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Ethylene in Postharvest Technology: A Review

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ABSTRACT
The plant hormone ethylene has become the focus of plant biology over the last 100 years. It is a gaseous plant hormone that is responsible for fruit ripening, growth inhibition, leaf abscission, aging and a wide range of other plant processes. Extensive reviews of ethylene biosynthesis have been described by researchers and the effects of ethylene in plants are well established. Most studies on fruit ripening and ethylene biosynthesis have been confined to climacteric fruits such as apple (*Malus domestica*), tomato (Lycoperscion esculentum), avocado (*Persea gratissima*), banana (*Musa acuminata*) and to a lesser extent in paw-paw (*Carica papaya*), kiwi fruit (*Actinidia deliciosa*) and breadfruit (*Artocarpus altilis*). Ethylene is also at the centre of postharvest technology acting as a key in the extension of shelf life and fruit quality during storage. In this review, the use of postharvest technology is examined to preserve the quality and shelf life of produce through the removal/inhibition of ethylene from storage systems and describe systems that primarily employ ethylene inhibition/removal as its core.

Key words: Ethylene, fruit ripening, postharvest technology

INTRODUCTION
Ethylene biosynthesis occurs in plants via a complicated cycle known as the methionine cycle. The portion of the pathway is represented below:

Methionine $\xrightarrow{1}$ S-adenosylmethionine (SAM) $\xrightarrow{2}$ 1-amino cyclopropane 1-carboxylic acid (ACC) $\xrightarrow{3}$ ethylene (Adams and Yong, 1979).

The first step is catalysed by s-adenosylmethionine transferease, the second (rate limiting step) by ACC synthase, a pyridoxal phosphate dependent enzyme and the third by ACC oxidase which needs Fe^{2+} as a cofactor and ascorbate as a cosubstrate. The final step is also oxygen dependent.

Inhibition of ethylene biosynthesis usually takes place at steps 2 and 3 in the biosynthetic pathway. ACC synthase which is a pyridoxal phosphate dependent enzyme is powerfully inhibited by AVG (aminoethoxyvinylglycine) and AOA (aminooxy-acetic acid). The free radical scavenger n-propyl gallate and oxygen consuing chemicals like sodium metabisulphite inhibits ACC oxidase. NBD (2, 5-norbornadiene) and Ag^{+} inhibits an ethylene response by binding and therefore blocking the ethylene receptor ETR1 (ethylene resistant 1).

The production of ethylene by *Carica papaya* (Dunkley and Golden, 1998) and *Artocarpus altilis* (Williams and Golden, 2002) have been demonstrated. Ethylene production at

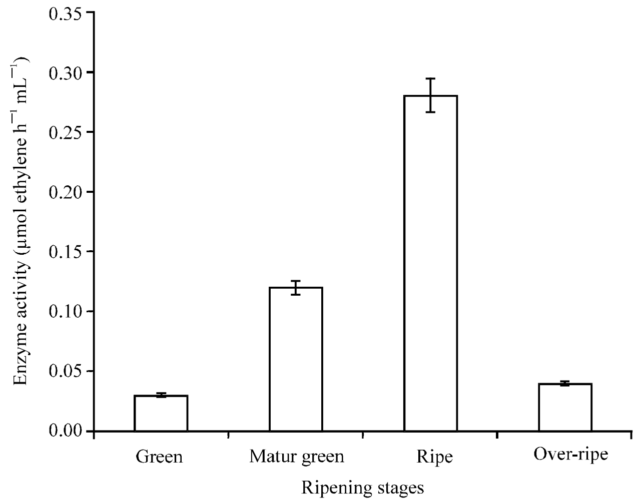

Fig. 1: Ethylene production during the ripening stages of papaya (*Carica papaya*)

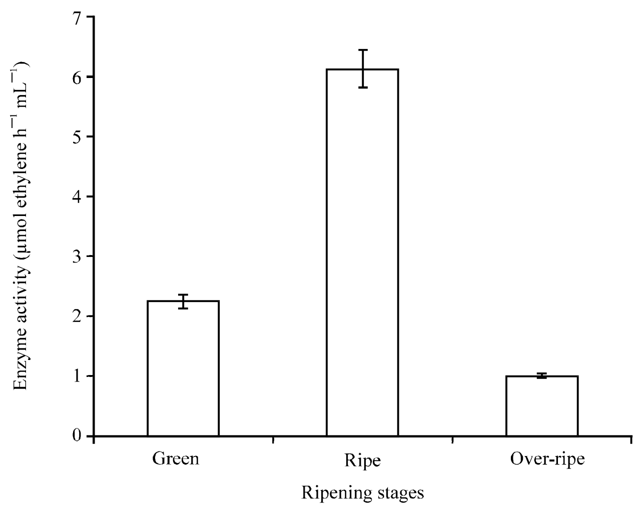

Fig. 2: Ethylene production during the ripening stages of breadfruit (*Artocarpus altilis*)

the various stages of ripening (green; mature green; ripe and over-ripe) was determined by assaying the enzyme ACC oxidase. Both fruits showed similar ethylene biosynthetic patterns during ripening; increasing from the mature-green stage to the ripe stage and decreasing at the over-ripe stage (Fig. 1 and 2). The ACC oxidase from *Carica papaya* upon purification and characterization displayed the following properties.

M_r was 27.5 kDa with a Km of 37 µM. The enzyme was inhibited by n-propyl gallate (0.240 mM), sodium dithionite (0.022 mM), sodium metabisulphite (0.021 mM) and cobalt sulphate (0.101 mM). Dithiothreitol (DTT) at a concentration of 3 mM enhanced the activity of the enzyme. Optimum pH was 7.0 and the optimum temperature was 30°C. The ACC oxidase form *Artocarpus altilis* (breadfruit) showed very similar characteriticts, the exception being the M_r which was 42.3 kDa for the breadfruit enzyme.

The ACC oxidase, from other sources, showed similar properties, Km values for tomato (Zhang *et al*., 1995) and avocado (McGarvey and Christoffersen, 1992) were 23.2 and 36 μM, respectively. A pH optima of 7.4 was reported for melon (Smith *et al*., 1992). Apple (Kuai and Dilley, 1992) and avocado (McGarvey and Christoffersen, 1992) displayed a pH optima of 7.0. *Carica papaya* (Dunkley and Golden, 1998) and *Artocarpus altilis* (Williams and Golden, 2002) have a similar pH optima (7.0).

The role of ethylene in the ripening process is well established (Marriott and Palmer, 1980; Yang and Hoffman, 1984). In climacteric fruits, ethylene production begins at the onset of the climacteric period (increase in respiration rate and metabolic activities) and thereafter, increases and decreases parallel with changes in respiratory climacteric towards the full ripe stage (Liu *et al*., 1999). The onset of ripening is marked by a number of critical metabolic and physical changes including acceleration in metabolic activity, rapid transformation of starch to sugars, increase in acids, decrease in tannins and hemicellulose and softening of the skin and pulp tissues (Wardlow, 1961).

MATERIALS AND METHODS

Postharvest storage systems: The objective of storage is to extend the shelf life of fresh products by preserving them in their most usable form for consumers and processing industries (Ratti *et al*., 1996; Agoreyo *et al*., 2007). Many forms of food preservation (refrigeration, drying, modified atmosphere storage, etc) have been studied and commercially implemented during the past years (Gariepy *et al*., 1984).

The primary factors in maintaining quality and extending the postharvest life of fresh fruits and vegetables are harvesting at optimum maturity, minimizing mechanical injuries, using proper sanitation procedures and providing optimum temperature and relative humidity during all marketing steps (Kader *et al*., 1989). Secondary factors include modification of O_2, CO_2 and/or ethylene concentrations in the atmosphere surrounding the commodity to levels different from those of air. This is referred to as CA (controlled atmosphere) /MA (modified atmosphere). The primary benefit that one can expect from the CA/MA storage system is that the produce will maintain its freshness and quality for a longer period of time than it would if stored under Regular Atmosphere (RA) (Ratti *et al*., 1996).

The ideal storage system should have the ability to provide low temperatures, high relative humidity and optimum gas composition in order to minimize the metabolic activity of the stored commodity. Under these conditions, the stored product is expected to maintain its freshness and nutritional quality for a much longer period of time (Bohling and Hansen, 1977; Smock, 1979).

The shelf life of different produce may be extended by controlling temperature and relative humidity, manipulating storage gas composition in CA/MA storage systems, reducing microbial infection during storage, minimizing the postharvest injuries that may occur during handling (Raghavan and Gariepy, 1984) and by application of inhibitors of respiration and ethylene production.

Controlled Atmosphere (CA)/ Modified Atmosphere (MA) storage systems: MA storage has been defined as a form of storage where the concentration of gas is initially modified according to optimal conditions and then maintained during the period of storage (Parry, 1993). In MA systems, only an initial change is made, consistent with the requirements of the commodity during transportation while in CA systems, the atmospheric composition is maintained throughout the storage process (Wolfe, 1980).

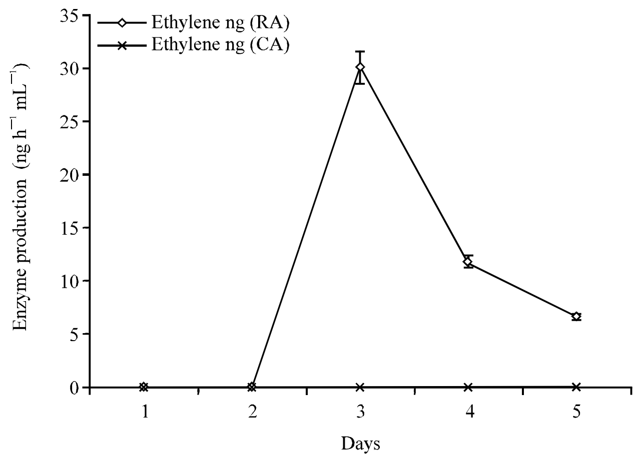

Fig. 3: Ethylene production in *Carica papaya* at RA and CA (silicone membrane)

Of the various beneficial effects of CA on fruits, prevention of ripening is the most important. Low temperature with high relative humidity and CA/MA is a common method used to maintain the quality of fresh produce (Ratti *et al*., 1996). The levels of both CO_2 and O_2 concentrations during storage affect the quality of the final product. Anaerobiosis (absence of oxygen) results in the development of off-flavours in the commodities (Lipton, 1975). Reducing O_2 concentrations below 8% and/or elevating CO_2 concentrations above 1%, retards fruit ripening. However, there are limits beyond which physiological injury will outweigh the benefits from retardation of ripening (Kader, 1980). In the case of vegetables, such as cauliflower and broccoli, the presence of CO_2 in high concentrations may cause physiological injuries to the commodities (Lipton, 1975). Reduced O_2 and elevated CO_2 concentrations delays fruit ripening, reduces respiration and ethylene production rates, retards softening and slows all the compositional changes associated with ripening (Kader, 1980). Prevention or delay of ripening has an indirect effect on the incidence of decay, since ripe fruits are more susceptible to attack by postharvest pathogens.

Modified atmospheres with low O_2 concentrations slow the deterioration of fruits by depressing respiration, ethylene production and tissue sensitivity (Kader *et al*., 1989). The extent of the depression of ethylene production depends on the oxygen concentration developed within the fruits internal atmosphere and the sensitivity of ethylene production to oxygen concentration (Elyatem *et al*., 1994). MA can also be achieved by using chambers fitted with silicone membranes or diffusion channels which reduce oxygen and ethylene concentration significantly (<6%) and hence retard ripening (Stewart *et al*., 2005). Figure 3 and 4 shows the production of ethylene under RA (Regular Atmosphere) and CA (Controlled Atmosphere) using the silicone membrane system or the diffusion channel system, respectively. Note the drastic reduction of ethylene production in both Fig. 3 and 4.

The potential benefits of properly used CA include, retarding of senescence (ripening and aging), reducing product sensitivity to ethylene, reducing the severity and incidence of decay and controlling insects. On the other hand, potential hazards include, aggravation of some physiological disorders, irregular ripening and potential of off-flavours and odours (Brecht, 1980).

Ethylene inhibition/removal: Inhibitors of respiration or ethylene production have been reported to be effective in preserving the postharvest life of produce. There is considerable use of

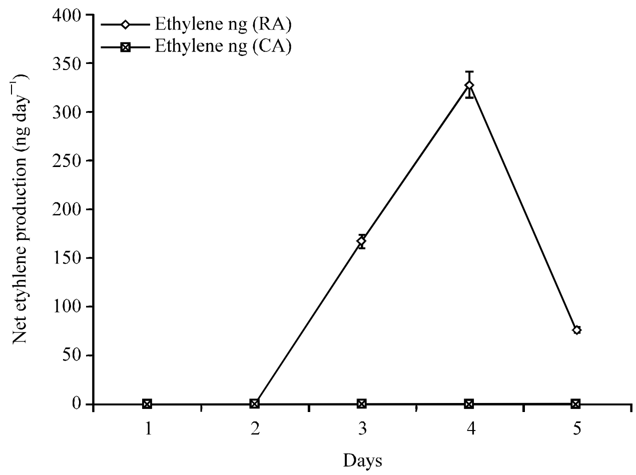

Fig. 4: Ethylene production in *Carica papaya* at RA and CA (diffusion channel)

chemicals in the postharvest stage particularly to protect produce from insects, bacterial and fungal pathogens and to reduce or inhibit ripening. The widespread application of these inhibitors is directly related to their cost and health implications (Marriott and Palmer, 1980).

The effect of exogenous ethylene on preclimacteric fruit is critical both to the understanding of the control of the climacteric and to the technology of bulk storage. Trace quantities of ethylene shorten the preclimacteric period, while higher-concentrations induce rapid initiation of the climacteric in most fruits (Marriott and Palmer, 1980). An atmospheric concentration of 0.1 ppm sets off internal ethylene production and shortens the green life (Marchal, 1998).

In commercial practice, ethylene accumulation is minimized by ventilation or by use of Ethylene Absorbers (EA) such as potassium permanganate (Marriott and Palmer, 1980; Chamara *et al.*, 2000). Ethylene absorbers are effective in lowering ethylene concentrations in packaging, thereby increasing the storage life of the produce. The removal of ethylene from storage environments extends the storage life by 100% (Chamara *et al.*, 2000; Truter and Combrink, 1990).

Shelf life may be further increased by using an EA such as potassium permanganate in combination with polyethylene films (Scott and Roberts, 1966; Jiang *et al.*, 1999; Chamara *et al.*, 2000). The use of a MA containing 2-3% O_2 and 5% CO_2 has been shown to extend the storage life of bananas 4-6 weeks (Truter and Combrink, 1990; Ke and Hwang, 1987). These processes were expensive, hence the extensive use of CA/MA has been hindered by the substantial costs it incurs (Marchal, 1998).

Ripening in fruit may be retarded by applying inhibitors of respiration and ethylene production. A complete absence of O_2 has an inhibitory effect on ripening while low concentrations of O_2 result in decreased respiration in the produce (Wardlow, 1961). Carbon dioxide inhibits endogenous ethylene synthesis and respiration, however if the level of CO_2 is too high, the effects are similar to those caused by the lack of O_2. An oxygen concentration of 2.5% slows respiration, peel coloration and changes in sugar. The aromatic quality is affected by anaerobic conditions (Marchal, 1998).

Other inhibitors of respiration and ethylene synthesis are ammonia (Von Loesecke, 1961), maleic acid (Wardlow, 1961), gibberillins (Leonce, 1974; Kapoor and Turner, 1976; Marriott and Palmer, 1980), auxins (Vendrell, 1970) and vitamin K (Beccari, 1969). Ozone and ultraviolet rays (Kao, 1971; Maxie *et al.*, 1974) diazocyclopentadiene (Blankenship and Sisler, 1992) and more recently salicylic acid (Srivastava and Dwivedi, 2000) 1-methylcyclopropene (Sisler *et al.*, 1996;

Jiang *et al*., 1999; Sisler and Serek, 2000; Macnish *et al*., 2000) and 3,3-dimethylcyclypropene (Golding *et al*., 1998) are shown to be effective ripening inhibitors. Factors affecting the widespread use of these methods for preserving bananas are mostly based on cost and effectiveness.

In some commodities, accelerated ageing and the initiation of ripening can occur following exposure to ethylene concentrations as low as 0.1 ml L^{-1} (Lee *et al*., 1995). As senescence begins, spoilage due to indigenous bacteria can be augmented. Ethylene is also a by-product of the aerobic combustion of hydrocarbons and it is therefore important during the handling of produce to maintain low levels of environmental ethylene, which are often increased by fork lifts and other machinery (Zagory, 1995).

Different biological structures of assorted produce varieties contribute to the product's sensitivity response to ethylene, as well as the response to O_2 and CO_2. Control measures taken to minimize perception and production of ethylene following harvest include storage in a modified atmosphere at optimal low temperatures (just above the chilling or freezing injury threshold) and oxidizing the ethylene by various chemical and physical means. Part of the success of MAP (Modified Atmosphere Packing) and the quality attributed to MAP products, depends on preventing the damaging effects of exposure to ethylene. To this end, CO_2 can inhibit ethylene action as well as autocatalytic production of ethylene by climacteric products such as apples and tomatoes. However, increased damage to whole leaf plants has been observed at CO_2 levels above 15-20%, thus reinforcing the importance of designing a specific MAP for each product (Lee *et al*., 1995).

ROLE OF ETHYLENE OXIDE AND SULPHUR DIOXIDE IN RIPENING

Ethylene oxide has been shown to be most effective in delaying banana ripening at low concentrations. At a concentration of 50-100 ppm the banana fruit was storable for 6 weeks and was of excellent quality (Williams, 2002). However, when the fruit was treated with 400 ppm EO this caused burning of the peel, however the damage did not extend to the pulp (Williams, 2002). Storage using sulphur dioxide (SO_2) was effective in maintaining banana quality and prevented fungal infection in treated fruit. By comparison, control fruit stored at regular atmosphere suffered fungal infection (Williams, 2002). In general, banana fruit treated with 2 and 8 μg kg^{-1} SO_2 were of excellent quality after storage irrespective of being stored under regular atmosphere or controlled atmosphere.

CONCLUSION

It is obvious that ethylene plays a major role in the aging process of plants. The enhancement of the shelf-life of many fruits can be achieved by the removal of ethylene from the atmosphere surrounding the fruit. The use of postharvest technology to produce controlled or modified atmospheres is the technology that has the potential of allowing the developing countries to earn more vital foreign exchange due to the increased shelf-life of tropical fruits.

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