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Eco-friendly shelf life enhancement of green vegetables by using synthetic cytokinin (6Benzylaminopurine): An overview

Meenakshi Kumari and SS Solankey

Abstract
Vegetables contain high amount of moisture and the rate of respiration is also high therefore vegetables are highly perishable in nature, so after harvest they require proper handling and storage to preserve their quality and get full advantage of their health benefits. In India, due to improper and poor postharvest practices nearly 30-35% of its total production is lost. The major factors of postharvest losses are physiological and biochemical processes, microbial decay, high perishability and sub-standard postharvest handling infrastructures. So, it is very important to reduce the postharvest losses of vegetable by applying modern technologies. Some of the major steps for checking postharvest losses are proper handling of the produce, pre-cooling, washing and disinfection before storage. Hot water treatment, irradiation and use of edible coating are also an effective way for reduction in quality loss and suppressed pathogen infection. Apart from these, various plant growth regulators have been used, most common being 1-MCP in okra, 6-benzylaminopurine (BAP) in cauliflower, MeJA, CaCl2, KMnO4 in many vegetables that reduce physiological loss in weight, delay senescence, maintains chloroplast activity, declines chlorophyll degradation and helps in production of protein and nucleic acid. Out of these 6-BAP, which is a first generation synthetic cytokinin is more eco-friendly and cost effective option of increasing shelf life of commercial vegetables. 6- benzylamino purine also known as benzyl adenine that elicits plant growth and development responses, setting blossoms and stimulating fruit richness by stimulating cell division. It increases post-harvest life of green vegetables by inhibiting the respiratory kinase of plants. Influence of cytokinin as 6-BAP in combination with other methods on postharvest green colour retention on different vegetables, showed positive results for quality retention. However, it should be popularized among people that can lead to effective saving of the 35% vegetables loss every year which can boost the farm income and also feed some more hungry mouths.

Keywords: Postharvest, shelf life, quality, treatments, vegetables

Introduction
Fresh vegetable endowed with almost all of the nutritional principles that our body requires for proper growth and development. They are good source of many vitamins, minerals, proteins, antioxidants and dietary fibre. All the green-yellow-Orange vegetables are rich in calcium, magnesium, potassium, iron, beta-carotene, vitamin B complex, vitamin-C, vitamin- A and vitamin K. After China, India is the second largest producer of vegetables in the world, accounting about 15% of global vegetable production. Vegetables cover an area of 9.39 million hectares with a total production of 162.89 million metric tons with productivity of 17.3 MT/ ha in the country. Diverse types of vegetables are grown in our country due to prevalence of tropical, subtropical and temperate climates. Unfortunately, due to improper management practices considerable proportion of the harvested produce is lost. Approximately, between 30 and 35% of India’s total vegetable production is lost due to poor postharvest practices. In Brazil 70% and in USA 65% of the total vegetable production is commercially processed but in India only less than 2% (Rahul *et al*., 2015) [11]. The main reasons for the losses are physiological and biochemical processes, microbial decay, high perishability, inadequate knowledge on harvesting, carrying, packaging, transport and storage techniques. The postharvest losses of vegetables are higher than any other cereal crops. Such losses are attributed to the perishable nature of vegetables, which causes deterioration more quickly. Losses can vary from 25 to 40% depending on the type of vegetables. In the vegetable marketing channels, traders suffer from maximum losses, because they handle and transport more quantities from one place to another than any other intermediaries (Rashid, 1998) [12].
All fruits, vegetables and root crops continue their living processes even after harvest because they are living plant parts and contain 65 to 95 percent water, their postharvest life depend upon the rate of consumption of reserved food and water loss. When food and water reserves are exhausted, they produce senescence and decays.

The instant a crop is removed from the ground, or separated from its parent plant, it begins to deteriorate. It is well established that the quality of the harvested commodities cannot be improved further but it can be retained till their consumption if the rate of metabolic activities are reduced by adopting the appropriate postharvest handling operations (Wu, 2010) [17].

Postharvest treatment largely determines final quality. The most important goals of postharvest handling are keeping the product cool, slow down undesirable chemical changes, avoid moisture loss, avoid physical damage such as bruising and delay spoilage. Vegetables and fruits are subject to the active process of senescence. The original composition of the crop changes continuously by the numerous biochemical processes until it becomes unmarketable. The period during which consumption is considered acceptable is defined as the time of "postharvest shelf life". These are affected by a number of factors leading to the postharvest spoilage. Besides, packaging, grading and transportation many factors contribute to these losses. Prevention of such losses is most important to make available quality vegetable. Proper postharvest treatments slow down the physiological processes of senescence and maturation, reduce/ inhibit development of physiological disorders and minimize the risk of microbial growth. It can be used to maintain quality, reduce losses and waste of fresh produce. Therefore, postharvest treatments are essential to minimize microbial spoilage and reduce the risk of pathogen contamination for vegetables.

Various postharvest physical, chemical and gaseous treatments are being used to maintain the quality of crops with high nutritional value and meet safety standards of fresh produce. These postharvest treatments are typically combined with appropriate management of storage temperatures (Mahajan *et al.*, 2014) [8]. To conserve the quality of produce and delay the senescence and ripening process, understanding the cause of deterioration in vegetable is the fundamental step, followed by utilizing appropriate and affordable technological procedure. Some of the important parameters determining the metabolic activity of vegetables are (1) respiration, (2) transpiration, (3) ethylene production, (4) ripening and (5) senescence.

1. Respiration

Respiration represents sum of total of all the metabolic activities of the tissue. After harvest respiratory rate of produce is reversely proportional to its storage life and is entirely dependent on its own food reserves (Wu, 2010) [17].

2. Transpiration

Transpiration is a physical term related to weight loss. The rate of water loss by intact plants via transpiration is generally far greater than with decapitated harvested products.

3. Ethylene Production

Ethylene is known as the “aging” hormone in plants. It effects fruit ripening, induction of flowering, loss of chlorophyll, abortion of plant parts, stem shortening, abscission (dropping) of plant parts, epinasty (stems bend) and dormancy. Ethylene can be either good or bad, depending on what commodity you work with (Blankenship, 2001) [3]. In higher plants, ethylene is produced from methionine via three enzymatic reaction.

4. Ripening

Ripening is a physiological process that comprises several physical, chemical, and biochemical changes which renders fruit attractive and palatable. These changes includes change in colour, firmness, taste, and flavor which results in the transformation of unripe fruits into ripe product.

5. Senescence

Senescence is defined as the period when anabolic (synthetic) biochemical process give way to catabolic (degradative) process, leading to ageing and finally death of the tissue. During senescence numerous biochemical processes continuously change the original composition of the crop until it becomes unmarketable. These are the some process occurred during senescence:

a) Oxidative stress
b) ROS production
c) Lipid peroxidation
d) Firmness loss
e) Membrane permeability
f) Microbial infection

a) Oxidative stress

Oxidative stress occurs when critical balance is disrupted by excess of reactive oxygen species (ROS), reduction in antioxidants, or both. Several environmental perturbations like extreme temperature, drought, flooding, salinity, ozone exposure, UV irradiation, heavy metal toxicity, herbicides, and environmental pollutants augment the development of oxidative stress in plants (Apel and Hirt, 2004) [1].

b) ROS production

Reactive oxygen species (ROS) are the partially reduced forms of molecular oxygen that result from either the excitation of O_2 to form singlet oxygen or the transfer of one, two, or three electrons to O_2 to form, respectively, superoxide (O_2^-), hydrogen peroxide (H_2O_2) or hydroxyl radical (OH) (Hodges *et al*., 2004) [7]. ROS are produced both during normal metabolism as well as stress conditions in a plant cell.

c) Lipid peroxidation

It is the oxidative degradation of lipids which leads to loss of membrane integrity, physical structure, and membrane fluidity that would in turn affect proper function of proteins either by direct attack on the proteins by reactive oxygen species or loss of activity due to an unfavorable lipid environment. Lipid peroxidation can be viewed as a consequence of life.

d) Firmness loss

Firmness is a visual attribute that directly represents the texture of fruits and vegetables. Firmness of fresh products deteriorates gradually during storage, due to the various physiological activities such as respiration and transpiration which largely influence the quality and facilitate the pathogen infection. The activity of many enzymes such as pectin methylesterase (PME) and polygalacturonase (PG) is responsible for the loss of firmness.

e) Membrane permeability

Cell membranes composed of lipids and protein, that play an integral role in the response of plant tissues to chilling and freezing. It has been proposed that the thermo tropic phase transition of membrane lipids might play an initiative role in the chilling sensitivity. It was measured by ion or electrolytic leakage from cells.

f) Microbial infection

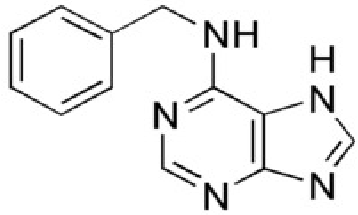
Microbial infection increases due to increase in membrane permeability and lipid peroxidation which ultimately decrease shelf life and quality of the produce. Postharvest treatments reduce the microbial load and thus extend shelf life.

Important Postharvest Operations:
There are many postharvest operations which determine the quality of the fresh produce; some of the most important operations are as follows:

1. Washing
It is an important operation which is follow immediately after harvest. Vegetables need washing for following main reasons i.e., to remove dirt and dust so that they can be presented to the consumer in a visually appealing manner, to increase the shelf life by controlling the postharvest plant diseases and to maintain the purpose of food safety so that harmful pathogens that may be present on the surface of vegetables are not passed on to consumers (Premier, 2013) [10].
A study conducted by Bayoumi (2008) [2] on the effect of hydrogen peroxide treatment on white pepper and he estimated that hydrogen peroxide treatments significantly reduced weight loss, rot rate index and nitrate content of fruits especially with 15 mM hydrogen peroxide as compared with control treatment (0 mM hydrogen peroxide) and also increased general appearance, ascorbic acid content and the activity of the antioxidant enzymes such as ascorbate peroxidase and dehydroascorbate reductase of white pepper stored at room temperature (20 ºC) for 2 weeks and in freeze (10 ºC) for 4 weeks.

2. Precooling
Precooling is second most important operations. It improves the performance of vegetables by reducing the compressor load and the head pressure therefore, ultimately increase shelf life and quality of the fresh product. It is essential to minimize microbial spoilage and increases the shelf life of green vegetables.

3. Anti senescence treatments (6-Benzylaminopurine)
6-Benzylaminopurine, benzyladenine or BAP is a synthetic cytokinin which promotes the cell division and ultimately increases the shelf life of green vegetables (Table 1). BAP delay the fruit senescence, decreases the loss of membrane integrity, decrease the weight loss of fresh produce, decreases the respiration rate, reduces the softening of green vegetables and ultimately improves the nutritional quality. Exogenous application of cytokinins to plant tissues results in delay in senescence, maintenance of chloroplast activity, decline chlorophyll degradation, production of protein and nucleic acid synthesis and mobilization of nutrients into cytokinin treated area Wingler (1998) [16] and Clarke (1994) [5].



IUPAC name: N-(Phenylmethyl)-7H- purin-6 amine
Chemical Formula: $C_{12}H_{11}N_5$
Appearance: White to off white powder.

Siddiqui *et al.* (2011) [13] estimated that treatments with 10 and 15 ppm 6benzylaminopurine can be used to extend shelf life of fresh-cut broccoli florets during storage at 6±1 °C at commercial level. Another study conducted by Siddiqui *et al.* (2015) [15] reported that 6benzylaminopurine (BAP) with 200 ppm and 300 ppm can be used to increase quality and enhanced antioxidant activity of cauliflower upto 12 days at 25ºC. In his study, fresh cauliflower curds were treated with 6benzylaminopurine at three concentrations (100, 200, or 300 ppm w/v) and its effects on lipid peroxidation, membrane integrity, bioactive molecules, antioxidant activity, soluble sugar, etc. were observed during storage at ambient conditions. BAP profoundly delayed lipid peroxidation and loss of membrane integrity of the tissue, which was associated with the ageing and senescence processes. A positive effect of BAP on maintaining higher bioactive molecules (ascorbic acid and total phenols), antioxidant activity, and soluble sugar was also observed at 200 and 300 ppm, which was decreased in control curds. Therefore, 200 ppm BAP is recommended for practical application considering the cost of 6-benzylaminopurine.
Zhang *et al.* (2018) [20] observed that 6-BAP significantly inhibited decay incidence of harvested litchi, associated with a direct inhibition on *Peronophythora litchii,* the major pathogenic fungi and also reduced H_2O_2 accumulation and lipid peroxidation, which may account for browning inhibition to an extent and higher contents of anthocyanin and total phenolic compounds. Furthermore, higher activities of SOD, CAT and APX and DPPH radical scavenging capacity in BAP-treated fruit possibly benefited reducing ROS accumulation and lipid peroxidation. Overall, application of BAP showed great potential to control decay and browning and extend shelf life of harvested litchi.

Table 1: Effect of 6- BAP treatment on important commercial vegetables

S. No.	Vegetable	Storage Period (Days) and Temperature (ºC)	Doses	Best Result	References
1.	Cauliflower	12 d at 25 ºC	100, 200, 300 ppm for 10 min.	• 200 ppm increases storage life • Decreases lipid peroxidation • Reduces weight loss • Reduces loss of ascorbic acid • Decreases senescence	Siddiqui *et al.* (2015) [15]
2.	Broccoli	4d at 15 °C	200 ppm for 1 min and Distilled water as control	• 200 ppm delayed the loss of chlorophyll • Improved the nutritional value • Prohibit loss of membrane integrity • Delay senescence	Xu *et al*. (2011) [18]
3.	Cabbage	8d at 6± 1º C	0, 5,10 & 15 ppm	• 15ppm reduces the loss of OQ	Siddiqui *et al.* (2011) [14]

4.	Summer squash	25d at 15º C	0 &1 mmol/L	• 1mmol/ L, reduces fruit deterioration • Decreases weight loss	Massolo *et al*. (2014) [9]
5.	Cucumber	16 d at 2º C	0, 10, 50 & 100 mmolL/1	• 50 mmolL/1 was most effective to restrain Chilling injury • Maintained higher levels of chlorophyll, ascorbic acid, total phenolics and total antioxidant capacity, ATP content and energy charge.	Chen and Yang (2013) [4]
6.	Green asparagus	10d at 12±2º C followed by 10 days at 2±2C	20 µg/g and submitted t o ultra sound wave (20 kHz) for 20 min	Enzyme activities of asparagus including catalase, polyphen ol oxidase, ascorbate peroxidase were enhanced.	Yun and Xing (2011) [19]
7.	Broccoli	(0, 6, 12, 24, 48, 60, 72, 84, 96, 120, 168 and 240 h) after harvest at 20 º C	50 ppm	Maintained soluble protein up to 160 h after harvest. Delay the yellowing of broccoli flore delayed the increase in glutamine concen- tration by 24-48 h. ts at 120 h after harvest	Downs *et al*. (1997) [6]
8.	Broccoli	9d at 6±1 °C.	0, 5, 10 and 15 ppm for 10 min	Delayed yellowing and chlorophyll degradation, consequently maintained organoleptic quality during storage.	Siddiqui *et al*. (2011) [13]

Conclusion

It is apparent from the above study that vegetables are very perishable in nature and they are metabolically active even after harvest, therefore needs proper handling and management to increase their shelf life. Implementation of efficient postharvest processing technologies were able to minimized the losses of the vegetables and enhance the food availability which can reduce the scarcity of the agriculture produces among the consumers. It is well established that the postharvest life depends on the rate at which they use up their stored food reserves and their rate of water loss. When food and water reserves are exhausted, the produce dies and decays and we know that the quality of the harvested commodities cannot be improved further but it can be retained till their consumption if the rates of metabolic activities are reduced by adopting the appropriate postharvest handling operations. Inadequate management of these processes can result in major losses in nutritional and quality attributes, outbreaks of food borne pathogens and financial loss for all players along the supply chain, from growers to consumers

Various postharvest treatment like physical treatments (hot water treatment, radiation), ethylene inhibiting treatments ($KMNO_4$, 1-MCP), transpiration inhibiting treatment (waxing), anti-senescence treatments PGRs (like 6-BAP), Methyl jasmonate and $CaCl_2$ can effectively increase shelf life of vegetables· We know that in a hungry and increasingly competitive world, reducing postharvest food losses is a major agricultural goal. But during literature reviewed, it has been observed that very little work has been done on the postharvest treatment of vegetables except on few crops like tomato, broccoli, cauliflower etc. So, there is ample scope to work on the postharvest treatments of vegetables. In cauliflower curd quality was associated with the increase in lipid peroxidation and loss of membrane integrity, moisture, bioactive molecules and antioxidant activity which were related to the senescence process. Delay of these undesirable changes was achieved by treatment of cauliflower curds with 6-benzylaminopurine. Compared to control, 6-benzylaminopurine (200 and 300 ppm) was found to significantly inhibit the degradative changes and preserve the curd quality and antioxidant status during storage of cauliflower at ambient conditions.

Therefore, maintenance of the physical appearance, flavour, market value and other characteristics of consumable commodities, the proper and scientific processing are required. This would help to enhance the per capita availability of vegetables by applying intensive and modern technologies because of reduction in losses automatically increased the availability of products without applying extra resources for enhancing the production and productivity to feed the millions of hungry people.

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