after the attainment of physiological maturity, a burst of ethylene production by the fruit triggers the respiratory climacteric and induces metabolic pathways responsible for the changes associated with ripening (Abeles et al., 1992). As a gas ethylene not only triggers ripening of climacteric fruits but also induces uniform ripening throughout the sphere of the fruit.

A causative role for ethylene in the ripening process was proved in the 1960’s with the advent of gas chromatography and during the last 40 years the biochemical pathways of ethylene synthesis have been determined. The enzymes involved with ethylene biosynthesis and their corresponding genetic sequences have been isolated and identified and progress has been made in understanding ethylene perception and signal transduction (Kieber, 1997).

Ethylene plays a critical role in ripening of climacteric fruit. It coordinates the expression of genes that are responsible for a variety of processes that include autocatalytic ethylene production and increased respiration rate, chlorophyll degradation, anthocyanin and carotenoid synthesis, conversion of starch to sugars, aroma production and increased activity of cell wall degrading enzymes (Abeles et al., 1992; Gray et al., 1992). In contrast, it is generally considered that the ripening of non-climacteric fruits is ethylene independent (Lelievre et al., 1997). However, in fruits new enzymes (proteins) are synthesized during ripening, indicating that changes in gene expression are involved. Little is known of the regulatory mechanisms underlying these biochemical changes (Lelievre et al., 1997).

The threshold concentration of exogenous ethylene required to trigger ripening has been extensively studied (Biale, 1960; Burg and Burg, 1967; Wang et al., 1972). It is not clear whether this threshold concentration causes ripening or it stimulates endogenous ethylene production to physiologically active levels which in turn causes the fruit to ripen (Sfakiotakis and Dilley, 1973). Sensitivity of ethylene is not consistent throughout the life of the fruit and the threshold level needed to cause the ripening of climacteric fruit is dependent on the age of the tissue.
Dilley (1980) has indicated that analysis of internal ethylene concentrations at harvest can be used to determine the optimum harvest date and can also be used to predict the potential storage life of fruits. However, there is large variability from fruit to fruit that is influenced by latitude, cultivar, harvest time and temperature (Blanpied et al., 1985; Saltveit, 1982) that has limited the commercial application of this technique.

Research is now focusing on the ways that plants perceive and transducer hormonal signals and the consequences of signal recognition for gene expression. An understanding of ethylene perception and signal transduction is being gained from genetic mutants of *Arabidopsis thaliana* and tomato where the genes for putative ethylene receptors have been cloned (Chang et al., 1993; Wilkinson et al., 1995).

Several types of ethylene inhibitors may also be applied exogenously to plants (Burg and Burg, 1967; Yang, 1985). Aminooxyacetic acid (AOA) and aminoethoxyvinylglycine (AVG) prevent ethylene synthesis (Yang and Hoffman, 1984). Recently the cyclopropenes, 1-MCP and 3, 3-dimethylcyclopropene (3, 3-DMCP), have been found to be effective antagonists of the ethylene response (Sisler et al., 1996).

**2:3.8 Minerals:**

The fruits and vegetables provide a milieu of phytochemicals, non-nutritive substances that possess health protective benefits. In contrast, fruits and vegetables may not usually be recognized as primary sources of mineral intakes from a nutritional point of view (Fairweather-Tait and Hurrell, 1996). Minerals are normally classified as macro or micronutrients, based on the relative concentration of each nutrient. The macro elements include N, P, K, Ca and Mg, while micro elements include elements such as Na, Fe, I, Zn, Cu and Mn.

Macro and trace elements have very varied functions: e.g. as electrolytes, as enzyme constituents and as building materials. The mineral content in fruits and vegetables can vary greatly depending upon genetic and climactic factors, agricultural procedures and composition of the soil and ripeness of the harvested crop. This applies to both macro and trace elements. Mineral supply depends not only on the intake in food but primarily on the bioavailability, which is essentially related to the composition of the food. Also, the redox potential and pH value determines the valency stage, solubility and
consequently absorption of minerals in plants. A series of constituents e.g. sugars, lignin, phytin, and organic acids, bind to minerals enhance and/or inhibit their absorption. The importance of minerals as food ingredients depend not only on their nutritional and physiological roles (Belitz et al., 2004). They also contribute to food flavour and activate or inhibit enzyme-catalyzed and other reactions and they affect the texture of food.

Nitrogen has a most recognized role as it is present in the structure of protein molecules. It is also a constituent of important biomolecules like purines and pyrimidines, which are found in DNA and RNA. Nitrogen is a structural component of coenzymes such as NAD$^+$, NADP$^+$, FAD$^-$ etc. Other compounds such as vitamins also contain nitrogen. Nitrogen is an essential component of nucleic acids, cofactors and other metabolites. Several plant hormones (indole-3-acetic acid, zeatin, spermidine, etc.) contain nitrogen or they are derived from nitrogenous precursors. Alkaloids and other secondary compounds contain nitrogen and various phenolics derive from phenylalanine and are therefore linked with amino acid metabolism. Moreover, nitrogen is a major constituent of chlorophyll.

Phosphorus is an essential constituent of nucleic acids. Both DNA and RNA have a sugar phosphate backbone in their structure. Triphosphate forms of nucleotides are precursors of nucleic acids. Important compounds like phospholipids or phosphoglycerides, which along with proteins are major component of cell membranes, also contains phosphorus. Moreover, phosphorus is also present in coenzymes like NAD$^+$ and NADP$^+$, which take part in most of the cellular oxidation reduction reaction involving hydrogen transfer. Important metabolic processes such as photosynthesis, respiration, nitrogen metabolism, carbohydrate metabolism, fatty acid metabolism etc. are dependent on the action of these coenzymes. Phosphorus is a constituent of ATP and other high energy compounds. The intermediates of glycolysis between glucose and pyruvate are all phosphorylated compounds. Phosphate occurs in phospholipids including those of membranes, in sugar phosphate, various nucleotides and coenzymes. Phosphate is also known to regulate many enzymatic processes. It is important both as a substrate and as a regulatory factor in oxidative metabolism and photosynthesis; it participates in signal transduction; and regulates the activities of an assortment of proteins by way of covalent phosphorylation/dephosphorylation reactions.
The primary function of potassium is its role in the maintenance of osmotic pressure and cell size, thereby influencing photosynthesis and energy production as well as stomatal opening, carbon dioxide supply, turgor and translocation of nutrients. As such, the element is required in relatively large proportions by the growing organs. The consequences of low potassium levels are apparent in a variety of symptoms causes: restricted growth, reduced flowering, lower yields and lower quality produce. High water soluble levels of potassium inhibit the uptake of other minerals and reduce the quality of the crop. Potassium is an important constituent of many proteins like ascorbic acid oxidase, cytochrome oxidase, diamine oxidase, and polyphenol oxidase (Anderson, 2000). Potassium is said to be linked to carbohydrate metabolism. Potassium is essential for translocation of sugar. It is also required for maximal activity of enzymes like pyruvate kinase, succinyl CoA synthetase, nitrate reductase, starch synthase. Also potassium has a role in ATPase activity, by involving in ion transport across the biological membrane.

Calcium has been associated in cell wall structure. It is required to bind pectate polysaccharides that form a new middle lamella in the cell plate that arises between daughter cells. Calcium influences cell wall rigidity through its effect on ionic bridging between cellular molecules. Calcium is involved in spastically three dimensional arrangement of the membranes. Calcium ions serve as a protective function and protects from the effect of hydrogen ions, high salt concentrations and toxic effects of other ions. Calcium ions are required for amylase activity as well as an activator of enzymes such as arginine kinase, adenylkinase etc. Calcium is necessary for normal mitosis, as it helps in formation of microtubule; it is also involved in chromatin organization. It is also believed to have a major influence on the rheological properties of the cell wall and consequently, on the texture and storage life of fruits and vegetables. Ca$^{2+}$ interacts with the anionic pectic polysaccharides, coordinating with the oxygen functions of two adjacent pectin chains to form the so-called “egg box structure” and cross-linking the chains (Rose et al., 2003). Intracellular Ca$^{2+}$ also occupies a pivotal role in cell signal transduction (Sanders et al., 1999). The plant signals thought to be transduced through cytosolic Ca$^{2+}$ include wounding, temperature stress, fungal elicitors, oxidative stress, anaerobiosis, abscisic acid, osmotic stress, red or blue light and mineral nutrition. Intracellular Ca$^{2+}$ transient increases are often associated with initiation of responses. Thus, Ca$^{2+}$ is a prominent second messenger, and it must be maintained in the cytoplasm at concentrations many orders of magnitude lower than the Ca$^{2+}$ in the cell wall.
Manganese plays an important role during the division of water to hydrogen and oxygen. Manganese also helps the oxidase enzyme in carrying oxygen and entering into the oxidation and reduction reactions needed in carbohydrate metabolism and in seed formation; as manganese has a strong connection with oxygen (Walters and Fenzau, 1996). Manganese is involved in oxidation reduction process together with decarboxylation and hydrolytic reactions. It has an important role in photosynthesis. It is also a predominant metal ion in the reactions of Kreb’s cycle. Manganese acts as an activator of enzymes such as nitrite reductase and hydroxylamine reductase. Also like magnesium, manganese is required in enzyme reactions involving carbon assimilation.

Zinc is associated most commonly with the biosynthesis of indole 3-acetic acid and is suggested that it prevents the oxidation of IAA. Zinc induces the de novo synthesis of cytochrome and participates in chlorophyll formation and prevents chlorophyll destruction. Zinc is a pervasive microelement that plays a catalytic or a structural role in more than 200 enzymes (Romheld and Marschner, 1991), but only a few of them (i.e. alcohol dehydrogenase, superoxide dismutase, carbonic anhydrase, RNA polymerase) contain the micronutrient. Zinc can affect carbohydrate metabolism because different Zn-dependent enzymes participate in biochemical reactions involving sugars. Zinc also plays a role in the maintenance of cell membrane integrity, in the protection from O_2^- damage, and the synthesis of RNA and tryptophan, a precursor of indole-3-acetic acid.

Copper containing protein called plastocyanin, are electron carriers during photosynthesis. In plants, copper is required for chlorophyll synthesis and in several copper containing enzymes in which oxygen is used for oxidation of substrates, enzymes such as tryosinase, laccase and ascorbic acid oxidase undergoes the cyclic oxidation and reduction reactions. Copper has an indirect role in chlorophyll production and also acts as a redox active metal that plays an important role in the oxidative defense system.

Sodium partially replaces potassium in many of the reactions known to require potassium. It is essential for C_4 carbon fixation in certain species. Sodium is known to influence the balance between PEP carboxylase and RUBISCO. Sodium plays a role in maintaining water balance. Sodium is a systemic ion. It is important in electrolyte balance and essential in coregulating ATP with potassium.
2.4 Antibacterial studies:

Nature has been a source of medicinal agents since times immemorial. The importance of herbs in the management of human ailments cannot be overemphasized. It is clear that the plant kingdom harbours an inexhaustible source of active ingredients invaluable in the management of many intractable diseases. Furthermore, the active components of herbal remedies have the advantage of being combined with many other substances that appear to be inactive. Antibiotic resistance has become a global concern (Westh et al., 2004). Increasing incidence of multiple resistances in bacteria in recent years, largely due to indiscriminate use of commercial antibacterial drugs commonly employed in the treatment of infectious diseases. This has forced scientists to search for new antibacterial substances from various sources. The screening of plant extracts and plant products for antibacterial activity has shown that higher plants represent a potential source of novel antibiotic prototypes (Afolayan, 2003).

Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, over the past few decades these health benefits are under threat as many commonly used antibiotics have become less and less effective against certain illnesses, not only because many of them produce toxic reactions, but also due to emergence of drug resistant bacteria. It is essential to investigate newer drugs with lesser resistance (Sarkar et al., 2003). Numerous studies have identified compounds within herbal plants that are effective antibiotics (Basile et al., 2000). Traditional healing systems around the world that utilize herbal remedies are an important source for the discovery of new antibiotics (Okpekong et al., 2004); some traditional remedies have already produced compounds that are effective against antibiotic-resistant strains of bacteria (Kone et al., 2004). It also facilitates pharmacological studies leading to synthesis of a more potent drug with reduced toxicity (Ebana et al., 1991; Manna and Abalaka, 2000). The need of the hour is to screen a number of medicinal plants for promising biological activity.
Plant-derived substances have recently become of great interest owing to their versatile applications (Baris et al., 2006). Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs (Hammer et al., 1999). Many plants have been used because of their antibacterial traits and the antibacterial properties of plants have been investigated by a number of researchers worldwide. Ethnopharmacologists, botanists, microbiologists, and natural-product chemists are still searching the earth for phytochemicals, which could be developed for the treatment of infectious diseases (Tanaka et al., 2006) especially in light of the emergence of drug-resistant microorganisms and the need to produce more effective antibacterial agents.

Traditionally used medicinal plants have recently attracted the attention of the pharmaceutical and scientific communities. This has involved the isolation and identification of metabolites produced by plants and their use as active principles in medicinal preparations (Taylor et al., 2001). Many of the plant metabolites are constitutive, existing in healthy plants in their biologically active forms, but others occur as inactive precursors and are activated in response to tissue damage or pathogen attack (Osbourne, 1996). The array of metabolites produced by plants is daunting, with wide ranging chemical, physical and biological activities. These constitute a source of bioactive substances and presently scientific interest has increased due to the search for new drugs of plant origin.

2:5 Underutilized fruits:

Plant biodiversity represents the primary source for food, feed, shelter, medicines and many other products that make life on earth possible and enjoyable (WCMC, 1992; UNEP, 1995). The number of plants used by humans around the world is only one-third of the total number of plants, which have been used to meet the specific needs. Increased reliance on major food crops has been accompanied by a shrinking of the food basket which humankind has been relying upon for generations (Prescott-Allen and Prescott-Allen, 1990). However, the shrinking of agricultural biodiversity has reduced both the intra and inter-specific diversity of crops, increasing the level of vulnerability among users, particularly the poorer sections, for whom diversity in crops is a necessity for survival rather than a choice.
The dependence of humankind on plant resources is inevitable. Since the dawn of agriculture, domestication and gathering of desired plant species have helped in the evolution of useful plant species and these resources have been exploited to our advantage. So far, out of the estimated 75,000 species of edible plants (Gautam and Singh, 1998), only about 150 have been widely used. Of these about 30 species provide 90% of the world’s food. Therefore, there has been focused attention by the researchers on exploiting alternative plant species or underutilized species for multifarious uses. Many of these occur in extreme environments and marginal or waste lands, and are being lost through rapid loss of natural habitats, especially in the tropics. The plants on which humans depend in adverse conditions/regions can be called the life supporting species (Paroda, 1988).

The 20th century has witnessed the undertaking of systematic collecting to rescue the genetic resources of staple crops (Pistorius, 1997), while the 21st century has started with the awareness to rescue and improve the use of those crops left aside by research, technology, marketing systems as well as conservation efforts. These underutilized crops are also referred by other terms such as minor, orphan, neglected, underexploited, underdeveloped, lost, new, novel, promising, alternative, local, traditional, niche crops etc. Improving the availability of information on underutilized crops is one of the most important areas demanding our immediate attention. At the formal level, individual studies on underutilized crops continue to need support to ensure their publication. Further studies that bring together the sparse and often inaccessible literature are also needed. At local level, there is a need to gather and document information that is maintained within farming communities. The recognition of the value of this by researchers and scientists can often act as a powerful stimulus to improve a community’s own valuation of the knowledge.

Underutilized plants constitute the lesser known species in terms of trade and research, often well adapted to marginal and stress conditions. Generally they possess promising nutritional and industrial importance for a variety of purposes for humankind. Their cultivation is restricted to specialized geographical pockets in different agro-ecological regions, mainly by poor farming communities which derive their sustenance and livelihood from such plants. But their commercial importance and market value is still unknown to the public. Therefore, research on underutilized crops holds
promise to attain sustainability, profitability and diversification in agriculture and to restore the balance of trade, reduce dependence on imports and to make more competitive in agricultural exports. This will make the global economy more sound and in many cases benefit the environment as well, since our depleting resources can be replaced with renewable ones.

The establishment of germplasm collections for underutilized species has been advocated in the Global Plan of Action of FAO (FAO, 1996) and is one key element of the promotion process pursued by several other international organizations, including IPGRI and ICUC. The goals of these organizations is to work on the underutilized species, including the improvement of food security; enhancement of nutritional balances, sustainability in agriculture and alleviation of poverty through income generation.

This global ‘opening’ towards underutilized species is the result of a gradual change of attitude towards biodiversity and plant genetic resources by many countries. The activity of ‘Promoting development and commercialization of underutilized crops and species’ has started since 1992 at the Convention on Biological Diversity and the FAO IVth International Technical Conference on Plant Genetic Resources for Food and Agriculture held in Germany during 1996 (UNEP, 1992; FAO, 1996). A Conference organized by IPGRI in 1998 in Aleppo, Syria, on ‘Priority Setting for Underutilized and Neglected Plant Species of the Mediterranean region’ was followed by the Global Forum on Agricultural Research (GFAR) in 1999, which also emphasized the role of underutilized species in raising income of the rural poor.

Concerted research efforts have been made in the recent past on domesticated and cultivated underutilized species, particularly for crop diversification for food and commerce (Paroda, 1988; Paroda and Bhagmal, 1992). Most challenges in the promotion of underutilized species are now well understood and appreciated and have been addressed in numerous works (Sankary, 1977; VonMaydell, 1989; Monti, 1997; Padulosi, 1999).
India, located between 8° and 38° N and 68° and 93.5° E, shows altitudinal variations ranging from below sea level to more than 3500 m above mean sea level and exhibits extreme diversity for edapho-climatic conditions, agro-climatic regions and ecosystems. The Indian sub-continent is a reservoir of several plant species, which have known uses of future potential for the benefit of human beings. India occupies a unique position among the major gene-rich countries of the world with a bounty of 49,000 species of higher plants known to occur. About 30% of these species are endemic: of which 17,500 species are known to occur in 16 major vegetation types of the country (Gautam and Singh, 1998).

Several systematic efforts have been made to compile information on lesser known food plants including wild resources used by farmers and tribal communities in different regions of the country. Ethnobotanical investigations have been made to record a large number of wild plant species used by native tribal and aboriginal people, to meet their varied requirements (MEF, 1994). Immensely rich landrace diversity occurs in major agri-horticultural crops, which is attributed to the farmer’s conscious or unconscious selection, inherited and perpetuated over generations.

There are several underutilized and introduced plant species that have naturalized well in the country and hold great potential to be exploited for various purposes. For strengthening of research on underutilized plants, an All India Coordinated Research Project on Underutilized Plants (AICRPUUP) was initiated in 1984 with its headquarters at the National Bureau of Plant Genetic Resources (NBPGR), New Delhi. At present the project operates at 21 centres in different agro-climatic zones of India. Concerted research efforts have been made in this project in respect of germplasm build-up, maintenance and evaluation of some prioritized underutilized crop plants.

The German Federal Ministry for Economic Cooperation and Development (BMZ) financed from 1993 to 1997 a project implemented by GTZ at the International Plant Genetic Resources Institute (IPGRI) with the title: “Genetic resources of neglected crops with good development potential: Their conservation, use and breeding status”. This project collaborated with numerous national and international research centers and contributed considerably to the IPGRI “Strategy for neglected and underutilized species and the human dimension of agrobiodiversity” was developed in 1998. A specific
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inventory on underutilized edible fruits and nuts published in 1998. Around 23 monographs on priority crops have been produced so far (Cowling et al., 1998). Bibliography of traditional African vegetables is provided by Mnzava et al., (1999). Neglected and underutilized species (NUS) for the Mediterranean region was held in Aleppo, Syria in 1998 (Padulosi, 1999). Realizing the benefits in neglected and underutilized plant species through technology transfer and human resources development is noted by Padulosi et al. (1999) during conference on technology transfer and capacity building in Norway. IPGRI has also published Bibliography on native fruits of Latin America in 2000. GRPC organized a conference on the theme “Enlarging the basis of food security: role of underutilized species was held in February 17th to 19th 1999 in Madras, India. Joshi et al. (2002) had described the conservation and use of underutilized crops in Indian perspective, while Padulosi et al. (2002) has mentioned about the trends, challenging opportunities of underutilized crops in the 21st Century. IPGRI has also published a book on edible plants of Kenya describing 800 indigenous plants used for food (Maundu et al., 1999) and ethnobotanic survey on more than 200 indigenous plants (Noun, 2003). “Underutilized fruits of Thailand” is a FAO published book written by Subhadrabandhu (2001).

The potential of underutilized fruit trees in Central Sudan has been studied by Gebauer et al. (2002). Underutilized edible plants of the Sikkim Himalaya - need for dosemication is published by Sundriyal and Sundriyal (2003). Characterization of underutilized fruits by molecular markers - A case study of loquat has been reported by Badeness et al. (2004). Production technology of underutilized fruits is a book published by Indian authors which includes description of fruits like Ber, Aonla, Custard apple, Ramphal, Jamun, Bael, Tamarind, Lasoda, Karonda, Timroo, Wood apple, Phalsa, Khirni, Kair, Mahua, Khejri, Drumstic, Pilu, Rambutan and Carambola (Dashora et al., 2004). A special review – fruits for the future, describing the need and use of underutilized fruits is reported by Banin (2006) in Journal of Biogeography. Antioxidant capacity and vitamin C content of some underutilized fruits is reported by Gunawardena and Silva (2006). Chemical composition of Black plum (Vitex doniana): an underutilized fruit is investigated by Agbede and Ibitoye (2007). Moreover a special issue is published by the Journal “Fruits”, an EDP science publication in 2008. Processing and marketing feasibility of underutilized fruit species of Rajasthan is described by Singh et al. (2008), while carotenoid content of underutilized tropical fruits is reported by Hock et al. (2008).
2:5.1 Karanda/ Christ thorn (*Carissa carandus* L.)

The plant of *Carissa carandus* is a native and common throughout much of India, Sri Lanka, Java, Malaysia, Myanmar and Pakistan. It is commonly known as Christ thorn, Carandas-Plum or Karanda in English, Karaunda in Hindi and Karamda in Gujarati. In traditional system of medicine, the plant is used as an anthelmintic, astringent, appetizer, antipyretic, in biliary, stomach disorders, rheumatism and disease of the brain (Kirtikar and Basu, 2003). In Western Ghats region of India, the decoctions and extracts of the roots of this plant are effective remedies in the management and/or control of convulsions and epilepsy.

Changes in some physical parameters of developing karanda fruit is studied by Uthaiah (1988), while some qualitative characteristics of karanda fruits grown in West Bengal have been reported by Mandal and Mazumdar (1997). Besides, the difference among fruit and seed size parameters of karanda is investigated by Singh and Singh (1998), while Misra and Jaiswal (1999) studied some quality characters of ripe fruits of some selected lines of karanda. A new isomer – ursolic acid have been reported by Naim et al. (1988) from the fruits and leaves of karanda, while the composition of amino acids in the fruit of karanda is reported by Hasnain and Ali (1990).

The plant of *C. carandus* has been screened by Sekar and Francis (1998) for energy, hydrocarbons and phytochemicals. Earlier studies have shown that the extract of the plant possesses cardiotonic, anticonvulsant activity (Hegde et al., 2009), anthelmintic properties (Pakrashi et al., 1968; Taylor et al., 1996), antiviral activity (Dhawan and Patnaik, 1985; Taylor et al., 1996; Rajasekaran et al., 1999) and nematicidal activity (Saleem et al., 1997). Various glycosides, triterpenoids, steroids, lignans and β-sitosterol were reported from the root extract of the plant (Pakrashi et al., 1968; Pal et al., 1975; Singh and Rastogi, 1975; Dhawan and Patnaik, 1985; Rastogi et al., 1996; Taylor et al., 1996; Wangteeraprasert and Likhitwitayawuid, 2009). Besides the plant of *C. carandus* has been reported by El-Mekkawy et al. (2009) to have a potent inhibitory effect on HIV-1.

Herregods et al. (2000) identified the fruits of karanda to have considerable commercial potential and nutritive value. Also certain medicinal and nutritional properties of karanda and its medicinal and culinary uses are highlighted by Singh (2001). Also the plant is said to be a potential alternative crops for renewable energy, oil, hydrocarbon and phytochemicals of Western Ghats (Augustus et al., 2003).
2:5.2 Lasora/ Indian cherry ((Cordia dichotoma Frost. f.))

The plant of *Cordia dichotoma* bears fruits during the months of March to June are drupe ovoid or rounded in shape measuring 1.5–2.2 cm across are bright-yellow with pinkish tinge and possesses mucilage (Shah, 1978). The plants are grown in India, Sri Lanka and other warmer regions. They are popularly known as Indian cherry, Clammy cherry or Fragrant manjack in English, Vadgundo or Gunda in Gujarati and Lahsora or Bokar in Hindi (Rathore, 2009). Ripe fruits are eaten raw, while green fruit are consumed as vegetable or used in curry and pickled. The fruits are reported to be demulcent, expectorant, tonic and refrigerant (Kirtikar and Basu, 2003). It is used to reduce the irritation of the urinary passages, alleviation of thirst and dry cough (Husain *et al.*, 2008).

The seed kernel of *C. dichotoma* has medicinal properties. Besides, the seeds of the species are anti-inflammatory. Besides, isolation of two important compounds \( \alpha \)-amyrin and 5-dirhamnoside has been reported. The bark is medicinal and several chemicals have been identified; Allantoin, \( \beta \)-sitosterol and 3', 5-dihydroxy-4'-methoxy flavanone-7-O-\( \alpha \)-L-rhamnopyranoside (Orwa *et al.*, 2009).

The floral anatomy of *C. dichotoma* flowers is studied by Joshi, (1978), while polarographic analysis of fixed oil obtained from seeds of *C. dichotoma* have been performed by Trivedi *et al.* (1990). Besides, Theagarajan and Prahbu (1977) examined the chemicals in the seed kernel of Lasora. Furthermore, the nutritional value of lasora fruit has been studied by Duhan *et al.* (1992), while Kuppasta and Nayak (2003) have realized the medicinal value of the plant and later reported the antihelmentic activity of *C. dichotoma* fruits. Recently, Alka (2003) has also reported the uses, sustainable harvesting methods and value addition of the Lesora crop for economic upliftment and biodiversity conservation.

2:5.3 Khirni/ Rayan [Manilkara hexandra (Roxb.) Dubard]

Fruits of *Manilkara hexandra* are berries measuring 1–1.7 cm long, ovoid or ellipsoid with smooth skin and plenty of latex are found to be in plenty during the months of November to April (Shah, 1978). They are commonly found in India, Pakistan, Bangladesh, Sri Lanka etc. It is commonly known as Khirni in English and Hindi, while is popularly known as Rayan in Gujarati.
A study on the blossom biology and fruiting of *M. hexandra* is reported by Divedi and Bajpai (1974), while seed germination studies were reported by Sathappan *et al.* (1998). As the plant possesses various medicinal values the indigenous knowledge about *M. hexandra* has been proved by Jadeja and Baxi (2004) in the Saurashtra region of Gujarat. Furthermore, Pant and Rastogi (1977) isolated a new compound called Hexandrin, a new triterpene from *M. hexandra*.Besides, the plant parts have also been used against experimentally-induced gastric ulcers by Shah *et al.* (2004).

### 2:5.4 Spanish cherry/ Bakul/ Rayan (*Mimusops elengi* L.)

The fruits of *Mimusops elengi* usually set in between October to March, usually yellow to orange in colour, ellipsoid in shape possessing smooth surface, it measures 2-4 cm in length and encloses 2 seeds (Shah, 1978). They are commonly called as Spanish cherry in English, Bakul or Bakula in Hindi and Borsali in Gujarati. The plant is commonly observed in countries like India, Sri Lanka, Andaman Islands, Myanmar, and China.

Medicinal properties of *Mimusops elengi*, a common tree species in India is not very well known. Besides, some of the workers have reported that the plant of *M. elengi* possess some active compounds. The chemical constituents reported from the seeds of *Mimusops elengi* are taraxerol, taraxerone, ursolic acid, betulinic acid, $\alpha$-spinosterol, $\beta$-sitosterol glycoside (Nusarat *et al.*, 1995), quercitol (Misra and Mitra, 1967), Lupeol (Misra and Mitra, 1968), pentacyclic triterpenes such as mimusopgenone and mimugenone (Sen *et al.*, 1995), triterpenoid saponins such as mimusopsides A and B, mimusopin, mimusopsin, mimusin, Mi-saponin A and 16$\alpha$-hydroxy Mi-saponin A (Sahu *et al.*, 1995, 1997, 2001; Sahu, 1996; Lavaud *et al.*, 1996; ).

Geneshan and Unnikrishnan (1999) reported the seed dry weight, leaf area and photosynthetic rate of spanish cherry. Distribution pattern of fatty acids in the seed oil of *M. elengi* is reported by Ahmed *et al.* (1975). The flowers of *M. elengi* have been screened by Gupta *et al.* (1976) to assess the chemical constituents present. Mitra and Yadav (1980) carried out pharmacognostical studies using the leaves of *M. elengi*, while Shah *et al.* (2003) has reported the bark of *M. elengi* to be effective against experimental induced gastric ulcers. Besides Kalita and Saikia (2004) studied the chemical constituents and energy content of spanish cherry. The plant of *M. elengi* has also been
worked out for its antibiotic activity (Hazra et al., 2007), antibacterial activity (Rana and Choudhary, 2005; Rangama et al., 2007), antifilarial activity (Ahmed et al., 2004) and antioxidant capacity (Boonyuen et al., 2009).

2:5.5 Sunberry/Gooseberry (*Physalis minima* L.)

The plant of *Physalis minima* is commonly observed in India, Bangladesh, China, Pakistan etc. They are commonly called as Gooseberry or Sunberry in English, Rusbhari in Hindi and Popta in Gujarati. The fruits are berries of 0.5–1.0 cm in diameter, usually covered by a papery calyx and observed to turn orange or light brown in colour when they ripens. The seeds are discoid, glabrous and muriculate (Shah, 1978). The fruits are juicy, mildly astringent and sweet with a pleasant blend of acid. They are juicy and as is evident from their chemical composition that they are a good source of vitamin C. The raw fruit can also be used as a vegetable. This plant is highly resistant to insect pests and diseases (Parmar and Kaushal, 1982).

Kirtikar and Basu (1935) have reported that the plants of *Physalis minima* bears the fruits, which are bitter, appetizing, tonic, diuretic, laxative, useful in inflammations, enlargement of the spleen and abdominal troubles. The fruit is also considered to be a tonic, diuretic and purgative in the Punjab. The mundas (a tribe) of Chhota Nagpur mix the juice of the leaves with water and mustard oil and use it as a remedy against earache (Parmar and Kaushal, 1982).

Some studies on seedling and nodal anatomy of *P. minima* has been reported by Raghava et al. (1994), while the general characteristics and compositions of *P. minima* seed and oil are investigated by Rao et al. (1984). Various important chemicals like cholinesterase have been reported by Gupta and Gupta (1997), cytotoxic withaphysalins is reported by Lei et al. (2007), while physalins have been isolated by Choudhary (2005). Furthermore, Azlan et al. (2005) studied the accumulation of physalin in the cells and tissues of *P. minima*. The anti-fertility effects and anti-inflammatory activity of *P. minima* is previously reported by Sudhakaran et al. (1999) and Sethuraman and Sulochana (1988) respectively.
2:5.6 Jamun/ Blackplum (*Syzygium cumini* L. Skeels)

The fruit formation takes place in the plant of *Syzygium cumini* between the months of February to May. The plant is common in India, Bangladesh, USA, China, Pakistan etc. They are commonly called them Blackplum or Javaplum in English and Jamun in Hindi and Gujarati. The fruits are berries 1-2 cm in diameter, ovate or oblong in shape, appears bright to dark-purple in colour when ripe (Shah, 1978).

The plant of Blackplum has been attributed in the Indian folklore medicine system to possess several medicinal properties (Warrier *et al.*, 1996). The bark of the plant is astringent, sweet, refrigerant, carminative, diuretic, digestive, antihelminthic, febrifue, constipating, stomachic and antibacterial (Kirtikar and Basu, 2003). The fruits and seeds are used to treat diabetes, pharyngitis, spleenopathy, urethrorrhea and ringworm infection. The leaves have been extensively used to treat diabetes, constipation (Bhandary *et al.*, 1995), leucorrhoea, stomachalgia, fever, gastropathy, strangury and dermopathy (Warrier *et al.*, 1996), and to inhibit blood discharges in the faeces (Bhandary *et al.*, 1995). The plant possesses acetyl oleanolic acid, triterpenoids, ellagic acid, isoquercitin, quercetin, kaempferol and myricetin in different concentrations (Rastogi and Mehrotar, 1990). Most of these compounds have been reported to possess antioxidant and free radical scavenging activities (Tanaka *et al.*, 1998; Zhang and Lin, 2001; Banerjee *et al.*, 2005; Benherlal and Arumugham, 2007; Ruan *et al.*, 2008; Rekha *et al.*, 2008), antibacterial (Tsakala *et al.*, 1996; Chattopadhyay *et al.*, 1998; Corine *et al.*, 2000; Shafi *et al.*, 2002), antifungal (Chandrasekaran and Venkatesalu, 2004; Ratnam and Raju, 2008), antidiabetic (Grover *et al.*, 2000) and anti inflammatory (Muruganandan *et al.*, 2001) activities. Looking into the importance of *M. elengi* the phytochemicals investigation has been carried by Kumar *et al.* (2009).

Fruit development in jamun has been studied by Geetha *et al.* (1992). Physiological and compositional changes during maturation and ripening of jambolan fruit is observed by Rao and Charyulu (1989), Mohammed and Wickham (1996), Venkitakrishnan *et al.* (1997), Garande *et al.* (1998) and Devi *et al.* (2002). The volatile flavour components of jamun fruits has been reported by Vijayanand *et al.* (2001), while the presence of pyrrolizidine alkaloids is reported by Singh *et al.* (2003). In addition Gomes *et al.* (1986) studied the polysaccharide components in the pulp and the seeds of *Syzygium cumini*. Furthermore, the post harvest stability of the Jamun fruits was tested by Raiz and Tabassum (1994) on the storage stability in two varieties of Jamun fruit.