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A study on fruit ripening

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Ripening is a process in fruits that causes them to become more palatable. In general, a fruit becomes sweeter, less green, and softer as it ripens. The requirement of fruit eating consumer is properly ripe fruit only. Semi ripe or unripe fruits are being consumed as processed products like, jam, jelly, candies, pickles, preserves, etc. The starch as reserved food in fruit is converted in to sugar during the ripening process and it reaches at its optimum stage at the end of ripening. Against that it was observed that acidity of the fruits reduce, even though the acidity of fruit increases as it ripens, the higher acidity level is not reflected in its flavor, which can lead to the misunderstanding that the ripen fruit is more 'sweeter' and not 'sour'. This curious fact is attributed to the brix-acid ratio. Initial colour of any fruit is green; this is attributing to the chlorophyll presence in the skin of the fruits. Magnesium (mg) is the main element in the chlorophyll, which reflects the green colour of all unripe fruits. During the process of ripening, the magnesium oxidized and became transparent and real ripen fruit colour appears. Calcium (Ca) and pectin keeps the structure of fruit. During the due course of ripening process, the calcium and pectin are disintegrating in the pulp. In absence of bonding agent and main structural component, fruit looses its firmness and became softer. After achieving fruit maturity, the ripening process continued even after harvest, this type of fruits falls under the category of climacteric fruit, but in case of non-climacteric fruit; it ripen only on the plant before harvest. Ripening stage of fruit could be identified by non-destructive physical method like, measurement of respiratory gases; *i.e.* oxygen (O_2) and carbon dioxide (CO_2).

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Biochemical changes during ripening:

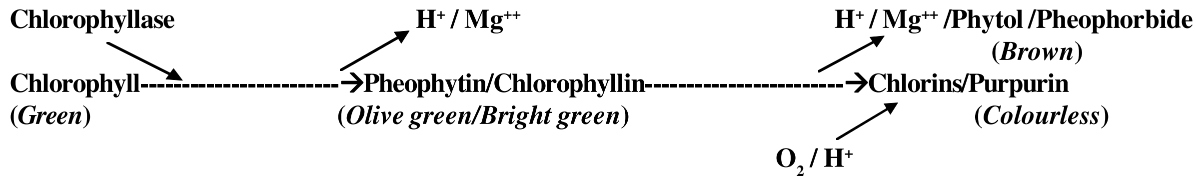
Ripening is a dramatic event in the life of a fruit – It transforms a physiologically mature but inedible plant organ into a visually attractive olfactory and taste sensation. Ripening is an irreversible process and marks the completion of development of fruit and the commencement of senescence. It is the result of complex of changes, many of them probably occurring independently of one another. The changes that occur during the ripening of fleshy fruits are as under.

– Seed maturation.
– Colour change.
– Abscission (Detachment from parent plant).
– Change in respiration rate.
– Change in rate of ethylene production.
– Change in tissue permeability and cellular compartmentation and softening,
– Change in composition of pectic substance.
– Change in carbohydrates composition.
– Change in organic acid.
– Change in protein.
– Production of flavour volatiles.
– Development of wax on skin.

Colour development:

Most of the fruits, vegetables and ornamentals are in green colour at early stage of developments and with the time it changes to its characteristic colour. The green colour is due to the presence of chlorophyll, which is a magnesium – organic complex. The loss of green colour is due to degradation of the chlorophyll structure. The principle agents responsible for this degradation are pH change, oxidative systems and chlorophylases enzymes.

Degradation of chlorophyll:



The disappearance of chlorophyll is often associated with the synthesis and/or revelation of pigments ranging from yellow to red. Many of these pigments are carotenoids, which are unsaturated hydrocarbons with generally 40 carbon atoms and which may have one or more oxygen atom in the molecule. The carotenoids are stable compounds and remain intact even when extensive senescence has occurred.

Three basic phenomenon of colour development:

– The carotenoids are synthesis during the development stage of the produce but remained masked by the presence of chlorophyll. Following the degradation of the chlorophyll, the yellow carotenoid pigments become visible. eg. banana

– With the other tissues, red carotenoid synthesis occurs concurrently with chlorophyll degradation. eg. tomato.

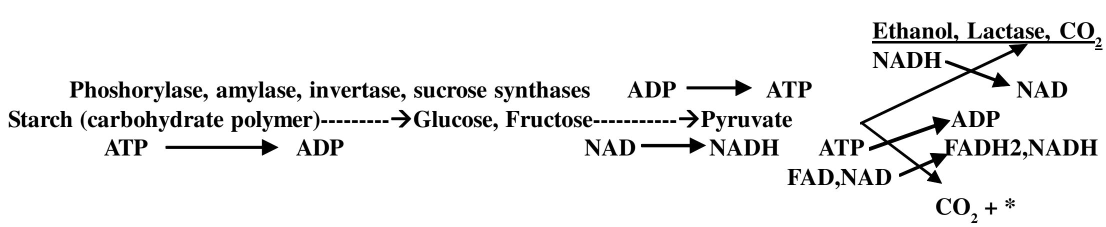
– Anthocyanins provide many of the red-purple colour of fruits, vegetable and flowes. Anthocyanins are water soluble phenolic glucosides that can be found in the cell vacuoles of the fruits, vegetables and flowers but are often in epidermal layer. They produced strong colours, which often mask carotenoids and chlorophyll. eg. beetroot, apple, graps.

Softening:

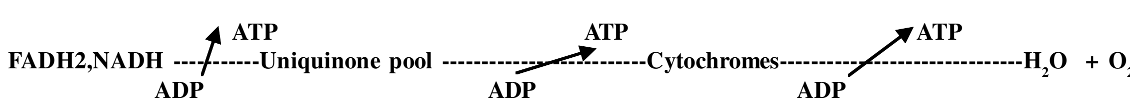
A number of different hydrolysis enzymes break down the carbohydrate polymers (e.g. pectins, celluloses, hemicelloloses) responsible for the structural integrity of cell walls. Among these is the enzyme ploygalacturonase (PG), which hydrolyses the a–(1-4) linkage between galacturonic acid residues in pectins. This could be the primary enzyme responsible for softening. Further, hydrolase enzyme, pectinesterase, which demethylates galacturonic acid residues in pectins, the release of Ca^{2+}, which is important in the cross linking the polymer chains in the cell wall, and the consequent swelling in middle lamella of the adjacent cell walls, allowing the cell to move apart. During ripening and maturation, protopectin is gradually broken down to lower molecular weight fractions, which are more soluble in water. This gives the softer mouth feel characteristics of a ripe fruit.

Sweetening:

The largest quantitative change associated with ripening is usually the breakdown of carbohydrate polymers, especially the near total conversion of starch to sugars. This changes both taste and texture of the produce. The increase in sugar renders the fruit much sweeter and, therefore, more acceptable. In non-climacteric fruits, the accumulation of sugar is associated with the development of optimum eating quality, although the sugar may be derived from sap imported into the fruit rather than from the breakdown of the fruit's starch reserves.



* = acetyl CoA, citrate, α-ketoglutarate, succinate, malate, oxaloacetate



Organic acid:

The organic acids decline during ripening as they are respired or converted to sugar. Acid can consider as a reserve source of energy to the fruit, and would, therefore, be expected to decline greater during the metabolic activity that occurs with ripening.

Nitrogenous compound:

Nitrogenous compound or free amino acids or protein are minor constituents of fruit and, as far as is known, have no role in determining eating quality. Change in nitrogenous constituents; indicate variations in metabolic activity during different growth stage. During climacteric phase of the many fruits there is a decrease in free amino acids, which often reflect in increase in protein synthesis. During senescence, the level of free amino acid increase, reflecting a breakdown of enzymes and decreased metabolic activity.

Aroma:

Aroma plays an important part in the development of optimal eating quality in most fruits. Aroma is due to the synthesis of many volatile organic compounds during ripening phase. The total amount of carbon involved in the synthesis of aromatic volatiles is less than one per cent of that expelled as carbon dioxide.

Factor affecting the ripening process:

There are many factors, which accelerate the ripening process in fruits. Stage of maturity (particularly climacteric fruits), respiratory gas concentration, ethylene concentration, temperature of ripening, mechanical damage, etc, are some of the major factors, which affect the ripening process. The factors affecting fruits ripening can be physiological, physical, or biotic.

Physiological factors:

Physiological factors relate to fruit maturity or environmental factors, which affect the metabolism of fruits. The upper limit and lower limit of parameters varies with maturity of fruits.

Fruit maturity:

The more mature plantain is at harvest, the shorter the ripening period. Studies show that False Horn plantain harvested 100 days after flowering ripened in 11 days. When the same cultivar was harvested 90 days after flowering, the ripening period increased to 15 days, and further increased to 22 days when the fruit was harvested at 80 days maturity. Similar in case of mango; the over mature mango ripen within 6 days against 15 days for the partial mature mango. Immature mango doesn't ripen until and unless accelerated ripening treatment given. However, an early harvest reduces yield, but also provide marketing time to fruit at distance market. Identified harvesting stage followed by correct ripening treatment can serve best quality fruits through out the sphere.

Table 1 : Ripening conditions for some fruits using ethylene gas

Fruits	Temperature (ºC)	Relative humidity (%)	Ethylen (ppm)	Treatment time (h)
Avocado	18-21	85-90	10	24-72
Banana	15-21	85-90	10	24-48
Honeydew melon	18-21	85-90	10	24-36
Kiwifruit	18-21	85-90	10	24-36
Mango	29-31	85-90	10	24-36
Papaya	21-27	85-90	Nil	6-12
Pear	15-18	85-90	10	24-36
Persimmon	18-21	85-90	10	24-36
Tomato	13-22	85-90	10	Till colour change

Temperature:

Physiological studies on bananas show that a 1ºC reduction increases storage period by 1-2 days. The relationship between ripening period and temperature is due to fruit respiration. Fruit respiration depends on many enzymatic reactions, and the rate of these reactions increases exponentially with increase in temperature. Studies show that ripe fruits respire at approximately 4 times the rate of unripe fruits. Consequently, ripe fruits lose sugar resources at a higher rate than unripe fruits. This explains why ripe fruits deteriorate quickly. It indicates that by reducing the temperature of ripening process, the fruit ripening index could be prolonged and vise-versa.

Water loss and humidity:

Water loss occurred in fruits during ripening is due to high water vapor pressure difference between surrounding air and fruit surface cells. Where fruit is sold on a weight basis, loss of water means economic loss. Additionally, water loss reduces visual quality. Water loss causes fruit to lose its firmness, the peel becomes soft and shriveled, and ripening period reduces. Detailed studies on plantain show a curvilinear or power relationship between fruit weight loss and ripening period. For a 2 per cent change from 2 per cent to 4 per cent weight loss per day, ripening period reduced by 9 days or 50 per cent. However, for the same 2 per cent change from 8 per cent to 10 per cent per day, only a 1 day or 5 per cent reduction in the ripening period occurred. Therefore, at a low rate of weight loss, a small increase in weight loss has a critical effect on ripening. At an ambient RH of 95-100 per cent, fruit loses little or no moisture, and ripening period is unaffected. However, as humidity decreases, the rate of water loss increases, and ripening period reduces. Excessive wetting can also be a problem. When fruit is stored in wet conditions, such as in moist coir, the uptake of water from the coir to the plantain leads to peel splitting.

Sunlight:

Exposure to direct sunlight reduces the ripening period of fruits. Sunlight increases fruit temperature above ambient temperature, which increases respiration, and possibly the rate of water loss. Shading provides protection from sun light and increase the ripening period.

Ethylene:

Ethylene (C_2H_4) is a gaseous plant hormone which determines the time between harvest and senescence. The time from harvest to the climacteric respiratory response is called the 'green life' or pre-climacteric period. Ethylene shortens the pre-climacteric period; at high concentrations, ethylene causes rapid initiation of the climacteric respiratory response and accelerates ripening. All fruits produce small amounts of ethylene during development and when damaged or stressed. During ripening, climacteric fruits produce larger amounts of ethylene than non-climacteric fruits. When ethylene is applied to climacteric fruits, at a concentration as low as 0.1-1.0 ml/l of space volume, for 1 day, ripening starts. Once ripening starts, climacteric fruits ripen within 1-2 days. When non-climacteric fruits are exposed to ethylene, fruits show an increased rate of respiration. However, respiration rate falls when ethylene is removed. A rise in respiration rate may occur more than once in non-climacteric fruits. However, for climacteric fruits, the climacteric is autocatalytic, that is, once started, and the process cannot be stopped until the fruit is ripe. Poor storage methods allow a build up of ethylene, stimulate the climacteric response, and reduce the ripening period. For example, plastic sheets placed over stacks of fruit for shade increase the level of ethylene within the fruits stack and increase the rate of ripening. Therefore, store fruits in thatched or ventilated areas to prevent the build up of ethylene. Also, do not store unripe fruits with ripe fruits. During the pre-climacteric period, fruits are less susceptible to physical damage and pathological attack. This is the best time for handling, transportation, and marketing. A 24- hour exposure initiates ripening and all mature fruits, both early and late season fruits will ripen in three to 4 days after the beginning of the ethylene treatment. Ethylene is generally recognized as safe (GRAS) by the United States Food and Drug Administration (FDA) with Good Manufacturing Practices (GMP).

Respiratory gas:

Respiratory gas includes oxygen (O_2) and carbon dioxide (CO_2). For the production of 2-oxoglutarate in fruit respiratory, concentration of oxygen and carbon dioxide plays important role during ripening. The high concentrations of CO_2 decreased the oxidative activity of NADP-IDH in an *in vitro* assay, indicating a decrease in production of 2-oxoglutarate. Higher level of carbon dioxide could lead the blackening and softening of the fruit flash during ripening. It is recommended to change the air of the ripening chamber (*i.e.* surrounding air of produce) at least after every 48hr; otherwise, CO_2 level increases significantly and that leads to non-aerobic respiration. Iodine (I) can be used to determine whether the fruit is ripening or rotting by showing whether starch in the fruit has turned into sugar. It was also observed that C_2H_4 effectiveness reduces with the increasing level of the CO_2.

Physical factors:

Physical factors include mechanical damage, or relate to dimensions of the fruit.

Mechanical damage:

Mechanical damage is a physical factor affecting ripening. Fruit damage during handling generates ethylene. If ethylene production is sufficient to start the climacteric respiratory response, fruit immediately starts to ripen. Damage can also reduce ripening period by causing moisture loss. The effect of damage can easily be measured by recording fruit weight loss over time. Cuts and abrasions on the surface membrane cause the most weight loss. After harvest, fruits lose the ability to repair ruptured peel. Harvesting techniques which damage fruit reduce storability. Studies on plantain show that an abrasion affecting 5-10 per cent of the peel can reduce the ripening period by 40 per cent. A trader selling abraded fruit has half the normal time to sell an already less attractive product. Damage can also lead to secondary infection, which increases the rate of water loss and further reduces quality.

Surface to volume ratio:

The ratio between surface area and volume (Physical dimension) determines the rate of water loss. Greater the surface to volume ratio, shorter the postharvest life. Large fruits lose less water than small fruits because of less surface–to–volume ratio.

Peel thickness:

Fruits with thin peel lose more water. Higher peel permeability leads to a higher rate of water loss and a faster ripening rate. Also, fruits with thick peel, for example melons, withstand damage better than fruits with thin peel, such as tomatoes.

Stomatal density:

A higher density of stomata may cause a higher rate of water loss, which accelerates ripening. Studies show that the french plantain cultivar Obino l'Ewai has a more dense arrangement of stomata than other cultivars.

Biotic factors:

Biotic stress includes attack from pests and diseases. Fungi, bacteria, viruses, and insects also account for a considerable proportion of total postharvest loss. Pests and diseases reduce both ripening period and overall quality. However, attack by pests and disease is often secondary because a pest exploits a damaged area of the fruit. Careful fruit handling often prevents such attacks.

Different methods of fruit ripening:

The ethylene plays important role in the ripening of the fruits. The most suitable condition for the ripening of fruit are; temperature: 18º to 25º C, relative humidity: 90 to 95 per cent, ethylene gas concentration: 10 to 100 ppm, duration of treatment: 24 to 72hr and CO_2 level should be less than 0.1ppm. These all parameters vary with the maturity index of the fruits. In all the methods of fruit ripening, ethylene is the main gas. The danger of explosion from the ethylene gas by spark or ignition of flame is always there, as it is the property of ethylene. But it could be eliminated by taking proper care in injection and fumigation of gas. Following methods are popular for the fruit ripening process.

Gas injection line in 'Shot' system

'Shot' system:

In this system, measured quantities of ethylene as gas is introduce into the room at regular intervals from ethylene-cylinder. The 'shot' may be applied by weight or by flow, using a gauge that registers the discharge of ethylene in cubic meter per minute. The required ethylene application is made by adjusting the regulator to give an appropriate flow rate, then timing the delivery of the gas. It is customary to apply a shot of ethylene twice each day. The room should be well ventilated before each new application, particularly if it is well sealed, by opening the room door for about half an hour. In large ripening room ventilation room should be provided.

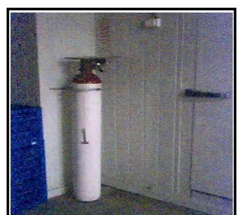
Trickle system:

C_2H_4 Gas analyzer-cum-controller

The ethylene as gas is introduced into the room continuously, rather than intermittently from ethylene-cylinder. As the flow of ethylene is very small, it has to regulate by reducing the pressure using a two-stage regulator and passing the gas into the room through a metering valve and flow meter. To prevent a buildup of CO_2, fresh air is drawn into the ripening room at a rate sufficient to ensure a change of air every 6 hours through small exhaust port. The ethylene bubbles pass through the water trap of a 'sight glass' on the way to the ripening room.

Explosion free ethylene mixture system:

The explosive C_2H_4 gas is mixed with non-ignited gases; and then mixture is injected in the ripening room using either 'shot' or trickle system. 6 –10 per cent ethylene gas concentration in CO_2 by weight is the most popular mixture as commercial ripening agent. The calculation of CO_2 is critical in this method as elevated CO_2 level may generate ripening disorder like; blackening or watery flesh.



C_2H_4 Gas cylinder

C_2H_4 Generator

Ethylene generator system:

In this system, ethylene is generated by heating a liquid in presence of a catalyst. The generator is placed inside ripening room with roper care of electrical insulation. Ethanol as liquid is dripped at the rate of 1pint per 8 hours in to the generator from a bottle. A heated platinized asbestos catalyst and the ethanol delivers about 12 – 16 liter of ethylene gas per hour. In another case, ethylene can be produced in the laboratory by heating absolute ethanol with concentrated sulfuric acid.

$$C_2H_5OH \xrightarrow{\text{Heated platinized asbestos}} C_2H_4 + H_2O$$

Ethaphone solution dipping:

Ethephon ($C_2H_6ClO_3P$) is the most widely used plant growth regulator. The toxicity of ethephon is actually very low, because any ethephon used on the plant is converted very quickly to ethylene. Ethaphon (2-chloroethane phosphonic acid) is strongly acidic in water solution. When the solution pH is above 5, the ethephon molecule spontaneously hydrolyses, and liberating ethylene. Ethaphone (Commercially available as Ethrel, Florel, Cepa) is registered for inducing ripening. The ethaphone solution has to apply to the fruit in water solution as a spray or as a dip, which may include extra step in handling with attendant dangers of microbial infection. In this method for fruit ripening, no extra capital investment is needed provided, the ambient temperature should within the range of ripening range (Burg and Burg, 1962). On small scale, commercially the 'shot' treatment could be generated in a close ripening room using ethephon solution. In this system, take approximately 200ml of active ingredient into in a stainless steel bowl, then, just before closing the door, add 85g of caustic soda pellets to completely neutralize the ethephon. It will generate 27 litre ethylene gas in one 'shot'. Here as a safety step, protective wears are recommended while handling the treatment.

Ethephone solution for 'shot' treatment

Calcium carbide:

Calcium carbide (Ca_2C), a grayish solid, is readily produced by heating calcium oxide with charcoal under reducing conditions. When hydrolyzed, calcium carbide produces acetylene, containing trace amount of ethylene and that are sufficient to be used in fruit ripening. Calcium carbide is used for ripening the fruit artificially in some countries. Industrial-grade calcium carbide may contain traces of arsenic and phosphorus, and, thus, use of this chemical for this purpose is illegal in most countries; included India. Rule 44-AA of the Prevention of Food Adulteration Rules, 1955 prohibits the use of carbide gas for ripening of fruits. Acetylene is believed to affect the nervous system by reducing oxygen supply to brain. Presence of acetylene in the closed room, fruit box and treatment chamber can be detected by keeping filter paper strips dipped in reagent solution (1:2 mixture of 1.5 g of cupric chloride and 3 g of ammonium chloride in 20 ml concentrated ammonia, dilute to 50 ml with water and 5 g of hydroxylamine hydrochloride in 50 ml water). Turning the colour of filter paper strip to red brown or brown violet indicates the presence of acetylene.

Recommendation for ripening conditions for some fruits:

Banana:

Recommended ripening temperature for babana pulp was 18-21ºC using ethylene at 10ppm (10 µl litre $^{-1}$) for 24 hours in 85-90 per cent Rh (Wills *et al.* 1998).

Mango:

Fruit ripened at 19-21°C gave better quality characteristics than those ripened at 8-30ºC(*Singh and Mathur, 1952*). Mango ripening at 15.5-18.5ºC was satisfactory but the fruit were a little tart and required up to 3 days' subsequent exposure to 21-24ºC to develop good flavour (*Hatton et al., 1965*). The recommended ripening temperatures for mango pulp were 29-31ºC using ethylene at 10ppm for 24 hours in 85-90 per cent Rh (Wills *et al* 1998). The ripening temperature varies with variety of mango.

Melon:

Recommended ripening temperatures for Honedue melon pulp were 18-21ºC using ethylene at 10ppm for 24 hours in 85-90 per cent Rh (Wills *et al.,* 1998). For most of the muskmelon cultivars since they are 'adequately self ripening'; except temperature, ethylene dose not required (*Pratt, 1971*).

Papaya:

Optimum ripening was achieved with papaya pulp temperature at 21-27ºC and no ethylene dose (Wills *et al.,* 1998).

Guava:

For the best flavour, allow fruit to ripen on the tree. The picked green-mature and allowed to ripen off the tree at room temperature of 21-27ºC. Placing the fruit in a brown paper bag with a banana or apple will hasten ripening (Morton, 1987). Recommended ripening temperature for mature green guava fruit pulp is 15-20ºC with 90-95 per cent Rh and 100ppm dose for 24-48 hours (Kader, 1996).

Conclusion:

Ripening is natural process in the fruits' life, which could be accelerated by ethylene gas. For better ripening of any fruits; maturity stage, ethylene concentration and storage environment plays important role. 'Shot' and 'Trickle' systems are not only high-tech and accurate, but also generate good quality ripen fruits compared to other methods. Calcium carbide (Ca_2C) should not be used for the ripening of fruits as this is punishable under PFA-1955, Rule nor 44-AA.

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