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Accelerated ripening of fruit-its positive and negative aspects: A Review

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Introduction

Tissue softening, color changes, the development of volatile flavors and aromas, a decrease in astringency, and many other biochemical changes are all part of the genetically programmed, highly coordinated, irreversible phenomenon known as ripening. The life of a fruit can be divided into three phases: fruit set, fruit development and fruit ripening. Fruit ripening is the initiation of fruit senescence which is a genetically programmed highly coordinated process of organ transformation from unripe to ripe stage to yield an attractive edible fruit. A number of biochemical, physiological, and organoleptic alterations are involved in this irreversible event. These changes include changes in carbohydrate content, increment of sugar content, changes in colour, texture, aroma volatiles, flavour compounds, phenolic compounds and organic acids. Respiration is a process of breakdown of complex material in cells to simpler molecules giving energy and some specific molecules which are used in different cellular reactions. Thus respiration is a good indicator of cellular metabolic activity and respiratory pattern is characteristic of the stages in the life cycle of a fruit such as development, ripening and senescence. Fruit ripening is closely linked to ethylene, a phyto-hormone that can trigger initiation of ripening and senescence. Based on regulatory mechanisms leading to fruit ripening, fruits can be divided into two groups: climacteric and non-climacteric fruits (Maduwanthi and Marapana, 2019).

In climacteric fruits, as ripening proceeds there is a strong respiratory peak with high level of ethylene production. As a result, climacteric fruits are known as ethylene-dependent fruits because they can ripen after harvest, frequently with the aid of exogenous ethylene. Apple, Banana, Mango, Tomato etc. are considered as climacteric fruits (Maduwanthi and Marapana, 2019).

In non-climacteric fruits respiration rate is almost constant or shows a steady decline until senescence occurs, with little or no increase in ethylene production. Non-climacteric fruits are said to only ripen if they stay linked to their parent plant. Grapes, Pineapple, Watermelon etc. are considered as non-climacteric fruits (Maduwanthi and Marapana, 2019).

Ripening Process

The ethylene biosynthesis pathway in higher plants has been well studied. Briefly, ethylene is synthesized from methionine. First methionine is converted to S-adenosyl-L-methionine (SAM) which is catalyzed by SAM synthetase. Next, 1-aminocyclopropane-1-carboxylic acid (ACC) is produced from SAM. This conversion is catalyzed by the enzyme ACC synthase and then ACC is converted to ethylene catalyzed by ACC oxidase. Methionine is regenerated from ACC through the Yang cycle (Maduwanthi and Marapana, 2019).

Changes during Ripening

Ripening causes structural and compositional changes that make the fruit edible and appealing. Among these changes, textural change is very important and a major event in fruit ripening. Some fruits, like bananas, mangos, and papayas, experience significant softening, whereas other fruits, like apples, often show less softening. These textural changes vary by species. Textural changes and fruit softening are due to de-polymerization and solubilization of cell wall components and loss of cell structure. Fruit softening is determined by changes in turgor pressure, the breakdown of cell wall polysaccharides, and the enzymatic breakdown of starch. Cell wall polysaccharides such as pectin, cellulose, and hemicellulose undergo solubilization, de-esterification and de-polymerization during ripening. Cell wall degrading enzymes, such as pectinmethylesterase, polygalacturonase, β-galactosidase, endo-1,4-β-d-glucanase and many others are involved in this mechanism. Cell wall alterations also involve additional loss of neutral sugars and galacturonic acid, followed by solubilization of the residual sugar residues and oligosaccharides. According to gene expression analysis it has been revealed that ethylene directly regulates the transcription of both a softening-related PpPG gene that encodes α-L-arabino furanosidase / β-xylosidase (PpARF/XYL) and an expansin (PpExp3). Enzymatic hydrolysis of starch plays a significant role in the softening of some fruits, including bananas, which have a high starch content in their flesh. In citrus fruit, softening is mainly associated with change in turgor pressure, a process associated with the post-harvest dehydration and/or loss of dry matter (Maduwanthi and Marapana, 2019).

For many fruits, color development is a crucial indicator of maturity and is linked to ripening. The unmasking of preexisting colors through the breakdown of chlorophylls and the synthesis of anthocyanins and carotenoids is frequently the cause of the color shift that occurs during fruit ripening. The tomato plant has been used as a model to study carotenoid production during ripening. Carotenoids are derived from terpenoids and are synthesized in fruit at a high rate during the transition from chloroplast to chromoplast. Anthocyanins are responsible for orange, red, pink, blue and purple colours in fruits and can be classified into two groups as flavonoids and phenolic compounds. They are synthesized in the cytosol and localised in vacuoles and synthesized via the phenyl propanoid pathway. Anthocyanin biosynthesis requires two groups of genes: structural genes that encode the enzymes directly involved in the production of anthocyanins and other flavonoids, and regulatory genes that regulate the transcription of structural genes. According to reports, anthocyanin biosynthesis-related genes are regulated by ethylene (Maduwanthi and Marapana, 2019).

Numerous fruits release volatile chemicals that give them their unique flavor and scent. Fruit aroma volatiles are produced by the metabolism of branched amino acids and fatty acids. Esters, alcohols, aldehydes, ketones, and terpenes make up the majority of fruits' volatile flavor and aroma profiles. The relationship between volatile synthesis and ethylene generation has been the subject of numerous investigations. Mangoes and honeydew melons can produce more volatile aromas when treated with ethylene. When ethylene biosynthesis is blocked using aminoethoxyvinylglycine (AVG) or 1-methylcyclopropane (1MCP), additional aroma generation is decreased, suggesting a connection between fragrance synthesis and ethylene production and action in fruits (Maduwanthi and Marapana, 2019).

Astringency, which is caused by tannins in fruits, tends to decrease as numerous fruits ripen. According to reports, the molecular structure of tannin, which controls cross-linking with proteins and glycoproteins, influences astringency. As a result, when tannins breakdown in saliva, they produce an astringent flavor. Because tannins are insoluble, ripening increases their molecular weight through polymerization, which results in a lack of astringency (Maduwanthi and Marapana, 2019).

Accelerated Ripening

It is the process by which ripening is controlled and product may be achieved as per requirement by controlling the different parameters. It is done to achieve faster and more uniform ripening. Generally 80% fruits are ripened artificially through ripening agents.

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Accelerated Ripeners

The chemicals or agents which are used to ripe fruits artificially are known as accelerated ripeners. Farmers, transporters, and traders use these for pre-harvest, post-harvest, transportation, and storage. This speeds up ripening and causes color changes. It is not appropriate to transport and distribute ripped fruits. Thus, producers harvest hard, green, unripe crops and use chemicals to ripen them.

6) Accelerated Ripening Methods

- Ethylene Gas
- Acetylene
- Ethephon / Ethrel
- Ethylene Glycol

Ethylene Gas

The most popular method practiced in developed countries is application of ethylene gas in ripening rooms. In addition to having adequate ventilation and exhausting systems, modern banana ripening rooms are designed with methods to regulate temperature, humidity, and ethylene gas concentration. Banana combs are properly packed and kept in these rooms and then ethylene is supplied at proper temperature and humidity. Mostly "catalytic generators" are used to generate ethylene in commercial ripening rooms. The concentration of ethylene required for different commodities to enhance ripening is different (Maduwanthi and Marapana, 2019).

However early in the banana history many researchers tested ethylene as an induced ripening agent. Von Loesecke (1950), commented that some scientists showed that ripening of bananas can be induced by exposing them to vapour of apples, which was confirmed when it was shown that the vapor that has already passed through ripe bananas can be used to speed up the ripening process. Ultimately, it was determined that exogenous ethylene treatment can cause bananas to ripen by increasing their respiration rate and endogenous ethylene levels. Burg and Burg (1962), reported that low ethylene concentration as 0.1 ppm is effective in accelerating the ripening of bananas. Dominguez and Vendrell (1994) showed that exogenous ethylene, 100ppm treatment for 12 hours, can immediately increase the endogenous ethylene and CO2 production similar to respiratory climacteric. Additionally, this study demonstrated that the duration of treatment affects the increase in respiration, with 12-hour treatment being marginally more effective than 6-hour treatment. Lohani (2004), noted 11-fold decrease in fruit firmness which occurred within two days after exogenous ethylene treatment.

Additionally, it was noted that ethylene treatment increased the activity of four cell wall hydrolases in "Dwarf Cavendish" bananas: cellulose, pectin methyl esterase, polygalacturonase, and pectate lyase.

The highly sensitive technique of laser photo-acoustic spectroscopy coupled with the development of specific apparatus to determine in plant ethylene production in fruits and flowers of strawberry during development and ripening has revealed an increase in ethylene production and a concomitant rise in respiration rate in red ripe strawberry fruits (Iannetta *et al.* 2006). Additionally, studies using the ethylene action inhibitor silver thiosulfate showed that a positive feedback mechanism in ripe fruits controlled this increased ethylene production, indicating that a type of autocatalytic ethylene production is active during strawberry ripening. The patterns of ethylene production usually linked to the ripening of crops like tomatoes are different from the timing of this ripening-related rise in ethylene production. For example, ethylene production increases at the onset of ripening in tomato, and ripening is severely disrupted in transgenic fruit where this phenomenon is blocked (Oeller *et al.*, 1991). On the other hand, the increase in strawberries was not noticed until 24 hours after the fruits had fully turned red. Therefore, it is yet unknown what physiological function the enhanced ethylene synthesis in strawberries serves. However, a growing body of molecular information about ethylene-related alterations in gene expression is supporting a function for ethylene in strawberry ripening. For example, increased expression of cystathionine-gamma-synthase (CGS) and ACC oxidase genes has been reported during ripening of strawberry (Aharoni *et al.*, 2002; Marty *et al.*, 2000; Trainotti *et al.*, 2005). Similarly, increased expression of ethylene receptor homologs in strawberry fruit was also observed with increasing ripeness. In particular, increased expression of FaETR2, a type II receptor homolog that is closely related to the ripening-related LeETR4, was associated with ripening (Trainotti *et al.*, 2005). In addition, the isolation and characterization of a peptide-methionine- sulfoxide-reductase (PMSR) gene that is expressed late in strawberry ripening were also recently described (Pedraza-Lopez *et al.*, 2006). This gene is homologous to the tomato ripening-related gene E4, whose expression is regulated by ethylene in tomato and may be involved in the methionine salvage pathway that operates during increased ethylene synthesis (Lincoln *et al.*, 1987). Examining the connection between ethylene and the expression of FaPMSR and other genes whose expression is induced at late stages of strawberry fruit ripening may be relevant, even though it has not yet been tested.

The emerging data could be consistent with either a regulatory role for ethylene in as-yet defined aspects of ripening in strawberry fruit or a response to the dramatic cellular changes associated with ripening and the concomitant loss in cellular integrity and senescence characteristic of this developmental process. Both answers would be intriguing because the former would show that ethylene plays a basic role in the ripening of most, if not all, fruits, while the latter might indicate a more primitive developmental response that may have been used by evolution as a signaling system to accelerate the ripening process in a subset of species. The creation of transgenic strawberry with reduced ethylene responsiveness, possibly by expression of a dominant mutant allele of an ethylene receptor, may help clarify this role. Small but significant increases in ethylene synthesis at the onset of ripening have also been detected in grape berries. Chervin *et al.*, (2004), demonstrated the presence of a transient peak of ethylene production in grapes just prior to the onset of ripening, and experiments with 1-MCP indicated that ethylene was required at this stage for the onset of anthocyanin accumulation, fruit swelling, and the decrease in acidity that is associated with ripening. Concomitant with an ethylene-stimulated rise in anthocyanin production, the abundance of four transcripts encoding enzymes involved in anthocyanin synthesis also increased following ethylene treatment (El-Kereamy *et al.,* 2003). In an additional study, 1-MCP treatment of grape berries was found to partially repress the ripening induced expression of the VvADH2 gene that encodes an alcohol dehydrogenase (Tesniere *et al.,* 2004). These studies suggest that ethylene may influence multiple aspects of ripening in grape. The significance of ethylene in grape berry ripening could be clarified using a similar approach as previously mentioned for strawberries. Citrus is also classified as a non-climacteric fruit, but studies with inhibitors of ethylene action revealed that ripening-related color changes in the flavedo are regulated by endogenous ethylene and that ethylene treatments can stimulate both chlorophyll breakdown and carotenoid accumulation (Goldschmidt *et al.,* 1993; Purvis and Barmore 1981; Stewart and Wheaton 1972). Furthermore, studies have shown some genes, including chlorophyllase, to be ethylene regulated in citrus fruit (Alonso *et al.,* 1995; Jacob-Wilk *et al.,* 1999). Recently, as in the case of strawberry, autocatalytic ethylene production has also been described in citrus fruit, but again with altered timing relative to typical ripening climacteric fruit. Whereas mature fruits exhibited no increased ethylene production associatedwith ripening, harvested immature fruits produce high levels of ethylene that can be further stimulated by ethylene and propylene treatments and inhibited by 1-MCP, suggesting that this event is autocatalytic (Katz *et al.,* 2004). This study unequivocally demonstrated that citrus fruits are capable of producing autocatalytic ethylene, even though this phenomenon may not have any bearing on the ripening process. To make sure that resources are available to support the development of mature fruits, citrus and other fruit trees go through a continuous process of fruit drop or selective abscission. Young citrus fruitlets' climacteric behavior might be related to this abscission process, or it might be the outcome of stress during detachment, as was shown in persimmon fruit.

Cost, convenience, and safety considerations determine the method used to apply ethylene. Use of diluted ethylene gas mixtures is safer than using pure ethylene, which is explosive and flammable at concentrations of 3% or higher. Fruit to be ripened ideally is placed in an airtight ripening room maintained at a constant temperature (18-21oC for most fruits, but 29-31oC in mango). Optimum storage and ripening temperatures for a few fruits are given below Table 1.

Table 1.: Optimum storage and ripening temperatures for a few fruits

Commodity	Ethylene conc.(ppm)	Ethylene exposure time (hr.)	Ripening temp. oC	Storage Temp. oC
Avocado	10-100	12-48	15-18	4.4-13
Banana	100-150	24	15-18	13-14
Honey dew melon	100-150	18-24	20-25	7-10
Kiwifruit	10-100	12-24	0-20	0.5-1
Mango	100-150	12-24	20-22	13-14
Orange de-greening	1-10	24-72	20-22	5-9
Stone fruit	10-100	12-72	13-25	-0.5-0

(Post-Harvest Technology of Horticultural Crops: Vol.07. Horticulture Science)

6.1.1) Positive aspects:

- Used for most of climacteric fruits.
- Does not pose any health hazard for consumers of fruits.
- It is used as de-greening agent in citrus fruits and bananas.
- High penetration power.
- Used to ripen the fruits when they are mature and not ripe and as a result these mature fruits are less likely to be damaged when transported.

6.1.2) Negative aspects:

- Unwanted fruit ripening and softening during storage.
- If inhaled highly, can cause drowsiness, unconsciousness.
- Accelerated senescence and loss of green colour in immature fruits.
- Sprouting (stimulation or retardation).

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Acetylene

Calcium carbide when hydrolysed produces acetylene which is an ethylene analogue. Although it is forbidden by official rules, calcium carbide is frequently used to artificially ripen bananas, particularly in poor nations like Sri Lanka. Hartshorn (1931), conducted a series of experiments to identify effects of acetylene on ripening process of bananas. This study demonstrated that acetylene released by calcium carbide can accelerate the ripening of bananas since, after 120 hours, treated fruits were consistently yellow, had a decent flavor, a medium starch content, and a somewhat soft texture, but control samples remained unripe. According to Burg and Burg (1967), acetylene has a lower biological activity than ethylene and it was reported in this study that concentration of acetylene should be 2.8 ml/L to enhance ripening of bananas. In contrast to bananas ripened by exposure to ethylene at the same concentration, Thompson and Seymour (1982) found that bananas do not react to acetylene at 0.01 ml/L and that treatment with acetylene at 1 ml/L resulted in indistinguishable color and soluble solids content. Further, it was shown in this study that there is no significant difference in sensory attributes between bananas treated with ethylene and acetylene at 1ml/L when they are compared at same stage of ripeness.

However, according to Section 26 of the food (labelling and miscellaneous) rule of 1993, calcium carbide is not widely regarded as safe and is forbidden in Sri Lanka as it is in the majority of other nations. Calcium carbide is considered as hazardous due to several reasons. Commercial calcium carbide contains traces of arsenic and phosphorous hydride and acetylene emitted from commercial calcium may also contain up to 3 ppm arsenic and up to 95 ppm phosphorous hydride. Arsenic and phosphorous hydride can be poisonous to humans and cause vomiting, diarrhoea with or without blood, burning sensation of the chest and abdomen, thirst, weakness, swallowing difficulties, skin and eye irritation or burning, long-term eye damage, and so on.Exposure to acetylene gas can cause headache, vertigo, dizziness, delirium, seizure and even coma (Maduwanthi and Marapana, 2019).

Positive aspects:

- Accelerates ripening of climacteric fruits with uniform colour, good flavour and required texture.
- Indistinguishable colour and soluble solid content as when compared with that of ethylene.
- No significant difference in sensory attributes as compared to that of ethylene ripening.

Negative aspects:

- Hazardous to health.
- Not effective as ethylene.
- Contains traces of Arsenic and Phosphorous hydride up to 3 & 95ppm respectively.
- Can cause vomiting, diarrhoea, burning sensation to chest, abdomen, eyes and skin.
- Also causes headache, dizziness, delirium, vertigo and even coma.

Ethephon / Ethrel:

Ethephon (2-chloroethylphosphonic acid), an ethylene releasing compound, is categorized as non-carcinogenic to humans by IARC (International Agency for Research on Cancer). It penetrates into the fruit and decomposes to ethylene and has been shown to hasten ripening of several fruits including bananas, apples, tomatoes, mango, peaches, citrus fruits and guava. Pendharkar (2011), treated bananas with different concentrations of ethephon. Here, it was shown that various ethephon concentrations had a substantial impact on the chemical changes that occur during ripening; the optimal ethephon concentration for early ripening was determined to be 1000 ppm. Ethephon (500 mg/L) prestorage treatments of mangoes (Mangifera indica L., Cv. Kensington Pride) increased TSS, TSS/acid ratio, and sugars and decreased chilling harm, according to research by Nair and Singh (2003). Adane (2015), compared ethephon treatment and traditional kerosene smoke treatment and their effect on ripening of “Cavendish” bananas, where it was shown that ethephon treated fruits demonstrated higher sensory quality. Apart from being used to initiate ripening, ethephon has been recorded as plant growth regulator which can be used to increase fruit size, induce flowering, enhance colour and induce flower abscission.

According to reports, studies on ethephon's health have indicated that it may be hepatotoxic. Ethephon is an organophosphorus compound and it has been reported to get rapidly absorbed in the gut. There is a possibility of converting ethephon into ethylene oxide, then to ethanediol and hydroxyethyl-glutathione and mercapturic acid. Further it has been studied that it can inhibit the growth Streptomyces and their antibiotic production (Maduwanthi and Marapana, 2019).

6.3.1) Positive aspects:

More acceptability, more shelf life and less harmful.

- It is also been recorded as plant growth regulator which can be used to increase fruit size, induce flowering, enhance colour and induce flower abscission.

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- Rapidly absorbed in the guts.
- Inhibits the growth of Streptomyces and their antibiotic production.
- More acceptable colour appearance than naturally ripened.

Negative aspects:

- Salivation, lacrimation, incoordination, and twitching of the muscles are indications of organophosphate intoxication.
- The most sensitive indicator is the inhibition of red blood cells and cholinesterase inhibition.
- Respiratory depression, tightness in chest, fluid in lungs, dark vision.
- In severe cases: seizures, incontinence, respiratory depression, loss of consciousness.

Ethylene Glycol

Ethylene glycol is C2H6O2 and commonly used as a coolant and antifreeze. Goonatilake (2008), experiments on the effectiveness of ethylene glycol as a fruit ripening agent have reported that when diluted with water, various fruits will ripen faster in colder climactic conditions. According to Stahler and Pont (1962), bananas can be artificially ripened by using alkyl alcohol containing between 6 and 14 carbon atoms. Further, it has been reported, in this patent, that lauryl alcohol is preferred to ripen green bananas and treatment with 0.01% by the weight of bananas can change bananas can become entirely yellow in 48 hours without losing any of its flavor.

Smoking is one of the customary practices in Sri Lanka and many other nations. In Sri Lankan traditional practice, bananas are laid in a pit, covered with banana leaves or a sheet cover and smoke, generated from burning of semidried leaves, is directed into the pit. In certain nations, smoke is produced during the commercial ripening of bananas using kerosene burners. Because smoke contains acetylene, ethylene, and other unsaturated chemicals that might promote ripening, it is known to do so (Goonatilake, 2008).

Background: Ethylene glycol is colorless, odorless and sweet tasting liquid which is found in everyday materials which are used in the present day, such as coolant, antifreeze, etc. $C_2H_6O_2$ is the molecular formula for ethylene glycol. Additionally, it is far less expensive than ethylene gas. at the United States, a single large bottle typically costs between fifteen and nineteen dollars at most home stores. The fact that ethylene glycol can be diluted with water is another factor contributing to its low cost. Most nations receive free water, and a single

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large bottle of ethylene glycol can be used as a ripening agent for a whole farm if it is diluted quite a bit (Goonatilake, 2008).

However, there is just one issue with ethylene glycol. In actuality, it is a very significant issue. There is proof that ethylene glycol is toxic when swallowed. Additionally, it has been demonstrated that consuming large quantities of this ethylene glycol agent might result in renal failure (Goonatilake, 2008).

One part ethylene glycol to four parts water (20% ethylene glycol and 80% water) is used to combine the ethylene glycol with the water (Goonatilake, 2008).

Positive aspects:

- It is very inexpensive as when compared with ethylene gas.
- It can be diluted in water.
- It can be used to ripe fruits in colder climatic environments.

Negative aspects:

- The main disadvantage of ethylene glycol is if swallowed, it is poisonous.
- The swallowing of liberal amounts can also cause the Kidney failures.

Conclusion

- Artificial ripeners are applied to fruits to accelerate their ripening. The usage of ripeners such as calcium carbide and ethephon needs to be closely monitored and controlled due to its dangerous effects.
- The government is not the only one responsible for it.
- The people must become aware and avoid consuming contaminated fruits.

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