AN OVERVIEW OF THE PHYSIOLOGICAL AND BIOCHEMICAL BASIS OF CA EFFECTS ON FRESH HORTICULTURAL CROPS

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Controlled atmospheres (CA) can influence, directly or indirectly, all causes of deterioration of harvested horticultural crops and consequently affect their quality and postharvest-life. Although much research has been done in order to identify the optimum oxygen and carbon dioxide levels for each commodity and sometimes each cultivar within a given commodity, the mode of action of reduced oxygen and elevated carbon dioxide concentrations remains largely unknown. Generally, the effects of CA on reducing rates of respiration and ethylene production have been assumed to be the primary reasons for reducing deterioration rate of fresh horticultural commodities. This may be true for some commodities, but it is not an adequate explanation for the responses of other crops to CA. In this paper, the possible effects of CA on each of the causes of postharvest deterioration of fresh horticultural commodities will be briefly discussed.

Respiratory Metabolism

Reducing the oxygen level around fresh horticultural crops decreases their respiration rate in proportion to the oxygen concentration, but a minimum of about 1 to 3% oxygen, depending on the commodity and duration of exposure, is required to avoid a shift from aerobic to anaerobic respiration. Although the oxygen level at which anaerobic respiration occurs may be as low as 0.2% within the plant cell, the gradient of oxygen concentrations from that point to the external atmospheres necessitates maintenance of about 1 to 3% oxygen around the commodity; the specific oxygen level depends on respiration rate and gas diffusion characteristics of the various tissues of each cultivar of a given commodity.

The decrease in respiration rate in response to reduced oxygen levels (of not less than about 2%) is not the result of suppression of the basal metabolism mediated by cytochrome oxidase, which has a Km value of 10^{-8} to 10^{-7}M oxygen (10,31). It appears that the reduction in respiration rate results from the decreased activity of other oxidases (e.g., polyphenol oxidase, glycolic acid oxidase, ascorbic acid oxidase) whose affinity for oxygen may be 5 to 6 times lower than that of cytochrome oxidase (31). Further reduction in oxygen concentration to a level influencing its uptake by cytochrome oxidase, could have marked effects upon the metabolism of the stored material, possibly beneficial though not necessarily so, and with danger of anaerobiosis in part of the material (10).

Elevated carbon dioxide concentrations also reduce respiration rate of fresh horticultural crops, but above a level of about 20% or higher (depending on the cultivar, oxygen concentration and duration of exposure), carbon dioxide can result in accumulation of ethanol and acetaldehyde within the tissues. The effects of reduced oxygen and elevated carbon dioxide on respiration rate are additive; a 10% carbon dioxide added to air influences respiratory metabolism to about the same extent as a 2% oxygen atmosphere and the combination of 2% oxygen + 10% carbon dioxide has approximately double the effect of either component alone.

Studies of the effects of high carbon dioxide levels on Krebs cycle intermediates and enzymes have shown accumulation of succinic acid due to inhibition of succinic dehydrogenase activity in apples (15,19,24), pears (13), and lettuce (4). Shipway and Bramlage (26) found that carbon dioxide levels above 6% stimulated malate oxidation and suppressed oxidation of citrate, α-ketoglutarate, succinate, fumarate, and pyruvate in mitochondria isolated from apple fruit. Monning (24) reported that elevated carbon dioxide inhibited glycolysis and succinic dehydrogenase activity and reduced formation of citrate/isocitrate and α-ketoglutarate, but malic dehydrogenase activity was not affected by exposing apple tissue to 10% carbon dioxide.

There are no published reports concerning the effects of elevated carbon dioxide levels on the glycolytic enzymes. Such information, especially for phosphofructokinase and pyruvic kinase as regulatory enzymes in the glycolytic pathway, would help elucidate the mode of action of carbon dioxide on the respiratory metabolism.

Ethylene Biosynthesis and Action

Reduced oxygen levels below about 8% decrease ethylene production by fresh horticultural crops and reduce their sensitivity to ethylene in proportion to the extent of reduction in oxygen concentration (17). Burg and Burg (7,8) demonstrated that oxygen is required for ethylene production and action. Under anaerobic conditions, the conversion of 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene is inhibited while earlier steps in ethylene biosynthesis from methionine can occur in the absence of oxygen (33). Lau et al. (22) found that storage of 'Golden Delicious' apples in 2.5% oxygen suppressed internal ethylene concentration and ACC accumulation.

Elevated carbon dioxide levels can reduce, promote, or have no effect on ethylene production rates by fruits, depending on the commodity, carbon dioxide concentration, and duration of exposure to carbon dioxide. Generally, the increase in ethylene production by some commodities during and/or following exposure to carbon dioxide occurs only when the carbon dioxide level is high enough to cause physiological injury to the tissue. Below the tolerance limit of each commodity to elevated carbon dioxide, ethylene production is reduced in proportion to the carbon dioxide concentration. Chaves and Tomas (11) observed a reduction in ethylene production by 'Granny Smith' apples following

exposure to 20% carbon dioxide for 2 hours. This treatment increased ACC accumulation in the tissue indicating a possible effect of high carbon dioxide levels on the enzyme system responsible for the conversion of ACC to ethylene.

The presence of 10% carbon dioxide abolishes the biological activity of 1 ppm ethylene (7), but at higher ethylene concentrations, the effectiveness of elevated carbon dioxide is reduced. In certain fruits, carbon dioxide accumulates within the intercellular space and functions as a natural ethylene antagonist (33). The mode of action of carbon dioxide in inhibiting or reducing ethylene effects is not known, but Burg and Burg (7,8) suggested that carbon dioxide competes with ethylene for the binding site. Beyer (3) reported that carbon dioxide can affect ethylene metabolism by inhibiting ethylene oxidation to carbon dioxide through a feedback inhibition mechanism.

Compositional Changes Affecting Color

Loss of chlorophyll and biosynthesis of carotenoids and anthocyanins are slowed down in horticultural crops kept in CA (5,16,30). Knee (20) reported that the rate of chlorophyll degradation in peel and cortex of apples was half maximal at 2.5 to 4% oxygen.

Elevated carbon dioxide can result in detrimental effects on color of some commodities during CA storage and after transfer to air. Examples include uneven red coloration of tomatoes picked at the mature-green stage and kept in more than 5% carbon dioxide, appearance of greyish-yellow color on cauliflower upon cooking following exposure to 15% carbon dioxide, and darkening of the red color of strawberries kept in 25% carbon dioxide, or higher. Brown discoloration of external and internal tissues can occur as a result of elevated carbon dioxide and/or reduced oxygen levels beyond those that are tolerated by each commodity.

Murr and Morris (25) reported that 0% oxygen was required to stop O-phenol oxidase activity and brown discoloration of the cap in mushrooms. Although carbon dioxide levels above 5% suppressed the activity of this enzyme, discoloration was enhanced. Buescher and Henderson (6) found that 10 to 30% carbon dioxide delayed brown discoloration of mechanically-damaged tissue in green beans via its reduction of phenolics content, phenolase activity, and oxidation of phenolics. The effect of carbon dioxide on inhibition of phenolics production and polyphenol oxidase activity was also observed in lettuce tissue (27). However, once the lettuce tissue was removed to air, browning resumed.

Compositional Changes Affecting Texture

Reduced oxygen and/or elevated carbon dioxide delay fruit ripening and softening. For example, Knee (20) found that the rates of flesh softening and soluble polyuronide formation in apples were half maximal at 2.5 to 4% oxygen. Goodenough et al. (14) reported that the appear-

ance of polygalacturonase was prevented by storing mature-green tomatoes in 5% oxygen + 5% carbon dioxide for up to 8 weeks at 12.5°C. The rate of kiwifruit softening during storage at 0°C for up to 24 weeks was significantly reduced in fruits kept in 2% oxygen + 5% carbon dioxide relative to those kept in air (1); elevated carbon dioxide had a greater effect on firmness retention than reduced oxygen.

CA can also influence the textural quality of non-fruit vegetables, such as retarding toughening of asparagus spears kept at 4°C in 12% carbon dioxide and tenderizing broccoli stored in 10% carbon dioxide for up to 2 weeks at 5°C (23). The mechanism of CA effects on textural quality is not clear and merits further investigation.

Compositional Changes Affecting Flavor

CA conditions may influence the rate of changes in carbohydrates, organic acids, proteins, amino acids, lipids, and phenolic compounds, which can influence flavor quality of fresh fruits and vegetables. Starch to sugar conversion, which is undesirable in table- and processing-potatoes kept at 2°C, can be slowed down by storage in 5 to 20% carbon dioxide or in less than 3% oxygen, but such treatments may increase sprouting (9). CA storage reduces losses in acidity in fresh fruits. In contrast, decreased acidity and increased pH following exposure to elevated carbon dioxide have been reported in several vegetables (23). Siriphanich and Kader (29) reported that immediately following removal of lettuce tissue from 5 to 15% carbon dioxide atmospheres, an increase in acidity and a decrease in pH occurred, but soon thereafter the reverse trend was observed.

Reduced oxygen can decrease the production rate of volatiles by fresh fruits. Knee and Hatfield (21) found that apples kept in 2% oxygen developed low levels of esters due to low rates of alcohol synthesis; this effect was reversible after removal of the apples from CA storage. Off-flavors can develop in any fresh fruit or vegetable if exposed to oxygen and/or carbon dioxide levels that result in anaerobic respiration and accumulation of ethanol and acetaldehyde.

Compositional Changes Affecting Nutritive Value and Safety

Generally, CA storage results in better retention of ascorbic acid in fresh fruits and vegetables than storage in air. For example, Wang (32) reported that 1% oxygen was very effective in retaining ascorbic acid content of Chinese cabbage, in delaying losses in sugars and chlorophyll contents, and in extending the storage-life at 0°C to 5 months as compared to less than 3 months in air. Aworh et al. (2) found substantial nitrite accumulation in spinach held in 1% oxygen at 10°C for 10-15 days relative to that kept in air, while 15-18% carbon dioxide reduced nitrite accumulation during 10 days of storage.

Growth and Development

Sprouting of potatoes is inhibited by exposure to 15% carbon dioxide at 10°C, but 2 to 5% carbon dioxide stimulated sprouting; a 2 to 4% oxygen atmosphere also encouraged sprouting (9). Sprouting and rooting of onions were inhibited when kept in 3% oxygen + 5 to 10% carbon dioxide at 1°C (16). Isenberg (16) suggested that CA may influence the levels of endogenous growth regulators which control sprouting and rooting of propagules. However, the mechanism involved is yet to be elucidated.

Elongation of cut asparagus spears is inhibited by elevated carbon dioxide (23). Cap opening of mushrooms is prevented by exposure to 1% oxygen and/or 5% carbon dioxide. Sorophore elongation was greatly reduced in mushrooms kept in 15% carbon dioxide or 0.25 to 1% oxygen (25).

Physical Injuries

While CA storage has no direct effect on incidence of physical injuries, it can influence symptom development and may interfere with the wound healing process. Suberization and periderm formation are reduced in potatoes kept in less than 5% oxygen or more than 10% carbon dioxide (23). Certain CA combinations inhibit or at least reduce brown discoloration of physically-injured tissues as a result of their effects on phenolic metabolism. Carbon monoxide at 2 to 3% has been shown to inhibit brown discoloration of cut surfaces in lettuce and other commodities (18).

Water Loss

CA does not directly influence rate of water loss, but its possible effects on reducing periderm formation on commodities such as potatoes and sweet potatoes renders them more susceptible to water loss. On the other hand, the need for a gas-tight environment for CA storage and transport facilities often results in significantly higher relative humidity around the commodity and consequently reduced water loss as compared to air storage.

Physiological Disorders

Fresh horticultural crops are subject to numerous physiological disorders which result from exposure to undesirable temperatures and/or levels of oxygen, carbon dioxide, and/or ethylene. CA conditions may alleviate, induce, or aggravate these physiological disorders (4,5,9, 10,16,23,30).

Elevated carbon dioxide (5 to 20%) has been shown to reduce severity of chilling injury symptoms in some commodities such as chili pepper, squash, okra, avocado, peach, and 'Fuyu' persimmons. In contrast, exposure of cucumber, bell pepper, and mature-green tomato to CA conditions at chilling temperatures aggravates the chilling injury symptoms.

CA conditions reduced the severity of certain physiological disorders such as scald on apple and pear (30) and ethylene-induced russet spotting of lettuce (5). On the other hand, elevated carbon dioxide in combination with ethylene induced white core inclusions in kiwifruit (1).

CA-induced disorders include impaired ripening of climacteric fruits; internal browning of many commodities; external browning of tomato skin, pepper calyx, and lettuce; and surface pitting of cucumber, mushroom, apple, and pear. However, the mechanisms by which reduced oxygen and/or elevated carbon dioxide induce these physiological disorders are not known.

Inhibition of succinic dehydrogenase and accumulation of succinate, a toxicant to plant tissues, do not provide an adequate explanation for carbon dioxide-induced brown stain on lettuce because Brecht (4) found the accumulation of succinic acid in lettuce kept in air + 5% carbon dioxide to be greater at 10° and 15°C than at lower temperatures, while carbon dioxide injury was much more severe at the lower temperatures.

In a study of carbon dioxide effects on phenolic metabolism in lettuce tissue, Siriphanich and Kader (27) found that 15% carbon dioxide induced phenylalanine ammonia lyase activity which correlated well with the development of brown stain. Phenolics production and polyphenol oxidase activity were reduced in the presence of carbon dioxide, but once the lettuce tissue was removed to air, phenolic oxidation resumed and brown stain symptoms became visible. Siriphanich and Kader (28) reported that 15% carbon dioxide prevented the development of cinnamic acid-4-hydroxylase in lettuce tissue. Subsequent removal of carbon dioxide did not allow the enzyme development to proceed, whereas total phenolic content increased and browning occurred. Thus, the effects of carbon dioxide on inhibition of lettuce tissue browning does not appear to involve this enzyme. Subjecting lettuce tissue to 15% carbon dioxide at 0°C for 6 days resulted in a decrease of about 0.4 and 0.1 pH units in the cytoplasm and vacuole, respectively. However, once the lettuce was removed to air an increase in pH was noted (29). They also found that lettuce kept in air had a higher glucose-6-phosphate content than carbon dioxide-treated lettuce. Exposure of lettuce at 0°C to light reduced carbon dioxide injury by about 50% relative to tissue kept in the dark. The possible involvement of reduced energy supply in carbon dioxide injury of lettuce merits further investigation.

Pathological Breakdown

CA conditions delay senescence of fresh horticultural crops and consequently reduce their susceptibility to pathogens. On the other hand, unfavorable CA conditions to a given commodity can induce physiological breakdown and render it more susceptible to pathogens. CA may also directly influence the pathogens and reduce postharvest decay problems. The oxygen and carbon dioxide concentrations required to inhibit growth and/or spore germination vary with the species of fungi, but generally oxygen levels below 1% and/or carbon dioxide levels above

10% are needed to significantly suppress fungal growth (12). However, not all horticultural commodities will tolerate such concentrations of oxygen and carbon dioxide without physiological injury.

Carbon monoxide at 5 to 10% is a fungistatic gas which suppresses fungal growth. The relative effectiveness of CO depends on the pathogen and is greatly enhanced when it is combined with less that 5% oxygen (12,18). However, the mode of CO action on pathogens is not known.

Conclusions and Future Research Needs

It is clear from this brief overview that CA can influence, directly or indirectly, all causes of postharvest deterioration of fresh horticultural crops. Knowing the relative importance of these deterioration factors for a given commodity and the potential effects of CA on each factor, it would be possible to predict the extent of benefit from using CA and to select the optimum range of oxygen and carbon dioxide levels. In order to better understand the mode of action of reduced oxygen and elevated carbon dioxide concentrations on fresh horticultural crops, further studies are needed to elucidate CA effects on the following aspects of postharvest deterioration:

1. Regulation of the glycolytic pathway and energy production associated with the respiratory metabolism.

2. Accumulation and metabolism of ethanol and acetaldehyde.

3. Site of carbon dioxide action in the ethylene biosynthetic pathway.

4. Mode of action in inhibiting or reducing ethylene effects and metabolism.

5. Effects on membranes of the plant cell and its organelles.

6. Influence on enzymes involved in cell wall metabolism.

7. Alteration of synthesis and metabolism of phenolic compounds, lignin, and aroma volatiles.

8. Effects on cell division and enlargement and levels of endogenous plant hormones which regulate growth and development in harvested plant organs.

9. Physiological and biochemical basis of CA-induced physiological disorders.

10. Mode of action of reduced oxygen, elevated carbon dioxide, and CO on postharvest pathogens.

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