1. INTRODUCTION

1.1. Definition of fruit

Fruits and vegetables are highly valued and important components of a healthy human diet as they are rich sources of vitamins, minerals, sugars and other biologically active compounds. According to Akin et al. (2008), sugars, organic acids, phenolics and carotenoids are naturally predominant in fruits and vegetables and these components play a major role in maintaining fruit quality and determining their nutritive value. The condition of fruit at the time of harvest has an important effect on the consumer’s level of satisfaction during consumption. All fruits reach their best eating quality when allowed to ripen on the tree or plant. The maturity indices are based on a compromise between those indices that would ensure the best eating quality to the consumer and those provide the needed flexibility in marketing. World wide availability of fruits continues to increase in terms of the number of species and cultivars as well as their expanded season of availability with the production in northern and southern hemisphere countries.

Fruits and vegetables have strongly been associated with reduced risk of cancer, heart disease, stroke and other chronic diseases (Prior and Cao, 2000; Knee, 2002). Some components of fruits and vegetables are strong antioxidants and function to modify the metabolic activation and detoxification of carcinogens. According to Prior and Cao (2000) and Knee (2002), even though the antioxidant capacity varies greatly among fruits and vegetables, it is better to consume a variety of commodities rather than limiting consumption to a few with the highest antioxidant capacity.

The word fruit is derived from Latin word “fructus” which means to enjoy the produce. Fruits are ripened ovaries that include seed of the plant that bore them. A broader definition is that fruit is anything which contains seeds. The fruit that is developed from the ovarian tissue is called as true fruit, while when fruit is formed from other parts excluding ovarian tissue, it is known as the false fruit (Singh and Sharma, 2000). Although the botanical term “fruit” refers to the seeds and surrounding tissues of a plant, the foods that are commonly referred to as “fruits” for culinary purposes are pulpy seeded tissues that have a sweet or tart taste (Pennington and Fischer, 2009).
Anatomically, fruits are described as swollen ovaries that may also contain associated flower parts. Fruit development involves fertilization, occurring simultaneously with seed maturation. In the initial stages, fruits enlarge through cell division and then by increment in the cell volume. The fruit then ripens. Fruits acquire many forms that have evolved to protect and disperse seeds. An attractive combination of color, aroma and flavor assist the dispersal of seeds from fleshy fruits (White, 2002).

Fleshy fruits undergo different developmental steps, including fruit set, fruit growth, maturation, and ripening. The first stage of the development after fertilization is represented by fruit set. This is followed by an active cell division and later cell expansion phase; both together contribute to the fruit growth phase. The growth phase causes fruits to attain their maximum size which is further followed by the final developmental stage where the fruit acquire the prerequisite competence i.e. ripening. Ripening signifies a very important phase change and results in conversion of a less palatable green fruit into a highly palatable, nutritionally rich, and colored fruit (Singh and Sharma, 2000).

1.2. Composition of fruits and their nutritive value

The carbohydrates in fruit have a moderate energy value. Fruit contain protective vitamins and minerals, and dietary fiber but very little protein. They are practically fat-free, both of which contain up to 15% free of fat. Fruit vary widely in their carbohydrate content (between 1.5% and 26%). Ripe fruit contain no starch; the main sugars are sucrose, fructose and glucose which are often present in equal proportions. Fruit contribute an appreciable amount of minerals like sodium, magnesium, potassium, iron and calcium. A very high percentage of the fresh weight of the fruit is their water content around 95%- 98%.

1.3. Biochemistry of Fruit Ripening

Botanically, fruit is ‘a seed receptacle developed from an ovary’. Reid (2002) indicated that the term mature is best described by the Webster’s dictionary and the definition for mature was given as “having completed natural growth and development.” Ripening is one of the most important processes in fruits which involve changes in color, flavor, texture and thereby making them the most acceptable for edible purposes. Fruits vary in their morphological structure and based on these morphological variations, up to a certain extent they vary in their
respective metabolisms (Tucker, 1993). Fruit ripening involves physiological, biochemical, and structural changes, such as cell wall hydrolysis, pigment synthesis and degradation, carbohydrate metabolism, and generation of secondary metabolic compounds which influence fruit appearance, texture, flavor and aroma, and the process of ripening is a genetically programmed process (Mworia et al., 2012).

The four common techniques used for fruit ripeness of melons in the field are ground spot color, hollow sound of the fruit, color of axial leaf and color of axial tendril, of which brown axial leaf in combination with a brown axial tendril was the most consistent indicator of fruit ripeness (Miles et al., 2004).

The major modification occurs during fruit ripening process are changes in color, flavor, texture etc. which makes the fruit acceptable. Moreover, enormous changes occurs in physiological, biochemical and structural processes which include starch degradation, synthesis of sugars, pigments, volatile compounds and dissolution of cell wall and cell wall polymer depolymerization (Tucker, 1993). Textural changes are the most significant phenomenon among all these above mentioned changes occurring in a fruit during ripening, as the texture directly affects the shelf life of fruits. Likewise, another important factor is its rate of respiration. Fruits show two distinct respiratory patterns during ripening and on this basis they are classified into climacteric and non-climacteric groups.

Ethylene plays a major role in ripening process of climacteric fruits. The pattern of growth, respiration and ethylene during development and ripening of climacteric and non-climacteric fruits are illustrated in Figure 1.1.

Ethylene is a natural plant growth hormone essential for ripening of climacteric fruits. Ripening factors such as fruit flesh softening (Haji et al., 2003; Hiwasa et al., 2003), color change (Flores et al., 2001), and production of aromas depends strongly on ethylene production. The role of ethylene as the ‘‘ripening hormone’’ in climacteric fruits such as tomato, apple, and banana has been firmly established. However, there is an increasing body of experimental evidence that implicates ethylene in the ripening of fruits that have been classically thought of as a non climacteric. There are also a number of species in which the fruits of different varieties and cultivars exhibit both climacteric and non climacteric behavior (Genard and Gouble, 2005).
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Figure 1.1: Role of ethylene in climacteric and non-climacteric fruits

Melon is also a fruit crop that exhibits great phenotypic diversity. Some fruits of melons produce large quantities of ethylene, whereas smooth skin type of melons produces little or no ethylene during ripening. Therefore, melons behave as both climacteric and non climacteric fruit, and the level of ethylene production produced by melon fruit is directly proportional to postharvest rates of decay (Barry and Giovannoni, 2007). The variation in ripening behavior among different types of fruits, among different cultivars and also deviations in plant attached and plant detached fruit is due to the differences in endogenous concentrations of O₂, CO₂ and ethylene (Paul et al., 2012).

1.3.1. Changes in sugars and organic acids

Sweet taste is an important quality parameter for fruits and is generally associated with glucose, fructose and sucrose which are often used as an index of ripening (Gomez et al., 2001). Glucose and fructose accumulates in the initial stages of fruit development, whereas sucrose accumulates during ripening and after harvest. Sugars are the major biochemical component that directly influences fruit quality and therefore it becomes necessary to elucidate the sugar metabolizing
enzymes (Ersoy et al., 2007). Sugar accumulation in fruit also contributes to fruit enlargement by the process of osmotic potential in fruit cells. Sucrose is the major sugar translocated from source to sink tissue (Kanayama and Odanaka, 2000). The accumulation of sucrose seems to be related to sucrose-phosphate synthase (SPS) and sucrose synthase (SS) activities (Gomez et al., 2001) and its metabolism is described in Figure 2 (Kanayama and Odanaka, 2000).

![Sugar metabolism in fruits](image)

**Figure 1.2: Sugar metabolism in fruits**

Flavor is compounded mainly of sugars, acids and of numerous volatiles aroma components which are present in minor concentrations in fruit. During ripening, increase in sugars, volatiles and a decrease in acids cause changes in flavor of fruit (Ezhilarasi and Tamilmani, 2009).

### 1.3.2. Color changes

Color associated with ripening is a key attribute for determination of eating quality. Color change can be brought about by chlorophyll degradation, biosynthesis of anthocyanins or carotenoids (lycopene and β-carotene). The color change from green to red is a consequence of degradation of chlorophyll and accumulation of carotenoids synthesized within plastids as chloroplasts are transformed to chromoplasts (Gross and Ohad, 1983; Ezhilarasi and Tamilmani, 2009). Another diverse range of pigments localized in vacuole are anthocyanins. The carotenoids distribution in fruit is enormously complex and variable, giving
patterns that are characteristic of a particular species or variety. During ripening, the carotenoids concentration increases between 10- and 14-fold, due mainly to the accumulation of lycopene (Seymour et al., 1993; Bramley, 2013).

1.3.3. Antioxidants

Generally, living organisms need ample amount of oxygen for their metabolism and energy production, but free radicals are generated as a consequence of respiratory process (Packer, 1999). During peroxidation of lipid and proteins, uncontrolled generation of free radicals are observed which further leads to cellular damage, gene mutation and finally health disorders like cancer, hypertension, ageing etc. (Mantle et al., 2000). Therefore, body defends against these free radicals with the help of antioxidants. Antioxidants refer to a group of compounds that are able to delay or inhibit the oxidation of lipids or other biomolecules and thus prevent or repair the damage of the body cells that is caused by oxygen (Ismail et al., 2010).

Antioxidant activity means that some antioxidants can protect cells against the damaging effects of reactive oxygen species (ROS), such as singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals (Zhang et al., 2012). The analysis of antioxidants and antioxidant activity has been an important parameter for the nutritional quality of foods and its quantification gives the real evaluation of this nutritional value rather than the analysis of each single antioxidant compound (Ilahy et al., 2011). Synergetic antioxidant system with the involvement of antioxidant enzymes such as catalase (CAT; EC 1.11.1.6), peroxidase (POD; EC 1.11.1.7), superoxide dismutase (SOD; EC 1.15.1.1) and polyphenol oxidase (PPO; EC 1.14.18.1) defend against the reactive oxygen species (ROS) (Agarwal and Pandey, 2004).

1.3.4. Lycopene

Lycopene is a red pigment which imparts red color to some fruits and vegetables. Lycopene confers antioxidant protection in photosystem of plants, appear during ripening in chromoplasts. It has been extensively studied for past many years and its intake reduces risk of cardiovascular diseases and cancer (Collins et al., 2006). Lycopene is a carotenoid of great interest because of its
antioxidant capacity in scavenging reactive oxygen species, which cause oxidative damage and loss of proper cell function (Tarazona-Diaz et al., 2011).

1.3.5. Phenolics

Phenolic compounds are widely distributed in plants and in recent years they have gained attention due to their antioxidant activity and free radical scavenging ability with potential beneficial implications in health of humans (Chirinos et al., 2010). The scavenging ability of phenolics is mainly due to their reducing properties which allow them to act as reducing agents, singlet oxygen quenchers and hydrogen donors (Babbar et al., 2011).

1.3.6. Polyphenols

Polyphenols are the abundant micronutrients in our diet, and the evidence for their role in prevention of degenerative diseases is emerging. Several thousand molecules having a polyphenol structure (i.e. several hydroxyl groups on aromatic rings) have been identified in higher plants, and several hundred are found in edible plants. These molecules are secondary metabolites of plants and are generally involved in defense against ultraviolet radiation or aggression by pathogens. These compounds may be classified into different groups as a function of the number of phenol rings that they contain and of the structural elements that bind these rings to one another and also these polyphenols may be associated with various carbohydrates and organic and with one another. The amount of polyphenols varies according to genetic, environmental and technologic factors among which some may be controlling the polyphenol content of foods.

1.3.7. Ascorbic acid

L-Ascorbic acid is the natural form of ascorbic acid in fruits. Ascorbic acid is a universal component of plant cells and is especially abundant in tissues of high metabolic activity and rapidly oxidized by at least five enzyme systems extractable from plant tissues (Mapson, 1967). The ascorbic acid content increases with increase in growth of the fruit and with the advancement of maturity (Singh and Sharma, 2000).

1.3.8. Amino acids and Proteins

The basic building blocks of all proteins are amino acids and most of them are in the free form in tissues and during the diseased conditions in plants,
there will be a change in total free amino acid composition. However, the analysis
of amino acids provides information about the physiological and health conditions
of plant (Thimmaiah, 1999). Citrulline is a natural and rich source of non-essential
amino acid isolated in cucurbitaceous fruits such as cucumber, muskmelon,
pumpkin, bottle gourd etc. Watermelon rind is a rich source of citrulline which is
an efficient hydroxyl radical scavenger and a strong antioxidant (Rimando and
Perkins-Veazie, 2005).

Plant proteins should possess desirable functional properties and provide
essential amino acids for their utilization in different food systems. These
properties are intrinsic physicochemical characteristics that affect protein behavior
in food systems during processing, manufacturing, storage and preparation (Wani
et al., 2011). Proteins are said to be ubiquitous components of all living tissues.
Proteins, even though they occur in small amount in fruits, are involved in
metabolism during growth, development and ripening of fruits (Patel and Rao,
2009).

1.3.9. Textural changes

A fleshy fruit is composed of thin walled parenchyma cells, which are
lignified mostly. The cell wall is a dynamic structure, with a consistent change in
its composition and physicochemical properties in response to its development and
environment (Redgwell and Fischer, 2002). The phenomenon “softening” refers to
changes in the physical properties of an intact fruit, generally determined by
resistance to uniaxial compression, or various biomechanical features of the fruit
“flesh”. Measurements of tensile strength, resistance to compression (firmness) and
extensibility can all be taken into account, and all contribute to textural
characteristics, including mealiness, melting characteristics, crispness, and
juiciness (Chaib et al., 2007; Ruiz-May and Rose, 2013). Textural change is an
important event in fruit softening and it is considered as an integral part of ripening
as it directly dictates shelf life and nutritional quality (Tucker and Grierson, 1987;
Tucker, 1993).

Fruit texture is influenced by various factors like structural integrity of
primary cell wall and middle lamella, accumulation of storage polysaccharides,
modification of cell wall polysaccharides such as pectins, cellulose, and
hemicelluloses. The increased interest in controlling textural qualities of fruit
stimulated further research on the biochemistry of the cell wall polysaccharides and their degradation (Jackman and Stanley, 1995). Cell wall hydrolases participate in changes in cell wall composition and also wall degradation. Among these hydrolases, pectin degrading enzymes like polygalacturonase (PG), pectin methyl esterase (PME), lyase and rhamnogalacturonase are mostly implicated in softening of fruit (Prasanna et al., 2007). During the ripening of fruits like muskmelon and other melons, solubilization followed by depolymerization and deesterification of pectic polysaccharides is the most apparent change occurring (McCollum et al., 1989; Rose et al., 1998).

1.4. Cucurbitaceae

Crops belonging to family Cucurbitaceae are generally known as cucurbits. As Cucurbitaceae family has a considerable economic importance, they comprise approximately 122 genera and 900 species (Jeffrey, 1990) in which 36 genera and 100 species are from India. Cucurbitaceae occupy a largest area in India and other tropical countries. Fruits belonging to Cucurbitaceae family are called as ‘pepo’ as its rind gets hardened during maturity. Most cucurbits are seed propagated and annual. The optimal temperature for the growth required range between 25°C to 30°C. Growth below 10°C is limited as the plants are frost sensitive. Soil conditions such as sandy or loam soils with a pH range between slightly acidic to slightly alkaline are preferred. A common cucurbit disease is blossom rot and other abiotic disorders such as hollow heart in watermelon (Rubatzky and Yamaguchi, 1999).

1.5. Production of melons in India

Of the species of Cucurbitaceae in India, at least nine are introduced among cultivated vegetables from Central and South America or Africa (Citrullus lanatus, Cyclanthera nedata, Kedrostis foetidissina, Sicyos edulis, and five species of Cucurbita). The most species rich Cucurbitaceae genera in India are Trichosanthes with 22 species, Cucumis with 11 (all but two wild), Momordica 8, and Zehneria with 5 (Renner and Pandey, 2013).

Melon fruit are larger, sweeter, and mature (ripe) when eaten. During ripening, fruit soften and fruit aromatic essences are formed. Muskmelons are said to be originated in Africa and secondary centers are India, China, Iran etc. In case of muskmelon fruit, it requires about 6-10 weeks after anthesis to mature which
can be determined by the development of abscission zone between the fruit and peduncle. Another indicator of maturity is aroma and high quality melons have a soluble solid content of 10% or more. Melon production in India is 9,48,869 tonnes of the world production constituting about 3.6%.

Melons are naturally low in minerals such as sodium, fat, cholesterol and provide essential nutrients such as potassium, in addition to being rich source of beta-carotene and vitamin C. However melons contain low quantities of other vitamins like vitamin E, folic acid, iron and calcium (Lester, 1997). The nutritional composition of melons per 100 gm is shown in Figure 3 as below:

![Figure 1.3: Nutritional composition of melons per 100gms.](image)

1.5.1. Muskmelon

*Cucumis melo* L. commonly called as cantaloupe or muskmelon is a member of the Cucurbitaceae family (Bailey and Bailey, 1976). Consumer preference for this fruit is determined largely by its sweetness (i.e sugar content), flavor or aroma, texture and more recently as a rich source of phytonutrients (Lester, 2008). Muskmelon fruit in addition to its superior consumer preference, is an extremely healthful food choice as they are rich in ascorbic acid, carotene, folic acid, and potassium as well as a number of other human health-bioactive compounds (Lester and Hodges, 2008). Recently, melons have become the fourth most commercially important fruit in the world after oranges, bananas and grapes.
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(Supapvanich et al., 2011). Cantaloupe appears to have different diverse groups which have orange flesh, green flesh and mixed ones (Norrizah et al., 2012).

1.5.2. Watermelon

Watermelon (*Citrullus lanatus* (Thunb.) Matsum & Nakai) is cultivated for their fleshy fruits which are juicy and sweet. The centre of origin of watermelon is Africa, but has been cultivated in the Mediterranean regions for centuries and when it was introduced early into Asia, a strong secondary centre of diversification developed in India (Pratt, 1970). India is the third largest producer of watermelon in the world and the main areas under cultivation are Uttar Pradesh, Gujarat, Maharashtra and Andhra Pradesh (Singh et al., 2009). In India, the watermelon production was 4, 000, 00 tonnes (FAOSTAT, 2014) which contributed 3.6% to the world production of 1, 02, 889, 076 million tonnes.

The principal attributes of eating quality in watermelon are good flesh color, flesh crispness, and sweetness (Pratt, 1970). Watermelon is an important source of carotenoids, including lycopene, β-carotene and also citrulline (Munisse et al., 2011). Almost 50 years ago, the first icebox watermelon variety was introduced in the U.S. but it is only recently these icebox watermelons have become commonly available in markets. Icebox watermelons are rapidly gaining in popularity due to their small size and also offer farmers a means of producing high quality watermelons locally.

1.6. Significance of the present study

A comprehensive study encompassing the histological and biochemical changes in the melon fruit is scarce. Such studies would help to correlate the quantitative and qualitative analysis of biochemical parameters with the histological observations as well as histochemical localization of melons during their development and ripening. The visible changes that occur during the growth and ripening are the result of histo-architectural changes in the underlying tissues due to changes in cell division and cell differentiation and further development of tissues. Hence, insight into the histo-architecture of the melons in conjunction with the biochemical and histochemical changes occurring would reveal the bewildering mechanisms of development and ripening of fruits. Moreover, the present study was carried out with an aim of determining the nutritional quality of the melons.
based on the biochemical composition and antioxidant potential of fruit concomitant with the enzyme activities.

Furthermore, an understanding of the biochemistry and key enzymes involved in various biochemical phenomenon during development and ripening of melons is a prerequisite to possible genetic improvement of sweetness and antioxidant potential of this species. The overall focus of this study was to bring additional scientific exposure to these types of cultivars of watermelon and muskmelon which have superior food quality attributes and to determine variation in marketable qualities among some commercially available melons.

1.7. Objectives

- Elucidation of histo-architectural features associated with the physiological and biochemical changes during growth, maturation and ripening of melons.

- Understanding the relationship between the texture and composition of pericarpic tissues of melons at different maturities and their nutritional quality.

- To establish the linkage between the major textural changes due to the enzyme mediated alterations in the structure of cell wall.

- To study the antioxidant activity and its related enzymes, hormonal role in respiratory pattern and its relation to ripening of melons.