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Biochemical and Physical Changes during Ripening

Ripening

Ripeness and maturity, when applied to fruit and vegetables, are often difficult to define. They usually are used for defining the appropriate state for harvesting and for eating. Fruit ripening is a highly coordinated, genetically programmed irreversible phenomenon which lead to the development of a soft and edible ripe fruit with desirable quality attributes via series of physiological, biochemical, and organoleptic changes. Ripeness is usually considered readiness for harvest. It is the result of a complex of changes many of them probably occurring independently of one another. Ripening marks the completion of development of a fruit and the commencement of senescence. Development and maturation of fruit are completed only when it is attached to the plant but ripening and senescence may proceed on or off the plant. Fruits are generally harvested either when mature or when ripe, although some may be consumed as vegetables may be harvested even before maturation has commenced. e. g. zucchini. In vegetables, ripening stage does not occur and they are harvested over a wide range of physiological ages. As a result it is more difficult to differentiate change from maturation to senescence in vegetables. During the ripening process there is change in respiration rate and biosynthesis and evolution of ripening hormone ethylene. Based on their respiratory pattern and ethylene biosynthesis during ripening, harvested fruits can be further classified as climacteric and non-climacteric type. Climacteric is defined as a period in the ontogeny of fruit during which a series of biochemical changes are initiated by autocatalytic production of ethylene making the change from growth to senescence and involving an increase in respiration leading to ripening of fruit. This phenomenon was first observed by Kidd and West in 1925.

Climacteric fruits: These are harvested at full maturity, can be ripened off the parent plant. These produce much larger quantities of ethylene in association with their ripening, and exposure to ethylene treatment will result in faster and more uniform ripening. The respiration rate is minimum at maturity and remains rather constant, even after the harvest. The rate will rise up abruptly to the climacteric peak only when ripening is about to take place, and then it will slowly decline. Fruit softening, colour

changes, development of taste and flavour and a number of other parameters of ripening process are associated with the climacteric cycle. Non-climacteric fruits: These are not capable of continuing their ripening process, once they are detached from the parent plant. Also, these fruits produce a very small quantity of endogenous ethylene, and do not respond to external ethylene treatment. Non-climacteric fruits show comparatively low profile and a gradual decline in their respiration pattern and ethylene production, throughout the ripening process. This division is not fully conclusive and both types may also be found.

ETHYLENE AND RIPENING

Ethylene is a fruit ripening hormone which in minute amounts can trigger many events of cell metabolism including initiation of ripening and senescence. It is produced by unripe fruit in minute quantities (0.01-0.05 parts per million of internal air space) but at the beginning of ripening there is a drastic rise in ethylene production thousand time greater than unripe fruits. Treatment of unripe climacteric fruits with low concentrations of ethylene induces the climacteric rise in respiration and the onset of ripening and also stimulates the fruit tissue to produce its own ethylene. Thus, if the external supply of ethylene is removed, the ripening process is not interrupted. In the non-climacteric fruits ethylene production does not rise with ripening but treatment of unripe fruit with ethylene stimulate an increase in respiration and promotes in some changes associated with ripening such as the rate of loss of green colour from the skin. However, it does not stimulate the citrus fruit tissue to produce its own supply of ethylene and thus, if the external supply of ethylene is removed, the rates of respiration and of loss of green colour return to their original levels. Ethylene does not play an important role in the maturation of most vegetables. Generally the gas is produced in small amounts throughout the life of the vegetable and there is no upsurge in production as it matures. In vegetables there are no abrupt breaks in the respiratory pattern. However, metabolic changes are noticed on treatment of vegetables with ethylene. i.e. the respiration rate of potatoes rises in the presence of small amounts of ethylene while carrots produce a bitter tasting compound isocoumarin.

CHANGES DURING RIPENING

Fruit ripening involves many complex biochemical changes, including seed maturation, change in colour, abscission from the parent plant, texture softening, production of flavour volatiles, wax

development on skin, tissue permeability and change in carbohydrate composition, organic acids and proteins. During ripening the composition of fruit is altered either due to formation of new compounds or degradation of others. Out of various biochemical and physical changes occurring, changes in flavour, colour and texture are of utmost importance, for the acceptability of the fruit.

Colour Changes

Pigments are essential for the attractiveness of fruits and accumulate most often in the skin during the ripening process. Color is often the major criteria used by consumers to determine whether the fruit is ripe or unripe. 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.

- **Formation of pigments:** During ripening there is the development of the enzymes to catalyse the formation of pigments. The main pigments formed and accumulated are: β -carotene, xanthophyll esters, xanthophylls and lycopene. The anthocyanins are formed partially from acetic acid units and partially from the amino acid, phenylalanine. Carotenoids are terpenoids compounds and as such derive from acetyl CoA via the mevalonic acid pathway. The primary carotene produced is phytoene which is further metabolized to give other carotenoid pigments.
- **Degradation of pigments:** Climacteric fruits show rapid loss of green colour with attainment of optimum eating quality. Some non-climacteric fruits also exhibit a marked loss of green colour with attainment of optimum quality. The green colour loss is due to the degradation of chlorophyll structure. The main factors responsible for chlorophyll degradation are: pH changes (mainly due to leakage of organic acids from the vacuole), oxidative systems and enzyme chlorophyllase.

FLAVOUR CHANGES

Flavour of a fruit depends upon synthesis of various flavour compounds which are unique to each fruit. Several of these compounds are complex and a large proportion of compounds is volatile in nature and gives the particular flavour. These flavour compounds are present in very low amount and includes alcohols, aldehydes, esters and other chemical groups. In both climacteric and non-climacteric fruits, the

most important aroma volatiles that increase during ripening are the esters. Formation of flavour compounds:

- **Biosynthesis:** The increase in flavor and aroma during fruit ripening is attributed to the production of a complex mixture of volatile compounds and degradation of bitter principles, flavanoids, tannins, and related compounds. Bio-synthesis of volatile molecules in an intact fruit is a complicated process. The alcohols and aldehydes are generated after metabolism of their corresponding amino acid or oxo-sugar. Some of the flavour volatiles are synthesized via mevalonate/ isoprene pathway. Various organic acids also act as substrate for flavour manufacture. Some other important class of flavour compounds i.e. monoterpenes, sesquiterpenes are generated from amino acid, sugars and lipids. Natural plant volatiles, such as aliphatic esters, alcohols, acids and carbonyls are derived from fatty acid metabolism. Most unripe fruits, e.g. apples, bananas and strawberries, produce a variety of fatty acids which, during ripening, are converted into esters, ketones and alcohols via β -oxidation.

- **Degradative reactions:** Some of the volatiles are produced upon disintegration of fruit/vegetable tissue i.e. the compounds responsible for the characteristic taste and flavour are not present in the intact cucumber but are formed by enzymic breakdown of the fatty acids of the cell membranes which are disrupted when the cucumber cells are cut or chewed. The tannins (secondary metabolite) and other phenolic compounds, present in fruits impart astringency. Small amount of astringency is essential to the taste of many fruits but the high levels found in unripe fruits make them unacceptable. During ripening the tannins, are either partially broken down or polymerised into products which are not astringent. The ripening induces the breakdown of carbohydrate polymers, by various carbohydrases and leads to near total conversion of starch to sugars. This has the dual effect of altering the taste and texture of the produce. The increase in sugar renders the fruit much sweeter and therefore more acceptable. However many exceptions are there. In oranges and grapefruits the acid content drops during ripening, while in lemons, there is an increase in acids. Synthesis of ascorbic acid also occurs in many fruits during ripening. Generally, the acidity decreases as organic acid are utilized in respiration of fruits. The ratio of sugar to acid and the absolute amounts of both sugar and acid are important in the flavour quality of many ripe fruit. The breakdown of polysaccharides by cellular enzymes not only gives the typical sweetness, but also precursors for many aromatic flavor compounds.

TEXTURE CHANGES

Textural change is the major event in fruit softening, and is the integral part of ripening, which is the result of enzymatic degradation of structural as well as storage polysaccharides. The process of textural softening is of commercial importance as it directly dictates fruit shelf life and consumer acceptability. Cell walls of fruit undergo a natural degradation during fruit ripening, reducing cell wall firmness and intercellular adhesion. This leads firstly to the attainment of a desirable eating texture and then, as senescence begins, to a loss of this desirable texture. Fruit texture is influenced by various factors like structural integrity of the primary cell wall and the middle lamella, accumulation of storage polysaccharides, and the turgor pressure generated within cells by osmosis. Change in turgor pressure, and degradation of cell wall polysaccharides and starch determine the extent of fruit softening. Cell wall polysaccharides that undergo modifications during ripening are pectin, cellulose, and hemicelluloses. Amylase activity increases to some extent during ripening of many fruits. Starch is almost completely hydrolyzed to free sugars, thus contributing to loosening of the cell structure and textural softening during ripening. Pectin is the key substances involved in the mechanical strength of the primary cell wall and middle lamellae and contributes to fruit texture. During ripening, softening of fruit is caused by the conversion of protopectin, the insoluble, high molecular weight parent pectin into soluble polyuronides. The solubilisation of pectin is followed depolymerisation and deesterification; These changes are accompanied by an extensive loss of neutral sugars and galacturonic acid, followed by solubilization of the remaining sugar residues and oligosaccharides. Pectin from ripe fruit exhibit a lower degree of esterification, molecular weight and decreased neutral sugar content compared to pectin from unripe fruits. Ultrastructural studies of ripened fruits have also shown that cell wall breakdown is accompanied by dissolution of middle lamella and gradual dissolution of fibrillar network of primary cell wall. A rapid rise in polygalactouronase enzyme occurs during ripening is responsible for solubilisation of pectin. The other enzymes involved in hydrolysis of pectin are: pectin methyl esterase, pectate lyase, pectin lyase, arabinanase and galactanase. Firmness is also related to the turgor properties of a tissue or organ. During fruit ripening, there is a decline in turgor which contributes to textural changes probably due partly to an accumulation of osmotic solutes in the cell wall space and partly to postharvest water loss from the ripening fruit. In citrus fruit, softening is mainly associated with change in turgor pressure. According to softening behaviour, fruits are divided in to two groups. These are:

1. Very soft fruits: These fruits are greatly softened after ripening and possess soft and melting texture. e. g. apricot, strawberry, peach, plum, kiwifruit, European pear and most berries.

2. Moderately soft fruits: These fruits are softened to little extent after ripening and have a crisp and fracturable texture. e.g. apple, quince, cranberry, Asian pear, bell pepper and watermelon. There exist no relationship between climacteric and non-climacteric status of fruit and its texture.

OTHER CHANGES

Change in protein

During the onset of ripening the actual concentration of protein increases but the protein has no role in imparting any effect to 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.

Change in cellulose and hemicellulose:

Ripening causes apparent dissolution of cell wall fibrillar network in many fruits. There is often little change in the cellulose structure in fruits during ripening and the activity of enzymes does not correlate with softening changes that occurs. Ripening also involves degradation of hemicellulose. There is a decline in characteristic monomers of hemicelluloses viz. glucose, xylose, and mannose during ripening of fruits but that does not have a very drastic influence on the texture of the product.

Changes in lipid

Little is known about changes in the lipid fraction. There are speculations about shifts in composition and quantity of phospholipid fraction during ripening.