Biochemical and physiological changes during ripening
INTRODUCTION

• Ripening is the process by which fruits attain their desirable flavor, quality, color, palatable nature and other textural properties.

• Ripening is associated with change in composition *i.e.*

  • Conversion of starch to sugar.
  • Change in colour
  • Change in firmness
  • Shape and size
  • Odour /smell
Physiological Changes

- Fruits and Vegetables are living entities and diverse in structure, composition and physiology.
- They have the typical plant cell system.
- The life of fruit and vegetables can be conveniently divided into three major physiological stages following germination.

These are: Growth → Maturation → Senescence
Physiological Changes

- **Growth** - involves cell division and subsequent cell enlargement, which accounts for the final size of the produce.

- **Maturation** - usually commences before growth ceases and includes different activities in different commodities. Growth and maturation are often collectively referred to as the development phase.

- **Senescence** - is defined as the period when synthetic (anabolic) biochemical process gives way to degradative (catabolic) process, leading to ageing and finally death of the tissue.
• **Ripening** - is a phase of qualitative change which occurs in fruits particularly, after completion of maturation, during which the fruit becomes acceptable for consumption in terms of taste and flavour.

• Ripening occur during the later stages of maturation and is the first stage of senescence.
Respiration

• One of the major physiological and biochemical change which occur in fruits and vegetables is a change in the pattern of respiration.

• If the respiration rate of a fruit or vegetable is measured as their $O_2$ consumed or $CO_2$ evolved during the course of the development, maturation, ripening and senescent period, a characteristic respiratory pattern is observed.

• The respiratory pattern also impacts the pattern of evolution of ethylene. Based on this pattern, fruits can be classified into ‘climacteric’ and ‘nonclimacteric’.
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Respiration

• Few fruits exhibit the pronounced increase in the respiration (increase in CO₂ and C₂H₄) coincident with the ripening, such increase in the respiration is known as respiratory climacteric, and this group of fruits is called climacteric.

• Respiration is a process in which stored organic materials (carbohydrates, protein, and fat) are broken down into simple end products with release of energy. Oxygen is used in this process and carbon dioxide is produced.

\[ C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{energy (686 k.cal)} \]
<table>
<thead>
<tr>
<th>Climacteric Fruit (CF)</th>
<th>Non-climacteric Fruit (NCF)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Normally they ripen after harvest</td>
<td>Fruit that does not ripen after harvest. Ripen on the plant itself.</td>
</tr>
<tr>
<td>2. The quality of fruit changes drastically after harvest characterized by softening, change in colour and sweetness. (except in avocado, which will ripen only after detached from the plant)</td>
<td>The quality do not change significantly after harvest except little softening. Do not change to improve their eating characteristics</td>
</tr>
<tr>
<td>3. Exhibits a peak in respiration</td>
<td>Does not exhibit a peak</td>
</tr>
<tr>
<td>4. More ethylene is produced during ripening</td>
<td>Little / No ethylene production</td>
</tr>
<tr>
<td>5. Significant increase in CO₂ production</td>
<td>No significant increase in CO₂ production</td>
</tr>
<tr>
<td>6. Significant increase in CO₂ production</td>
<td>Slowly</td>
</tr>
<tr>
<td>7. Decrease in internal oxygen concentration</td>
<td>More</td>
</tr>
<tr>
<td>8. Low concentration of ethylene 0.1-1.0 μL/L/day is sufficient to hasten ripening</td>
<td>Not much response is seen to exogenous application of ethylene.</td>
</tr>
<tr>
<td>9. Eg - Many except in the opposite column</td>
<td>Eg- Bell pepper, Blackberry, Blueberry, Cacao, Cashew apple, Cherry, Citrus sp., Carambola, Cucumber, Eggplant, Grape, Litchi, Loquat, Okra, Olives, Pea, Pineapple, Pomegranate, Pumpkin, Raspberry, Strawberry, Summer squash, Tart cherries, Tree tomato and rin &amp; nor tomato, Watermelon</td>
</tr>
</tbody>
</table>
Fig. 3.1 Growth, respiration and ethylene production patterns of climacteric and non-climacteric plant organs

Fig. 3.2 Respiratory pattern of harvested climacteric fruits
### Classification of horticultural commodities according to their respiration rate

<table>
<thead>
<tr>
<th>CLASS</th>
<th>Range at 5°C (mgCO₂Kg⁻¹ hr⁻¹)</th>
<th>COMMODITIES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very low</td>
<td>&lt; 5</td>
<td>Dates, Dried fruit and vegetables, Nuts, etc.</td>
</tr>
<tr>
<td>Low</td>
<td>5 - 10</td>
<td>Apple, Beet, Celery, Citrus Fruits, Garlic, Grapes, Kiwi Fruit, Onion, Papaya, Pineapple, Potato (Mature), Sweet Potato, Watermelon etc.</td>
</tr>
<tr>
<td>Moderate</td>
<td>10 - 20</td>
<td>Apricot, Banana, Cabbage, Carrot (Topped ), Cherry, Fig, Lettuce (Head), Mango, Peach, Pear, Plum, Potato (Immature), Radish (Topped), Tomato, Summer squash</td>
</tr>
<tr>
<td>High</td>
<td>20 - 40</td>
<td>Avocado, Carrot (with tops), Cauliflower, Leeks, Lettuce (Leaf), Radish (with tops), Raspberry</td>
</tr>
<tr>
<td>Very high</td>
<td>40 - 60</td>
<td>Artichoke, Bean Sprouts, Broccoli, Brussels sprouts, Cut flowers, Green Onion, Okra</td>
</tr>
<tr>
<td>Extremely high</td>
<td>&gt; 60</td>
<td>Asparagus, Mushroom, Parsley, Peas, Spinach, Sweet corn</td>
</tr>
</tbody>
</table>
## Respiratory Pattern

- Climacteric
- Non climacteric

<table>
<thead>
<tr>
<th></th>
<th>Climacteric</th>
<th>Non climacteric</th>
</tr>
</thead>
<tbody>
<tr>
<td>Respiration</td>
<td>Increase</td>
<td>Not shows respiratory climacteric</td>
</tr>
<tr>
<td>Ethylene</td>
<td>More production</td>
<td>Less amount</td>
</tr>
<tr>
<td>Detachment ripening</td>
<td>occurs</td>
<td>On tree only</td>
</tr>
<tr>
<td>Fruits</td>
<td>Apple, Apricot, Banana, Guava, Kiwifruit</td>
<td>Cherry, Cucumber, Grape, Grapefruit, Lemon</td>
</tr>
</tbody>
</table>
Factors responsible for the respiration

1. Temperature
2. Relative Humidity
3. Gas composition in the ambient and in the cell
4. Moisture content of the tissue
5. Wounding or injury
6. Type of the plant parts
7. Stage of development of tissue
8. Surface area to volume of the produce
9. Pre-harvest treatments and PH methods
10. Chemical composition of tissue
11. Size of the produce
12. Presence of natural coating on the surface
Ethylene

- Ethylene is a natural plant hormone released by all plant tissues and microorganisms.
- It is also called ‘Ripening hormone’, as it plays an important role in ripening process. Low concentration of 0.1-1.0 microlitres is sufficient to trigger the ripening process in climacteric fruits.
Ethylene Biosynthesis

SAM = S-Adenosylmethionine
ACC = Aminocyclopropane carboxylic acid

- Malonyl ACC
- ACC synthase

ACC
- ACC oxidase
- Keto butyrate

- Oxidation products

C₂H₂

Fruit ripening
- Texture
- Colour
- Taste

Yang and Hoffman 1993

At the onset of fruit ripening, expression of multiple ACC synthase genes are activated, resulting in increased production.

Deamination of ACC to α-ketobutrate by over expressing ACC deaminase enzyme also inhibited ethylene formation and fruit ripening.

Mechanism of Ripening
Pathway of ethylene Biosynthesis and Metabolism
Biochemical Changes

1. Cell wall changes
2. Degradation of chlorophyll
3. Conversion of starch into sugars
4. Decrease in tannins and phenols
5. Decrease in acidity of the fruit
6. Development of characteristic flavour
Degradation of Chlorophyll and Pigment Synthesis

- Degradation of chlorophyll due to Chlorophyllase enzyme.
- Splitting of chlorophyll into Phytol chain and porphyrin.
- Loss of Mg ++ ion and conversion of porphyrin into Phaeophytin.
- Change in tetapyrolic chain and it becomes bilviridin.
- Oxidation or saturation of double bonds.

Chlorophyll (green) → Chlorophyllase → Chlrophyllin (bright green) → Phaeophytin (Olive green) → Phytol → Pheoporphide (Brown) → Chlorins, Purpurins (colourless product)
Metabolic Changes

- Increases in biosynthesis
- Evolution of ripening hormone
- Increase in respiration mediated
- Alternation of cell structure
- Hydration of cell wall
- Decrease in structural integrity
- Increase in intercellular space
Hydrolysis and Aroma

Starch to sugars ...........
Aroma volatile after ripening ....

<table>
<thead>
<tr>
<th>Product</th>
<th>Compound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>Ethyle 2-methyle butyrate</td>
</tr>
<tr>
<td>Banana</td>
<td>2 hexanol</td>
</tr>
<tr>
<td>Lemon</td>
<td>Citral</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Mode of action</th>
</tr>
</thead>
<tbody>
<tr>
<td>α Amylase</td>
<td>Mixture of glucose and maltose</td>
</tr>
<tr>
<td>Starch phosphorylase</td>
<td>Glucose 6-phosphat</td>
</tr>
<tr>
<td>α (1-6) glucosidase</td>
<td>Amylopectin</td>
</tr>
</tbody>
</table>
Texture and Cell wall changes

Textural changes
- Enzyme degradation of polysaccharides
- Different rates i.e. Degree
- Breakdown of starch
- Pectic substance
- Hydrolysis starch - firmness

Cell wall breakdown
- PG (Polygalacturonase)
- PME (Pectin methylesterase)
- Other hydrolases

Cell wall Degradation
- Depolymerisation
- Deesertification
# Phenolic Compound

Astringency to fruit e.g. Tannins.

Involve in **oxidative browning** due to **Polyphenoloxidase**

<table>
<thead>
<tr>
<th>S NO</th>
<th>Crop</th>
<th>Phenol</th>
<th>Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>01.</td>
<td>Grapes</td>
<td>Flavon-3-ol monomers</td>
<td>Decreases</td>
</tr>
<tr>
<td>02.</td>
<td>Loquat</td>
<td>Hydrobenzoic acid</td>
<td>Decreases</td>
</tr>
<tr>
<td>03.</td>
<td>Citrus</td>
<td>Limonoids</td>
<td>Decreases</td>
</tr>
</tbody>
</table>
Enzyme Activity

Many of the chemical and physical affects during ripening and after ripening processes are attributed to the enzyme action.
Physical condition – Chemical cause

Unripe Fruit
- Green - Chlorophyll
- Hard - Pectin
- Sour - Acid
- Mealy - Starch
- Odourless - Large orgs

Hydrolase

Chemical cause - Physical condition

Ripe fruit
- Anthocyanin - Red
- Less pectin - Soft
- Neutral
- Sugar sweet + juicy
- Small orgs + odor

Pectinase

Enzymatic action

Kinase

Amylase

Hydrolysis
Regulation of Ripening

- Ethylene regulation
- Regulation of O₂ and CO₂
  - MAS
  - CAS
- Chemical treatment
  - Calcium
  - 1MCP methylcyclopropane
- Bioregulators
  - Auxin GA, CK
  - Ethylene, ABA
- Ionized radiation
  - Gamma
Wishing to be friends is quick work, but friendship is a slow ripening fruit.

Aristotle