



# Conventional and Biotechnological Approaches for Enhancing the Shelf-life of Fruits and Vegetables: A Review

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## ABSTRACT

Due to highly perishable nature and less shelf-life, post-harvest losses of fruit and vegetables results in high gap between production and availability. Various plants traits which need to be genetically modified for higher shelf-life includes lowered rate of respiration and ethylene production, less sensitivity to ethylene, lowering ripening rate, reduced browning, decreased chilling sensitivity and increased postharvest disease resistance. The importance of understanding the biochemical process of softening and the use of such information for retarding the ripening process has been demonstrated in this paper. After reviewing the development made in extending the shelf-life of fruits, it becomes evident that although success in this field has been inadequate, there are possibilities that fruit breeders will succeed in near future in evolving superior cultivars with longer shelf-life.

**Key words:** Biotechnological, Conventional, Horticultural crops, Post-harvest, Shelf-life.

Twenty years ago, consumers only ate fruits and vegetables that was “in season”. Because there were no proper technologies to store the fruits for longer period. It is estimated that around 35-40% of all fresh fruits and vegetables are lost due to excessive softening, but the exact figure is hard to determine (Meli *et al.* 2010). Fruits and vegetables need to be stored as they have short post-harvest shelf-life. Shelf-life of fruits and vegetables means how long they will last. Shelf-life of fruit is directly related to ripening process. Ripening is an irreversible process which makes the fruits edible and constitutes, physiologically and commercially, the most significant phase in their life. As ripen, fruits become very soft and more prone to injuries, which make them highly perishable. Physiologists and biochemists attempted to extend the shelf-life of fruits by different means though the results were not satisfying. Pre-harvest factors and post-harvest treatments both contribute to shelf-life of fruits and vegetables. Earlier various treatments were given to fruits and vegetables to enhance the shelf-life. Now a day, biotechnological techniques also used for enhancing the shelf-life of horticultural produce. As we know main hormone responsible for the ripening is ethylene. Methionine is precursor of ethylene responsible for ripening of the fruits. Methionine is converted into S-adenosyl methionine (SAM) by adometsynthetaseenzyme. SAM is further converted into 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase enzyme and into methylthioadenosine. Now methylthioadenosine is converted into methionine again which continue the synthesis of ethylene. ACC is converted into ethylene by ACC oxidase enzyme (Abano and Buah, 2014).

## Effect of ethylene on fruit

Ethylene affects quality of fruit by different ways. Acids are neutralized by kinase enzymes. Starch is converted into sugar by amylase enzyme. Chlorophyll is degraded by

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hydrolase enzyme. Pectin which is present in hard form first is converted into softer pectin by pectinase enzyme. Large molecules lead to produce increased fragrance by hydrolase enzymes. Means ripening of fruits is coordinated by function of various enzymes. All these activities of ethylene in ripening process makes fruit edible and leads to perishability of the fruit (Shankar, 2016).

## Conventional approaches

### Breeding methods recommended for improving Shelf-life of fruits and vegetables

#### Domestication

It involve bring out the wild plant species under human management. It is observe that domestication of fruits involves a combination of genetic events including an increase in storage and shelf-life as compared to their wild counterparts (Singh and Singh, 2011).

#### Introduction

Plant introduction consists of taking a genotype or a group of genotypes of plants into a new area or region where they were not being grown before. It is of two types:

**a) Primary introduction:** When the introduced variety is well adapted to the new environment, it is released from commercial cultivation without any alternation in its genotype, it constitutes primary introduction.

**b) Secondary introduction:** The introduced variety may be subjected to selection or used in a hybridization programme to develop a superior variety, it constitutes secondary introduction.

Apple var. Jonathan, Early Grande Peach, Kinnow mandarin, Solo papaya *etc* are good examples as they all have better shelf-life.

#### **Chance seedlings**

A chance seedling is the name given to a fruit cultivation discovered by chance seedling without any sustained breeding efforts. In fact, chance seedlings are superior naturally occurring variety in a plant population. Mango var. Alphonso, Banganpalli, Dashehari, Ambri apple and Guava variety Apple colour and Harijha are originated as chance seedlings having higher shelf-life.

#### **Selection**

Selection is basic to plants for longer shelf-life. It is the oldest procedure in which the individual plant or group of plants are sorted out from mixed population, thus eliminating undesirable one's.

#### **Hybridization**

Mating or crossing of two plants or lines of dissimilar genotype is known as hybridization. The main objective of hybridization is to create genetic variations for better fruit quality and better shelf-life of fruits. In certain cases, hybrids show higher shelf-life and better fruit quality traits than parent.

#### **Polyploids**

It is an important approach to confer desirable characteristics in some fruits. Sunny Rouge, a new early ripening and tetraploid grape (*Vitis labruscana* × *Vitis vinifera*) was obtained by crossing Pione × Red Pearl. It has tolerance to major pre and post-harvest diseases and exhibits good shelf-life. Dark Ridge, a new tetraploid grape obtained from cross between Kyoho and 301-1 (Kyoho × Niabell). Dark Ridge fruits rarely crack and have good keeping quality (Singh and Singh, 2011).

#### **Mutation breeding**

Spontaneous and induced mutations may results into potentially novel genotypes with considerably higher shelf-life. Mutants with increased sugar content and extended shelf-life have been reported in pears.

Castel Gala is a low chilling apple mutant with very early fruit ripening. Although their fruits ripe much earlier than those of Gala, fruits of both cultivars have almost similar storage characteristics and shelf-life (Singh and Singh, 2011).

## **TWO TYPES OF FACTORS INFLUENCE THE SHELF-LIFE OF FRUITS AND VEGETABLES**

### **Pre-harvest factors**

#### **Environmental factors**

##### **Temperature**

During fruit development, high temperature can affect photosynthesis, respiration, aqueous relations, membrane stability as well as level of plant hormones, primary and secondary metabolites which affect the quality as well as post-harvest life of fruit. Warm days and cool nights during growth are necessary for the full development of color at the ripe stage (Asrey and Barman, 2011).

##### **Sunlight**

Fruits which are exposed to sun are having thinner peel, higher soluble solid content, lower in acidity than those fruits which are grown in shade inside the canopy. Further, the intensity and duration of light affect the quality of fruit after harvest.

##### **Wind**

It may cause damage to the fruits. Mild wind velocity causes wind scarring disorder when the fruits are rubbed against twigs. This leads to poor keeping quality of the fruits.

##### **Frost and hail damage**

Hail damage is sporadic but worldwide more extensive than it is generally thought and it directly affect physical quality of horticultural produce and also increases the incidence of diseases.

#### **Cultural factors**

##### **Mineral nutrition**

Excess or deficiency of certain elements can affect shelf-life of fruits. High levels of nitrogen lead to poor keeping quality of fruit. Phosphorus level in soil and plant does not have much effect on internal fruit quality but it certainly affects the fruit appearance also. Calcium sprays have beneficial effects. Iron and zinc deficiency results into reduced fruit size and poor color development (Karemera *et al.* 2014).

##### **Organic production**

Market for organically produced food is increasing. Organic production excludes the use of synthetic pesticides and fertilizers and allows use of animal and green manure, compost and botanical insecticides *etc*. There is conflicting information on the effects of organic production of fruits on their post-harvest characteristics but mainly organically produced fruits have poor keeping quality.

##### **Irrigation**

Careful manipulation of water supply may well decrease water usage and improve fruit quality without compromising sustainable plant growth.

### Pruning, thinning and girdling

Pruning can improve the light penetration into the canopy of the fruit tree. Fruitlet thinning increases size of fruit but reduces the yield. So, a balance between fruit size and yield must be maintained. Girdling has been found beneficial in grape and jackfruit. Heavy pruning of Kinnow improved yield as well as quality (Ahmad *et al.* 2006).

### Rootstock

Fruit trees grafted on to rootstocks for different reasons. 'Jonagold' apples grown on rootstock PB-4 and M-26 showed lower ethylene production and delayed ripening during storage (Tomala *et al.* 2010).

### Tree age

'Aroma' apples produced on young trees was higher in acid/SSC ratio, better coloration and flavor quality as compared to old trees. But, fruit produced in old trees were more firm, and better storage potential (Tahir *et al.* 2007).

### Canopy position

It also affects the quality of the fruit. Fruits which grow inside canopy remain green whereas fruits outside canopy develop red color.

### Genotype and cultivar

Genotype and cultivar selection are major factors involved in post-harvest quality and shelf-life outcomes of the fruits and vegetables.

### Growth regulators

Pre-harvest spray of GA3 application extended storage life of persimmon fruit by delaying both black spot development and fruit softening when stored at -10°C. It also delayed ripening and climacteric rise of respiration (Eshel *et al.* 2000).

### Post-harvest treatments

These are the treatments which are given to after harvesting which enhances their shelf-life. This includes following treatments:

#### Ionizing radiations

Irradiation has been used for years in food preservation with different applications depending upon the applied dose. Low doses alter physiological processes such as sprouting or life cycle of insect pest and fruit ripening. The dose of ionizing radiation *i.e.* 1.0 to 3.0 kGy extends shelf-life of fresh fish and seafood, fruits and vegetables and 0.5 to 1.0 kGy delays ripening in fresh fruits (Loaharanu, 2007).

#### Heat treatments

Heat treatments are like hot water, hot air and vapour heat treatment. Hot water treatment is mainly used for fungal organisms, vapour heat treatment is used for insects and hot air can be used for both. 14 days of shelf-life was observed in strawberries at 0°C with hot air treatment at 45°C for 3 hr (Vicente *et al.* 2002).

### Edible coatings

These are thin layer of material which can be consumed and provides barrier to moisture, oxygen and solute movement for the food. All these coatings have different advantages and disadvantages. These coatings can be adopted depending upon the need of the fruits *eg.* in strawberries, papayas, apples and pears. Mainly edible coatings are like cellulose derivatives, starch, lipid films, shellac resins, protein films, chitin and chitosan.

### Modified atmosphere packaging

MAP was first recorded in 1927 as an extension of the shelf-life of apples by storing them in atmospheres with reduced O<sub>2</sub> and increased CO<sub>2</sub> concentrations. MAP is a cheap and convenient packaging system that has the capacity to extend shelf-life of fruit if it is used properly (Sandhya, 2011).

### Shrink wrapping of high value temperate fruits for enhancing shelf-life

Fruit	Shelf-life at room temperature (days)		Shelf-life in zero energy cool chamber (days)	
	Unwrapped	Wrapped	Unwrapped	Wrapped
Apple	21	30	35	45
Kiwifruit	9	14	11	20

(Sharma and Pal, 2009).

### Calcium treatments

High calcium content in fruits has been related to longer post-harvest life as a result of reduced rates of respiration and ethylene production. This effect has been attributed to decrease in activity of 1-aminocyclopropane-1-carboxylic acid (ACC).

### Gibberellic acid

The use of Gibberellic acid as anti senescent regulator has been found to enhance the shelf-life in many fruits by their antagonistic effect on ethylene biosynthesis. Gibberellic acid inhibits ripening in fruit by decreasing respiration, delaying anthocyanin synthesis, chlorophyll degradation (Martinez *et al.* 1994).

### Salicylic acid

Application of salicylic acid has been determined to delay ripening in a number of fruits by reducing the activities of major cell wall degrading enzymes like cellulase, polygalacturonase and xylanase and by suppressing ACC synthase and ACC oxidase (Asghari and Aghdam, 2010).

### Polyamines

Ethylene and polyamines exhibits an opposite effect on fruit ripening and senescence, since reduced levels of PAs have been correlated with increased ethylene production, fruit ripening and senescence while high endogenous concentration of PAs associated with delay in these processes.

### 1-methylcyclopropene

The ethylene action inhibitor 1-MCP has become an important tool to maintain the quality of selected fruit in supply chain. Delayed softening and firmness retention are the most notable responses of climacteric fruit to 1-MCP. It was commercially approved on July 2002 in apples, apricots, avocados, kiwifruit, mangoes, nectarine, papayas, peaches, pears, persimmons, plums and tomatoes (Almeida, 2011).

### Biotechnological

#### Need of biotechnology

There was need of biotechnology because one of the most recent methods by which an effective shelf-life extension could be achieved by genetically modified plant whereby expression of gene responsible for ripening could be regulated. Ability to maintain shelf-life of fruits and vegetables during post-harvest storage is highly related to physiological, biochemical and molecular traits of the plants from which they derive. These traits are genetically determined and can be manipulated using genetic breeding and biotechnology. Genetic transformation provides the means for modifying single horticultural traits without altering the phenotype (Abano and Buah, 2014).

#### History: Flavr Savr tomato

The delayed ripening tomato fruit 'Flavr Savr' was first commercially grown genetically engineered food to be approved for human consumption. It was produced by Cal gene, USA in 1992 and was first sold in 1994 and was only available for a few years, there after production of this tomato was stopped because of its high cost and low yield. The Flavr Savr tomato was genetically altered to synthesize an antisense RNA of a ripening related gene polygalacturonase (PG), which is involved in dissolving cell-wall pectins, leading to fruit softening therefore, the fruit stays firm for longer periods (Dias and Ortiz, 2012).

#### Biotechnological techniques being used

##### Antisense RNAs

It means to effectively turning off gene expression of a key enzyme of ripening process by means of its antisense RNA. Antisense RNAs are long stretch of sequences that are complimentary to the sense RNA. They are very specific to the target genes and cause reduction of sense RNA by interfering with transcription and translation process (Surendranathan, 2005). PG-ase is one of the key enzymes involved in ripening-associated softening of fruits. This known to have effect on PG activity which is involved in dissolving pectins and causing softening therefore fruit stay firm for longer period. The cDNA from ACC oxidase gene when expressed in an antisense orientation caused a decrease in ethylene biosynthesis and delayed post-harvest ripening of tomato fruit.

Modification of ethylene biosynthesis through genetic engineering: In this, two types of genes may be used.

### Over expression of ACC deaminase gene

ACC deaminase metabolizes ACC to  $\alpha$ -ketobutyrate. This gene has been isolated from *Pseudomonas* sp. and was expressed in transgenic tomato plants. This approach leads to 90-95% inhibition in ethylene production during ripening reduction in ethylene synthesis does not cause any vegetative phenotypic abnormalities, however fruits from these plants show delay in ripening and they remained firm for at least 6 weeks longer than the non-transgenic control fruits (Klee *et al.* 1991).

### Over expression of SAM hydrolase gene

In this approach gene from bacteriophage T3 which encodes the enzyme s-adenosylmethionine hydrolase (SAMase) has been utilized to generate transgenic tomato plants that produced fruits with a reduced capacity to synthesize ethylene. SAM is the metabolic precursor of 1-aminocyclopropane-1-carboxylic acid, proximal precursor to ethylene. SAMase catalyzes the conversion of SAM to methylthioadenosine and homoserine thereby reducing synthesis of ethylene (Good *et al.* 1994).

### Use of polyamine genes

The common PAs are putrescine, spermidine and spermine. Two pathways of polyamines and ethylene biosynthesis are seen to regulate each other, which is a decisive factor in determining the predominance of either of two pathways. Over expression of yeast spermidine synthase has been found to increase shelf-life in tomato (Nambeesan *et al.* 2010).

### RNAi approach

RNAi, being a novel approach has great potential to modify the gene expression in plants for better quality traits and nutritional improvement in different crops. The post-harvest life can enhance by knocking-out genes responsible for ethylene production in tomato. This was achieved through introducing dsRNA and blocking the gene expression of ACC-oxidase which significantly reduced the ethylene formation and enhanced shelf-life in tomato. Use of n-glycan processing enzymes and cell wall modifying enzymes. Suppression of two ripening specific N-glycoprotein modifying enzymes Alpha-mannosidase and Beta-D-N-acetyl hexosaminidase resulted in down regulation of cell wall degradation and ripening related genes in transgenic fruits. In tomato down regulation of these enzymes resulted in extended shelf-life of fruits (Meli *et al.* 2010).

### VIGS approach

VIGS is a type of gene silencing approach. It has become an important tool in rapid and efficient functional analysis of genes by silencing action brought about by interfering RNA molecules delivered through modified viral infection of plant. In this when virus infect the plant there is dsRNA in virus which is broken into small nucleotides (23 nucleotides) called as siRNA (small interfering RNA) by a dicer present in the

**Engineering quality and delayed ripening of fruits**

Crop	Gene	Function	References
Tomato	ACC deaminase	Ethylene suppression	Chadha <i>et al.</i> 2000
Papaya	Polygalacturonase	Suppressing synthesis and activity of PG	Laurena <i>et al.</i> 2002
	Pectin methyl esterase	Delay in softening of fruit	Chadha <i>et al.</i> 2000
Mango	Endoglucanase	Suppress mango softening	Chourasia <i>et al.</i> 2008
	Alcohol dehydrogenase	Delay in ripening and aroma production	Vasanthaiah <i>et al.</i> 2006
Apple	Polygalacturonase	Suppressing degradation of cell wall	Atkinson <i>et al.</i> 2002
Pear	ACC oxidase	Ethylene suppression	Gao <i>et al.</i> 2007
Strawberry	SAM transferase	Ethylene suppression	Chadha <i>et al.</i> 2000

RISC (RNA induced gene silencing complex) mechanism of host plant. Now the antisense strand of dsRNA is identified by RISC and antisense RNA combines with argonaute protein of RISC and forms a complex. This complex binds with complementary genome in the host cell and silences the gene. VIGS has been used as an alternative approach to transgenic development and it was incorporated to delay ripening in tomato by suppressing the LeACS2, a member of ACC synthase gene by using tobacco rattle virus (TRV)-based VIGS method to knock down LeACS2 in tomato fruit after harvest, at mature green fruit stage (Xie *et al.* 2006).

**TILLING approach**

It is a technique that can identify polymorphisms (more specifically point mutations) resulting from induced mutations in a target gene by heteroduplex analysis. A variation of this technique, EcoTILLING represents a means to determine the extent of natural variation in selected genes in crops. This approach was used in melon plants for improving shelf-life by using gene ACC oxidase 1 (Dahmani-Mardas *et al.* 2010). It allocates the rapid and cost-effective detection of induced point mutations in populations of physical/chemically mutagenized individuals. Ethyl methane sulfonate (EMS) is mostly used and produce G/C to A/T transition by alkylating the G residues and the alkylated G residues to base pair with T instead of pairing with C. The crop species whose genome sequence has been finished; TILLING can be used to find the alleles in gene of interest for biotic and abiotic stresses.

EcoTILLING, an extension of TILLING, is an inexpensive and rapid method of discovery and analysis of single-nucleotide polymorphism (SNP) in natural populations. In TILLING parental DNA sequence whereas in EcoTILLING genomic DNA sequences used to identify the mutations created using endonuclease (CEL I). These techniques are independent of genome size; ploidy level and reproductive system of plants. They can be applied not only to model organisms but also to economically important crops. These provide a powerful approach for gene discovery, DNA polymorphism assessment and linkage disequilibrium. It is concluded that the induced mutations and natural polymorphism can be identified by TILLING and EcoTILLING approaches and the next task is their implementations in crop breeding.

**Case studies**

Elitzur *et al.* (2016) conducted a research study on the influence of transcriptional factors to promote shelf-life in banana (*Musa acuminata*, AAA Cavendish subgroup, Gr and Nain are used for transformation by three transgenic genes such as RNAi MaMADS1, RNAi MaMADS2 and AS MaMADS2). They found that reducing the transcript levels of either MaMADS1 or S2 by RNAi approach which leads to decrease in ripening progression and delay shelf life of fruits.

Das *et al.* (2016) evaluated the expression of SAMDC gene for enhancing the shelf-life for improving the fruit quality using biotechnological approaches into litchi chinensis cultivars. They observed that S-adenosylmethionine decarboxylase acts on SAM and convert into polyamines so that very small amount of SAM is available to produce ethylene and consequently ripening process can be delayed and fruits shelf life can be enhanced.

Tanase *et al.* (2016) stated that berries are characterized by a shorter shelf-life than other fruits because of higher susceptibility to microbial spoilage, increased respiration rate and ethylene production which stimulate wounding of tissue. Simple and best method to keep them in refrigerated state at 10°C but this method is not always optimal *i.e* modified atmosphere packaging, edible coatings and films, UV light, ozone, dipping berries in different solutions.

Krishna *et al.* (2012) conducted an experiment for extension of shelf-life of apple cv. Oregon Spur by applying combination of mineral nutrition (Ca and B) and bioregulator (salicylic acid and GA3) during 60 days of storage under ambient conditions. They concluded that shelf-life and quality of apple was improved significantly by treating apple with combinations of nutrients and bioregulators rather than treating with single nutrient or bioregulator.

**CONCLUSION**

Both conventional and biotechnological approaches are used for enhancing shelf-life of fruits. It is evident that developed biotechnological approaches have the potential to enhance shelf-life of fruits and to meet demand of 21<sup>st</sup> century. Post-harvest treatments are mainly used in case of conventional approaches. In biotechnological approaches genetic engineering is the main technique. Both these approaches are known to have beneficial effects in enhancing shelf-life of horticultural crops in future.

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