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Respiration and Gas Exchange

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I. AN OVERVIEW OF RESPIRATORY METABOLISM

Respiration (i.e., biological oxidation) is the oxidative breakdown of complex substrate molecules normally present in plant cells—such as starch, sugars, and organic acids—to simpler molecules such as CO_2 and H_2O. Concomitant with this catabolic reaction is the production of energy and intermediate molecules that are required to sustain the myriad of anabolic reactions essential for the maintenance of cellular organization and membrane integrity of living cells. Maintaining an adequate supply of adenosine triphosphate (ATP) is the primary purpose of respiration. The overall process of aerobic respiration involves the regeneration of ATP from ADP (adenosine diphosphate) and P_i (inorganic phosphate) with the release of CO_2 and H_2O. If hexose sugar is used as the substrate, the overall equation can be written as follows:

$$C_6H_{12}O_6 + 6\ O_2 + 38\ ADP + 38\ P_i \rightarrow 6\ CO_2 + 6\ H_2O + 38\ ATP + 686\ kcal$$

The components of this reaction have various sources and destinations. The 1 mole of glucose (180 g) can come from stored simple sugars (e.g., glucose, sucrose) or complex polysaccharides (e.g., starch). Fats and proteins can also provide substrates for respiration, but their derivatives (e.g., fatty acids, glycerol, and amino acids) enter at later stages in the overall process and as smaller, partially metabolized molecules. The 192 g of O_2 (6 moles $\times$ 32 g/mole) used to oxidize the 1 mole of glucose diffuses into the tissue from the surrounding atmosphere, while the 6 moles of CO_2 (264 g) diffuses out of the tissue. The 6 moles of water (108 g) produced is simply incorporated into the aqueous solution of the cell.

There are three fates for the energy (686 kcal/mole of glucose) released by aerobic respiration. Around 13 kcal is lost due to the increase in entropy when the complex glucose

molecule is broken down into simpler molecules. Of the remaining 673 kcal capable of doing work, around 281 kcal (about 41% of the total energy produced) is used to produce 38 ATP molecules (38 ATP × 7.4 kcal/ATP). The remaining 392 kcal (57%) is lost as heat. In actuality, most of the energy is lost as heat, since even the energy transferred to ATP is released and a portion lost every time a subsequent reaction occurs in which energy is transferred. These values have been verified by calorimetric measurements on harvested plant organs.

Aerobic respiration involves a series of three complex reactions, each of which is catalyzed by a number of specific enzymes that either (a) add a phosphate group to a molecule, (b) rearrange the molecule, or (c) break down the molecule to a simpler one (Biale, 1960; Davies, 1980; Forward, 1965; Kays, 1991). The three interconnected metabolic pathways are glycolysis, the tricarboxylic acid (TCA) cycle, and the electron transport system.

A. Glycolysis

Glycolysis (i.e., the breakdown or *lysing* of glucose), which occurs in the cytoplasm, involves the production of two molecules of pyruvate from each molecule of glucose. Each of the 10 distinct sequential reactions in glycolysis is catalyzed by one enzyme. A key enzyme in glycolysis is phosphofructokinase (PFK), which cleaves fructose 1,6-diphosphate into two triose phosphate molecules. Cells can control their rate of energy production by altering the rate of glycolysis, primarily through controlling PFK activity. One of the products of respiration, ATP, is used as a negative feedback inhibitor to control the activity of PFK (Davies, 1980; Solomos, 1983). Besides pyruvate, glycolysis also produces two molecules of ATP and two molecules of NADH (reduced nicotinamide adenine dinucleotide) from the breakdown of each molecule of glucose.

B. Tricarboxylic Acid (TCA) Cycle

The TCA cycle, which occurs in the mitochondrial matrix, involves the breakdown of pyruvate into CO_2 in nine sequential enzymatic reactions. Pyruvate is decarboxylated to form acetate, which condenses with a coenzyme to form acetyl CoA. This compound then enters the cycle by condensation with oxaloacetate to form citric acid. Citric acid has three carboxyl groups, from which the cycle derives its name. Through a series of seven successive rearrangements, oxidations, and decarboxylations, citric acid is converted back into oxaloacetate, which is then ready to accept another acetyl CoA molecule. Besides producing the many small molecules that are used in the synthetic reactions of the cell, the TCA cycle also produces one molecule of $FADH_2$ (reduced flavin adenine dinucleotide) and four molecules of NADH for each molecule of pyruvate metabolized.

C. Electron Transport System

The electron transport system, which occurs in the cristae of the mitochondria, involves the production of ATP from the high-energy intermediates $FADH_2$ and NADH. The energy contained in a molecule of NADH or $FADH_2$ is more than is needed for most cellular processes. In a series of reactions, one NADH molecule produces three ATP molecules, while one $FADH_2$ molecule produces two ATP molecules. However, since production of ATP is not directly coupled to specific enzyme reactions but proceeds through the chemiosmotic process, the exact number of ATP molecules produced during electron transport

depends not only on the energy contained in NADH and $FADH_2$ but also on the chemical environment (i.e., pH and ion concentrations) within the cell and mitochondria.

In the chemiosmotic process, the movement of hydrogen ions (H^+) across the inner membrane in the mitochondria (and the subsequent countermovement of electrons) establishes an electrical potential gradient across the membrane. The energy to establish this gradient is furnished by the NADH and $FADH_2$ generated in the TCA cycle. Specific transmembrane enzyme complexes called ATPases bridge the membrane and establish a conduit (a proton channel) by which the protons (H^+) can flow across the membrane to reestablish electrical neutrality. This flow drives the synthesis of ATP.

In the absence of O_2, NADH and $FADH_2$ accumulate, and as their oxidized forms (NAD^+ and FAD) are consumed, the TCA cycle comes to a halt and glycolysis becomes the sole source of ATP production. Regeneration of NAD^+ is absolutely essential for the survival of the anaerobic cell.

Anaerobic respiration involves the conversion of hexose sugars into alcohol and CO_2 in the absence of O_2. Pyruvate produced through glycolysis (a series of reactions that do not require O_2) is decarboxylated by the enzyme pyruvate carboxylase to form CO_2 and acetaldehyde. The acetaldehyde is converted by the enzyme alcohol dehydrogenase to ethanol with the regeneration of NAD^+. Two moles of ATP and 21 kcal of heat energy are produced in anaerobic respiration (i.e., alcoholic fermentation) from each molecule of glucose.

The oxygen concentration at which a shift from predominately aerobic to predominately anaerobic respiration occurs varies among tissues and is known as the extinction point, or the anaerobic compensation point. Since O_2 concentration at any point within a large vegetable will vary due to differing rates of gas diffusion and respiration, some parts of the commodity may become anaerobic while other parts remain aerobic. In some nonacidic vegetables, NAD^+ is regenerated during the conversion of pyruvate to lactate. Unlike the anaerobic production of ethanol, the anaerobic production of lactate does not involve a decarboxylation; therefore no CO_2 is released.

II. SIGNIFICANCE OF RESPIRATION IN POSTHARVEST BIOLOGY

A. Shelf Life and Respiration Rate

In general, there is an inverse relationship between respiration rates and the postharvest life of fresh vegetables. The higher the respiration rate, the more perishable (shorter postharvest life) the commodity, as shown in Table 1. Respiration plays a major role in the postharvest life of fresh vegetables for the reasons given below.

1. Loss of Substrate

Use of various substrates in respiration can result in loss of food reserves in the tissue and loss of taste quality (especially sweetness) and food value to the consumer. For certain commodities that are stored for extended periods of time, such as onions (*Allium cepa* L.) for dehydration, the loss of dry weight due to respiration can be significant. When hexose sugar is the substrate, 180 g of sugars are lost for each 264 g of CO_2 produced by the commodity.

2. Oxygen Requirements

An adequate O_2 concentration must be available to maintain aerobic respiration. This should be considered in selecting the various postharvest handling procedures, such as

Table 1 Classification of Vegetables According to Their Relative Rates of Respiration and Degrees of Perishability

Class	Range of respiration rates (ml CO_2/kg · h) at 5°C	Vegetables	
		Intact	Fresh-cut
Very low	<5	Cassava, garlic, honeydew melon, onion, parsnip, potato (mature), radish (topped), rutabaga, sweet potato, taro, turnip, watermelon, winter squash, pumpkin	
Low	5–10	Beet, cabbage, cantaloupe, carrot (topped), celeriac, celery, chayote, Chinese cabbage, cucumber, head lettuce, Jerusalem artichoke, jicama, kohlrabi, pepper, potato (immature), radish (with tops), rhubarb, summer squash, tomatillo, tomato	Diced pepper, grated red beet, potato slices
Moderate	11–20	Carrot (with tops), cauliflower, Chinese water chestnut, eggplant, giant garlic, green lima beans, green snap beans, kale, leaf lettuce, leek, okra, salsify	Cantaloupe cubes, carrot sticks and slices, cucumber slices, onion rings, peeled garlic, shredded cabbage and head lettuce, squash slices
High	21–30	Artichoke, bean sprouts, bitter melon, Brussels sprouts, Chinese chives, endive, green onions, spinach, watercress	Cauliflower florets, leek rings, cut-salad mixes of leafy lettuces, chicory, endive, arugula, and/or radiccio
Very high	>30	Arugula, asparagus, broccoli, mushrooms, parsley, peas, sweet corn	Broccoli florets, sliced mushrooms, shelled peas

Source: Gorny, 1997; Hardenburg et al., 1986; Murata et al., 1992; Peiris et al., 1997; Robinson et al., 1975; Ryall and Lipton, 1979; and van den Berg and Lentz, 1972.

waxing and other surface coatings, film wrapping, and packaging. On the other hand, reduction of O_2 concentration to less than 10% provides a tool for controlling respiration rate and slowing down senescence (see Chapter 9, "Atmosphere Modification").

3. Carbon Dioxide Production

Accumulation of CO_2 produced by the commodity in its ambient atmosphere can be beneficial or harmful, depending upon each commodity's tolerance to elevated CO_2 levels. For some vegetables, increasing the CO_2 concentration around them in a controlled or modified atmosphere can be used to delay senescence and retard fungal growth.

4. Release of Heat Energy

The heat produced by respiration (vital heat), which is about 673 kcal for each mole of sugar (180 g) utilized, can be a major factor in establishing the refrigeration requirements during transport and storage. Vital heat must be considered in selecting proper methods for cooling, package design, method of stacking packages, and refrigerated storage facilities (i.e., refrigeration capacity, air circulation, and ventilation).

B. Meaning of the Respiratory Quotient (RQ)

The composition of a commodity frequently determines which substrates are utilized in respiration and consequently the respiratory quotient (RQ). The RQ is defined as the ratio of CO_2 produced to O_2 consumed (measured in moles or volumes). Depending on the substrate being oxidized, RQ values for fresh vegetables range from 0.7 to 1.3 for aerobic respiration. When carbohydrates are being aerobically respired, the RQ is near 1, while it is <1 for lipids and >1 for organic acids. Very high RQ values usually indicate anaerobic respiration in those tissues that produce ethanol. In such tissues, a rapid change in the RQ can be used as indication of the shift from aerobic to anaerobic respiration.

III. GAS EXCHANGE

A. Barriers to Diffusion

Gas exchange between a plant organ and its environment follows Fick's first law of diffusion. The sequential steps are (a) diffusion in the gas phase through the dermal system (i.e., cuticle, epidermis, stomata, lenticels, etc.); (b) diffusion in the gas phase through intercellular spaces; (c) exchange of gases between the intercellular atmosphere and the cellular solution (cell sap) or vice versa; and (d) diffusion in solution within the cell to centers of O_2 consumption and from centers of CO_2 production. This exchange is a function of the resistance of the dermal system to gas diffusion, the distribution of the intercellular spaces, the tortuousness of the diffusive path, the surface area across which diffusion can take place, the solute concentration of the tissue, and the gradient in gas concentration established by the respiratory activity of the tissue.

Carbon dioxide produced within each cell will raise the local concentration and the gradient produced will drive diffusion of CO_2 outward, toward the lower concentration near the cell-wall surface adjacent to the intercellular space. Diffusion of CO_2 into the intercellular space continues toward regions of lower concentration until it reaches the intercellular space below the dermal system. From there, CO_2 moves through the cuticle or openings in the commodity's surface to the ambient air (Burton, 1982).

Gradients of O_2 within plant tissues are established in a reverse but analogous process to that mentioned above for CO_2. In senescent tissues, O_2 diffusion may become so impeded if the intercellular spaces become filled with cellular solution that anaerobic conditions develop within the tissue.

The rate of gas movement depends on the properties of the gas molecule, the magnitude of the gradient, and the physical properties of the intervening barriers (thickness, surface area, density, and molecular structure). Both the solubility and diffusivity of each gas are important for its diffusion across barriers. Carbon dioxide moves more readily than O_2, while diffusion rates of C_2H_4 and CO_2 are similar.

In leaves, gas diffusion is regulated by control of the stomatal aperture by guard cells, but most bulky organs have no functional stomata or other active controls of gas exchange. A number of other factors influence gas diffusion in bulky organs: they have a much lower surface-to-volume ratio than leaves; the distance over which gases must diffuse in the tissue is relatively large compared to leaves; and respiration, not photosynthesis, is the major metabolic process (i.e., reactions producing CO_2 and consuming O_2, rather than the reverse).

Internal concentrations of O_2 and CO_2 in plant organs depend upon the maturity stage at harvest, the current organ temperature, the composition of the external atmosphere, and any added barriers. Maturity stage influences the respiration rate and the components of the dermal system that affect gas diffusion, such as the development, composition, and thickness of the cuticle, epidermal hairs, trichomes, and lenticels. Increased temperatures raise the rate of respiration and, in response, the internal CO_2 concentration increases as the O_2 concentration decreases. If all other factors are held constant and if the gradient in gas concentrations is the driving force for diffusion, then the concentrations of O_2 and CO_2 within the tissue will fluctuate in accord with fluctuations in the external atmosphere. For example, a change in the external concentration from 21% to 15% for O_2 (i.e., a decrease of 6%) and from 0.03% to 3% for CO_2 (i.e., an increase of 3%) would cause a concomitant decrease in internal O_2 by 6% and an increase in CO_2 by 3%. However, these changes could affect respiration and produce different outcomes, especially if the gas concentrations exceed the tolerance limits (see Chapter 9, ''Atmosphere Modification'').

B. Methods to Alter Rates of Gas Exchange

There are three types of barriers to gas exchange that affect the postharvest handling of fresh produce (Fig. 1). At the level of the commodity, the structure of the dermal system (e.g., thickness of the cuticle; wax composition and arrangement on the surface; number and distribution of stomata, lenticels, and breaks in the epidermis) represent the first significant barrier to gas diffusion. Resistance to gas diffusion can be increased by added barriers such as wax coatings and wrapping with polymeric films. The package in which the commodity is shipped can be an additional barrier to gas diffusion. Its significance will depend upon the permeability of the package materials, extent of ventilation openings, and use of plastic liners within the package. Furthermore, the degree of gas tightness of the transit vehicle or storage room will also affect gas exchange with outside air. All these barriers must be considered from the standpoint of providing the optimum O_2 and CO_2 concentrations within each commodity that will maximize its postharvest life.

Fick's first law of diffusion states that the movement or flux of a gas in or out of a plant tissue depends on the concentration drop across the barrier involved, the surface area of the barrier, and the resistance of the barrier to diffusion. A simplified version of Fick's law can be written as follows (for CO_2):

$$J_{CO_2} = \frac{A \cdot \Delta C_{CO_2}}{R_{CO_2}}$$

where J = total flux of CO_2 ($cm^3 \cdot s^{-1}$)

A = surface area of the barrier (cm^2)

ΔC = concentration gradient across the barrier

R = resistance to diffusion of CO_2 ($s \cdot cm^{-1}$)

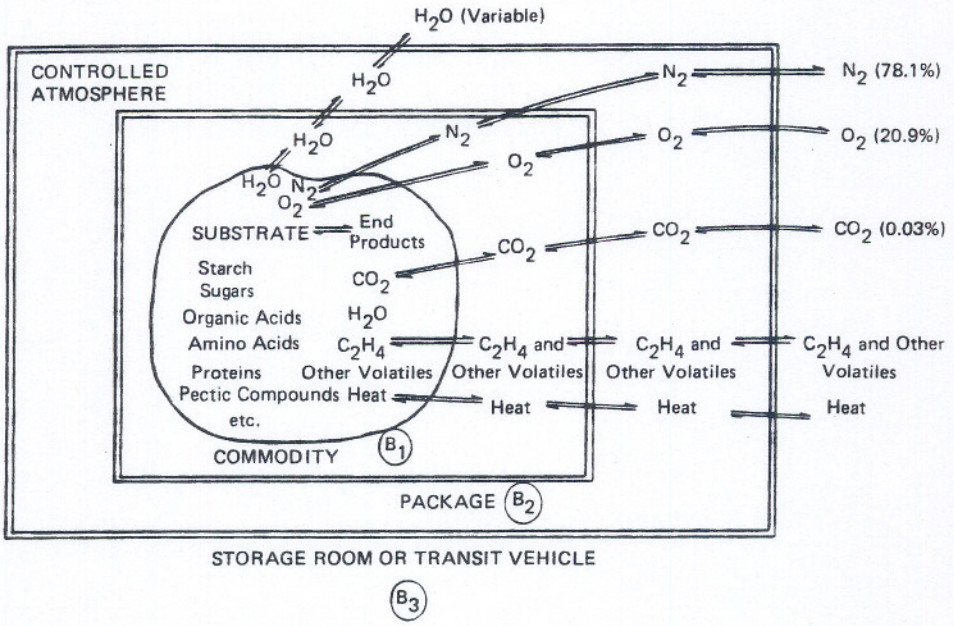

Figure 1 Schematic model of a commodity and its environment illustrating three levels of barriers to gas exchange: B_1 = structure of the commodity's dermal system and added barriers (e.g., waxing, film wrapping), B_2 = the package's permeability to gas diffusion, and B_3 = the degree of gas tightness of the storage room or transit vehicle.

The resistance of tissues and organs to diffusion of CO_2, O_2, and C_2H_4 has been investigated using the steady-state approach (Burg and Burg, 1965; Cameron and Reid, 1982). The production (or consumption) rate of the gas by the organ and the concentrations of the gas in the internal and external atmospheres are determined, then the resistance is calculated as follows:

$$R = \frac{\text{Concentration gradient}}{\text{Production (or consumption) rate}}$$

Accurate measurement of the internal concentration of gases is often difficult. Although internal samples are easily withdrawn from fruits with internal cavities, such as cantaloupe melons (*Cucumis melo* L. Reticulatus group), extraction methods (Saltveit, 1982) used to determine the internal atmospheric composition of some bulky organs, such as potatoes (*Solanum tuberosum* L.), are less satisfactory and can yield inconsistent results.

IV. MEASUREMENT OF RESPIRATION RATE

A. Intact Tissues

Measurement or estimation of respiration rate can be based on determination of the loss of dry weight, O_2 consumption, CO_2 production, heat production, or loss of energy content (Biale, 1960). Determination of losses in dry weight and energy content are destructive to the tissue and are difficult to carry out. Thus, these methods are seldom used. Heat

production can be measured using a calorimeter, but the complexity of the instrument, the small sample size that most instruments will accommodate, and the time required for setup and analysis make this method mainly of research interest. Measurement of the production and consumption of respiratory gases by the commodity is the most convenient and widely used method for measuring the respiration rate of fresh produce. Following is a brief description of methods for measurement of respiration (i.e., O_2 consumption or CO_2 production) rates in harvested vegetables.

1. Closed System

Commodity samples are placed in a sealed container and the concentrations of CO_2 and/or O_2 in the atmosphere are measured at the beginning and end of a specified period of time (usually 1 h) (Fig. 2). The respiration rate (expressed as ml $CO_2 \cdot kg^{-1} \cdot h^{-1}$ and ml $O_2 \cdot kg^{-1} \cdot h^{-1}$) can be calculated knowing the change in gas concentration, the time interval, the weight of the commodity, and the effective volume of the container into which the gases diffuse. Since the solubility of CO_2 in water is close to 1 ml $\cdot$ ml^{-1} at biological temperatures (0 to 30°C), very little error is introduced into the calculations by taking the effective volume of the container as its void volume. In contrast, the much lower solubility of O_2 and C_2H_4 require that the volume of the commodity be subtracted from the void volume to give the effective volume of the container.

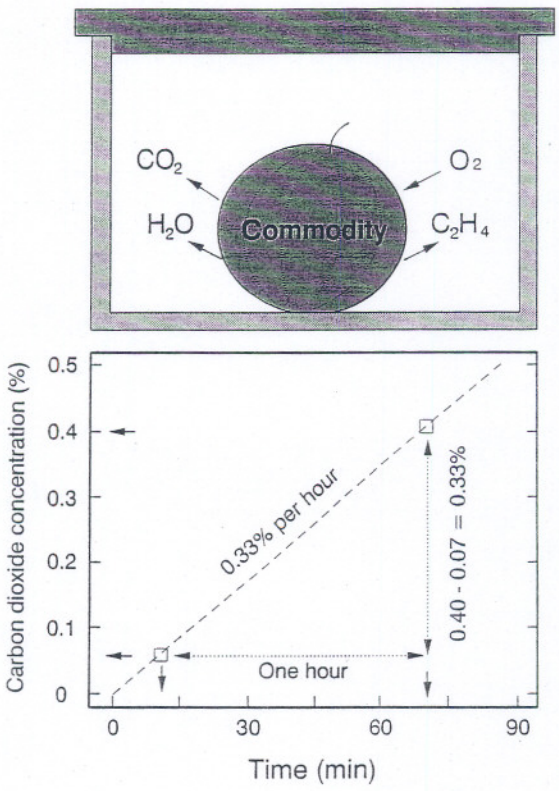

Figure 2 Diagrammatic representation of a closed system to measure respiration. The graph shows the increase of CO_2 in the container over time.

The advantages of the closed system are that it is easy and fast to set up and use. With the proper selection of tissue weight and container volume, the respiration rate can be measured within an hour. Its disadvantages are that it is a nonequilibrium system and that the depletion of O_2 and accumulation of CO_2 or other gases (especially C_2H_4) may affect the tissue and its respiration rate. These problems can be minimized by preventing CO_2 from accumulating above 0.2% and by keeping the length of the test to the shortest duration necessary to accumulate sufficient CO_2 to measure accurately. Gases that may alter the tissues' respiration rate and interfere with accurate measurements, such as CO_2 and C_2H_4, may be absorbed using KOH and $KMnO_4$, respectively.

2. Flowing System

Commodity samples are placed in a sealed container, which is ventilated at a known flow rate with humidified, CO_2- and C_2H_4-free air (Fig. 3). A period of time is required for the system to come to equilibrium; 95% of equilibrium is usually reached after three container volumes of gas have flowed through the system. The gas streams are periodically sampled and the concentration of gases in the inlet and outlet streams is measured by gas chroma-

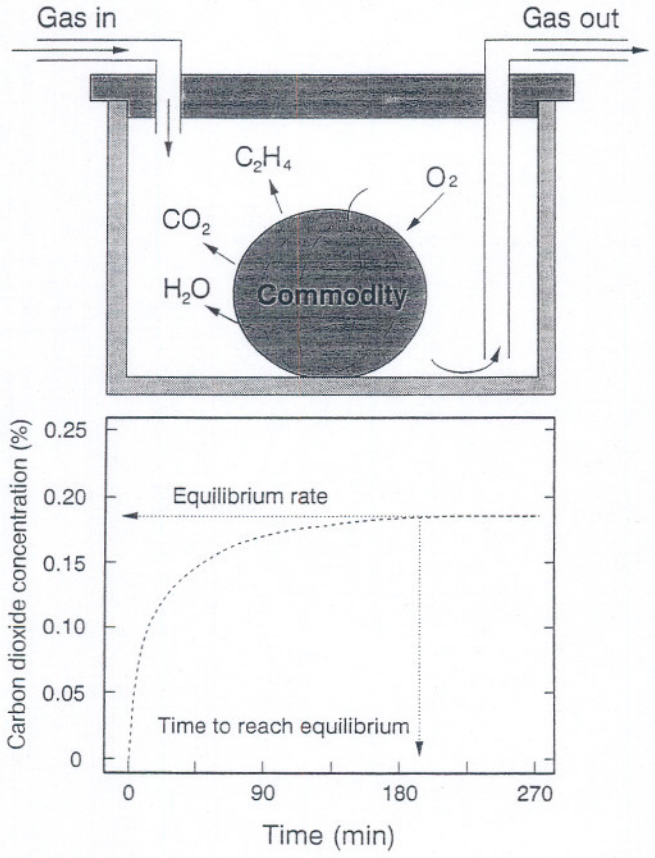

Figure 3 Diagrammatic representation of a flowing system to measure respiration. The graph shows the increase of CO_2 in the container over time as it reaches equilibrium.

tography (GC), infrared spectroscopy for CO_2, or paramagnetic, polarographic, or electrochemical oxygen analyzers for O_2. Differences in the concentrations of CO_2 and O_2 between the inlet and outlet gas streams are then used to calculate the respiration rate using commodity weight, flow rate, and concentration of CO_2 or O_2. Alternatively, the exit stream can be passed through a column containing a suitable CO_2 absorber, such as NaOH, which absorbs the respired CO_2. The amount of CO_2 produced during a specific duration is determined by subsequent titrimetric or gravimetric analysis of the absorbing material. Respiration rate is usually expressed as the volume or weight of CO_2 evolved or O_2 consumed per kilogram of fresh weight of the commodity per hour.

The advantages of the flowing system are that it can be operated for extended periods of time and the composition of the flowing gas mixture can be actively modified for prolonged periods. Its disadvantages are that it takes much longer to set up than the closed system, the gas flow must be humidified, the rate of flow must remain constant for the duration of the experiment, and it often takes a long time for the system to come to equilibrium.

B. Internal Gas Concentration

The concentration of gases within a commodity is usually different from that in the exterior atmosphere because of the combined effects of tissue respiration and significant internal resistances to gas diffusion. Because rates of respiration and barriers to gas diffusion differ among commodities and even within the same commodity over time, a specific combination of gases can rarely be equated with a specific rate of respiration. However, internal gas concentrations are also important to know because they are more indicative of the biological activity of the gases within the commodity than are their rates of evolution from the commodity. This is especially true for CO_2 and C_2H_4.

Internal gases can be sampled by a number of destructive and nondestructive methods (Saltveit, 1982). A thin hypodermic needle attached to a syringe can be used to penetrate the tissue and take samples at any location within the tissue. Clogging of the needle with tissue or fluids is much less of a problem when samples are taken from cavities within the commodity. Flexible cups sealed to the surface of the commodity can be used to obtain samples of gases in equilibrium with gases immediately below the surface. Vacuum extraction in air or under an aqueous salt solution can provide a composite sample of the gases within the commodity. These methods are replete with many opportunities for artifacts to compromise the observations, and care must be taken in their implementation. For example, too high a vacuum or too long an immersion can significantly alter the concentrations of the gases extracted, while insertion of a needle can lead to wound responses and microbial inoculation of the tissue.

C. Mitochondrial Respiration

Mitochondria can be extracted from plant tissue and their respiration rate measured as O_2 uptake, using an oxygen electrode. The tissue is gently broken in a buffer containing an osmoticum, a sulfhydryl reagent (e.g., dithiothreitol), bovine serum albumin, and polyvinyl pyrrolidone. The homogenate is filtered, centrifuged at 4000 $\times$ g for 10 min to remove large cell debris, and then the supernatant is centrifuged at 10,000 $\times$ g for 10 min. The pellet (largely mitochondria) is resuspended in the extraction buffer, and the centrifugation steps are repeated. The final mitochondrial pellet is resuspended in buffer and is then ready for examination.

Respiration of mitochondria is measured by placing a small amount of the mitochondrial suspension in a small, temperature-controlled cell fitted with a polarographic electrode to measure the O_2 content of the solution. Substrate (usually succinate or ketoglutarate) is added and O_2 uptake from the solution is recorded on a chart recorder connected to the electrode. This system has been used to determine many of the important control points in respiratory metabolism and to study the effects of environmental factors on respiration rate.

V. FACTORS AFFECTING RESPIRATION RATE

A. Commodity (Internal) Factors

1. Type of Commodity and Genotype

Vegetables vary greatly in their respiration rates (Table 1). Root, tuber, and bulb vegetables have low respiration rates. Fruit-type vegetables that are picked mature, such as tomato (*Lycopersicon esculentum* Mill.) and melons (*Cucumis melo* L.), respire at a lower rate than those picked immature, such as green beans (*Phaseolus vulgaris* L.), peas (*Pisum sativum* L.), sweetcorn (*Zea mays* L. var. rugosa Bonaf.), and okra [*Abelmoschus esculentus* (L.) Moench.]. Plant parts with vegetative floral meristematic tissues, such as asparagus (*Asparagus officinalis* L.), broccoli (*Brassica oleracea* L., Botrytis group), and green onions, have very high respiration rates. In general, the degree of perishability of fresh vegetables is directly proportional to their respiration rates.

Differences among plant parts in the surface area–to-volume ratio and in the nature of their surface coatings (e.g., cuticle thickness, stomata, lenticels) influence their gas-diffusion characteristics and consequently their respiration rates. Such differences are also responsible for genotypic variation in respiratory activity within a given commodity, as shown for lettuce (*Lactuca sativa* L.) types in Figure 4. Preharvest factors, such as climatic conditions and cultural practices, can also affect the morphological and compositional characteristics of a given genotype, which, in turn, influences its respiration rate.

2. Stage of Development at Harvest

The respiration rate is usually very high during the early stages of development and decreases as plant organs mature. Thus, vegetables harvested during the active growth phase—such as leafy, floral, and immature fruit-type vegetables—have high respiration rates. Generally, the respiration rate declines steadily after harvest; the decrease is slow in mature fruit-type vegetables and rapid in vegetative tissues and immature fruit-type vegetables. This rapid fall reflects depletion of respirable substrates, which are typically low in such tissues.

An exception to the declining pattern of postharvest respiration is the respiration of certain fruit-type vegetables that undergo a definite ripening phase on or off the plant. These vegetables show a marked rise in respiration, which has been called the "climacteric pattern" and is normally considered as having four phases: preclimacteric minimum, climacteric rise, climacteric peak, and postclimacteric phase (Fig. 5). Fruit-type vegetables that exhibit a climacteric pattern of respiration include cantaloupe melons, Crenshaw and honeydew melons (*Cucumis melo* L. Inodorus group), chili pepper (*Capsicum annuum* L. Longum group), tomato, and watermelon [*Citrullus lanatus* (Thunb.) Matsum. & Nak.]. In contrast, nonclimacteric fruit-type vegetables—which include green bean, pea, cucumber (*Cucumis sativus* L.), summer squash (*Cucurbita pepo* L.), casaba melon (*Cucumis melo*

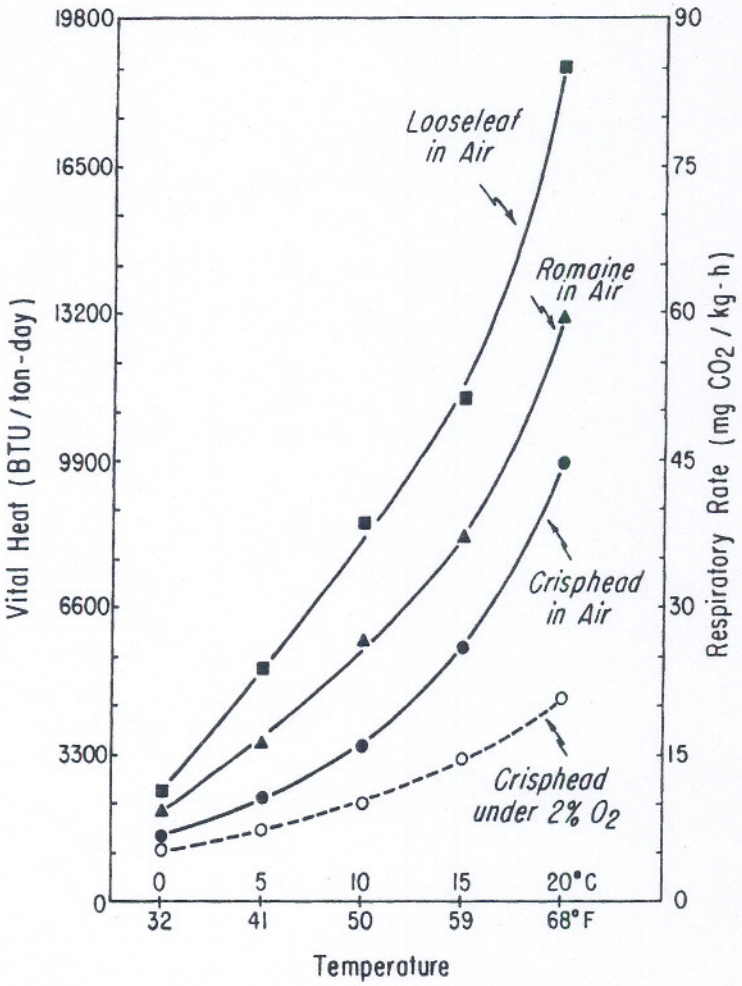

Figure 4 Effect of temperature on respiration rate and vital heat production by three types of lettuce. Also shown is the effect of 2% O_2 on reducing respiration rate of crisphead lettuce. (From Morris et al., 1974).

L. Inodorus group), eggplant (*Solanum melongena* L.), bell pepper (*Capsicum annuum* L. Grossum group), okra, and some tomato mutants (e.g., *rin* tomato)—exhibit a steady decline in respiration after harvest. The division between climacteric and nonclimacteric fruits is not absolute, as the magnitude of the respiratory rise varies from less than 20% to over 100% among cultivars and species. This compares with the hundred- to thousandfold increase in the production of the plant hormone C_2H_4, which usually accompanies the respiratory rise in ripening climacteric (see Chapter 29, "Mature Fruit Vegetables," for a more detailed discussion of the climacteric).

In climacteric fruit-type vegetables, the climacteric rise in respiration, which reflects enhanced metabolic activities, occurs at the transition from the growth phase of the fruit to its senescence phase. It coincides with an increase in the rate of C_2H_4 production and

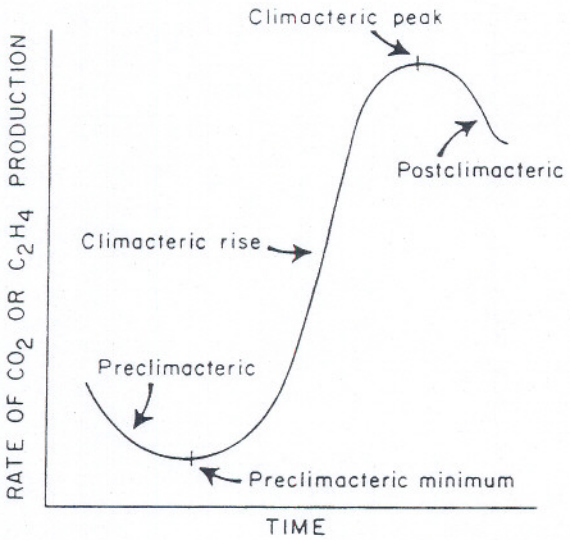

Figure 5 Phases of the respiratory climacteric in a ripening climacteric fruit. (From Watada et al., 1984.)

with changes associated with ripening, such as color changes, softening, increased tissue permeability, and development of characteristic aroma. The respiratory climacteric appears to be regulated by changes in the compartmentation of substrates, activators, and inhibitors rather than by de novo synthesis of glycolytic enzymes or by mitochondrial biogenesis (Biale and Young, 1981; Rhodes, 1980).

Exposure to C_2H_4 stimulates the respiration of vegetative tissues and both climacteric and nonclimacteric fruit tissues. At a certain phase in the maturation of climacteric fruit, C_2H_4 switches from being an inhibitor of its own production to being a promoter of its own synthesis (i.e., autocatalytic C_2H_4 production). This switch from a negative to a positive feedback of C_2H_4 synthesis may better account for the respiratory differences between climacteric and nonclimacteric fruit than other proposed mechanisms.

3. Chemical Composition

Generally, the respiration rate decreases with a decrease in water content of the tissue. The substrate (e.g., carbohydrates, proteins, lipids, organic acids, etc.) that is predominately utilized in respiratory metabolism usually dictates the value of the RQ.

B. Environmental (External) Factors

1. Temperature

Temperature is the most important environmental factor in the postharvest life of fresh vegetables because of its dramatic effect on rates of biological reactions, including respiration (Fig. 4). Within the physiological temperature range, the velocity of a biological reaction increases two- to threefold for every 10°C rise in temperature (Van't Hoff rule). The ratio of reaction rates at two dissimilar temperatures is called the temperature coeffi-

cient or Q_{10} if the interval between the two temperatures is 10°C. To allow easy comparisons among Q_{10} values obtained from measurements made over temperature intervals of not exactly 10°C, the Q_{10} can be calculated by applying the following equation:

$$Q_{10} = (R_2/R_1)^{10/(T_2-T_1)}$$

where R_2 = rate of respiration at T_2
R_1 = rate of respiration at T_1
T_2 and T_1 = temperatures in °C

If the difference between T_2 and T_1 is 10°C, then the Q_{10} will be the quotient of the two rates.

The Q_{10} concept allows calculation of expected respiration rates at a given temperature from a known rate at another temperature. Since Van't Hoff's time, scientists have found that the Q_{10} is not constant for most biological processes over a wide range of physiological temperatures (Tables 2 and 3). Usually, Q_{10} values range from 1 to 5, although higher values may occur. For most biological reactions, the Q_{10} is between 2 and 3 for temperatures between 10 and 30°C. That means that the reaction rate will double or triple with every 10°C rise in temperature. While normally constant over a limited temperature range, the Q_{10} of complex phenomena can change greatly over wide temperature fluctuations. These dramatic changes could be caused by different reactions dominating the overall reaction at different temperatures. The shift from one limiting reaction to another could therefore cause a rapid shift in reaction rates and Q_{10} values.

Typically, Q_{10} values are highest between 0 and 10°C, are commonly around 2 to 3 between 10 and 30°C, and continually decline to around 1 at higher temperatures. A Q_{10} of 1 indicates that the reaction rate is not changing with changes in temperature, while a Q_{10} below 1 means that the rate is actually decreasing with increasing temperature. While Q_{10} values differ widely among vegetables over a given temperature range (Table 3), a high rate of respiration does not necessarily mean that the Q_{10} will be high. For example, respiration of asparagus has a Q_{10} of 2 between 20 and 25°C when respiration is at 387 and 550 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$, respectively, while onions have a Q_{10} of 3 between 15 and 20°C when respiration is at 11 and 19 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$, respectively. The Q_{10} values

Table 2 Effect of Temperature on Respiration Rate of Vegetables

Temperature (°C)	Assumed Q_{10}	Relative velocity of respiration	Relative shelf life
0		1.0	100
	3.0		
10		3.0	33
	2.5		
20		7.5	13
	2.0		
30		15.0	7
	1.5		
40		22.5	4

Table 3 Respiration Rates (mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$) and Q_{10} Values for Selected Vegetables

Temperature	Asparagus		Cauliflower		Kale		Lettuce		Onions		Tomatoes	
(°C)	Resp.	Q_{10}	Resp.	Q_{10}	Resp.	Q_{10}	Resp.	Q_{10}	Resp.	Q_{10}	Resp.	Q_{10}
0	54		19		—		23		3		—	
		3.2		1.3		—		1.7		1.7		—
5	96		22		22		30		4		8	
		3.0		2.7		3.5		1.7		4.0		5.1
10	167		36		41		39		8		18	
		2.1		2.3		3.6		2.6		1.7		2.4
15	244		54		78		63		11		28	
		2.5		2.5		3.1		2.5		3.0		2.1
20	387		86		138		100		19		41	
		2.0		2.6		2.7		2.2		2.3		1.6
25	550		140		226		147		29		51	

for respiration rates of vegetables can vary among temperature ranges as follows:

Temperature range (°C)	Q_{10} values
0–10	2.5–4.0
10–20	2.0–2.5
20–30	1.5–2.0
30–40	1.0–1.5

Using this information, the effect of temperature on the respiration rates of vegetables is as shown in Table 2.

Significant changes in the relative rates of glycolysis and mitochondrial respiration may occur at chilling temperatures in chilling-sensitive vegetables (see Chapter 20, "Temperature Extremes"). These changes may result in higher respiration rates at chilling temperatures than at nonchilling temperatures for certain commodities, such as okra and cucumber. Another important symptom of chilling injury is the abnormally high respiration rate upon transfer to a nonchilling temperature. A sustained increase in the respiration rate usually indicates the occurrence of irreversible damage to the tissue. This enhanced respiration may be due to the tissue's efforts to repair damage to membranes and subcellular structures and/or to eliminate toxic metabolic intermediates that may have accumulated during the exposure to chilling temperatures (Wang, 1982).

As shown above, the rate of increase in respiration rates declines with an increase in temperature up to 40°C. Above 40°C the Q_{10} becomes less than 1 as the tissue nears its thermal death point (e.g., at about 50 to 55°C), when enzyme proteins are denatured and metabolism becomes disorganized. While most fresh vegetables may tolerate exposure to such high temperatures for short durations (e.g., a heat shock of a few minutes), longer exposures cause physiological injury and can lead to tissue collapse. The production of heat-shock proteins in response to brief, sublethal exposures to lethal temperatures increases the tolerance of the tissue to subsequent exposures to a number of biotic and abiotic stresses (see Chapter 20, "Temperature Extremes").

Even though they change with temperature, Q_{10} values are useful because if the reaction rate is known at 10°C intervals between 0 and 40°C, any intermediate rate can be calculated with sufficient accuracy for the prediction of refrigeration or ventilation requirements. However, Q_{10} values based on initial respiration rates at given temperatures could lead to incorrect calculated values if applied to vegetables after storage, because the produce would no longer be at the same physiological age or at the same stage of development.

a. Calculation of the Q_{10}

The easiest example to calculate is for reactions at 10°C intervals. For example, if cauliflower produces around 36 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$ at 10°C and 86 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$ at 20°C, the Q_{10} is simply calculated by dividing the respiration rate at 20°C by the rate at 10°C, giving a Q_{10} of 2.4. A more complicated example is when the temperature interval is not exactly 10°C. For example, cauliflower produces around 54 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$ (R_1) at 15°C (T_1) and 86 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$ (R_2) at 20°C (T_2). Since the temperature interval is less than 10°C, the following equation must be used:

$$Q_{10} = (R_2/R_1)^{10/(T_2-T_1)}$$
$$= (86/54)^{10/(20-15)}$$
$$= 1.59^2 = 2.53$$

The equation can also be used to calculate respiration rates at different temperatures from a known Q_{10} and a respiration rate. For example, what would the calculated respiration rate be for cauliflower at 0°C? Assume that the previously calculated Q_{10} value applies over this wide temperature range. The new values are $R_2 = 54$, $T_2 = 15°C$, $R_1 = ?$, $T_1 = 0°C$. Substituting these new values into the same equation results in the following:

$$Q_{10} = (R_2/R_1)^{10/(T_2-T_1)}$$
$$2.53 = (54/R_1)^{10/(15-0)}$$
$$= (54/R_1)^{0.67}$$
$$R_1 = 13.4$$

The actual value for CO_2 production by cauliflower at 0°C is around 19 mg $CO_2 \cdot kg^{-1} \cdot h^{-1}$, not the 13.4 calculated above. This error resulted from the fact that the Q_{10} is not constant over the temperature range from 20 to 0°C. The Q_{10} has actually been calculated to be 1.3 for 0 to 5°C, and 2.7 for 5 to 10°C.

While the temperature quotient (i.e., Q_{10}) is usually used to compare reaction rates at different temperatures (e.g., rates of chemical reactions and respiration or enzyme activity), it can also be used to express more subjective evaluations of horticultural quality (Table 3). The rate of objective and subjective changes in quality with time and temperature can also be expressed as Q_{10} values (Table 4).

2. Oxygen Concentration

Storing crisphead lettuce in 2% O_2 atmosphere significantly reduces its respiration rate, and the extent of reduction is greater as the temperature increases from 0 to 20°C (Fig. 4). The respiration rate of broccoli heads kept at 2.5°C also decreases as the O_2 concentration in their ambient atmospheres is reduced (Fig. 6). Relative to heads held in air, the reduction in respiration rate is about 28%, 36%, and 45% for broccoli heads in 2, 1, and 0.5% O_2, respectively.

Table 4 Temperature Quotient (Q_{10}) for Rate of Vegetable Deterioration

	Temperature range (°C)		
Commodity	0 to 10	10 to 20	20 to 30
Asparagus			
Appearance quality	2.7	2.4	1.8
Sugar loss	5.8	2.7	1.4
Fiber increase	10.0	2.0	2.0
Brussels sprouts (visual)	3.8	2.7	1.9
Celery (visual)	4.1	2.3	1.9
Head lettuce (visual)	2.5	2.2	1.9
Peas			
Appearance quality	3.3	2.8	2.0
Sugar loss	2.7	2.6	1.5
Spinach (visual)	3.3	2.5	1.8
Sweetcorn (sugar loss)	3.9	3.6	1.5

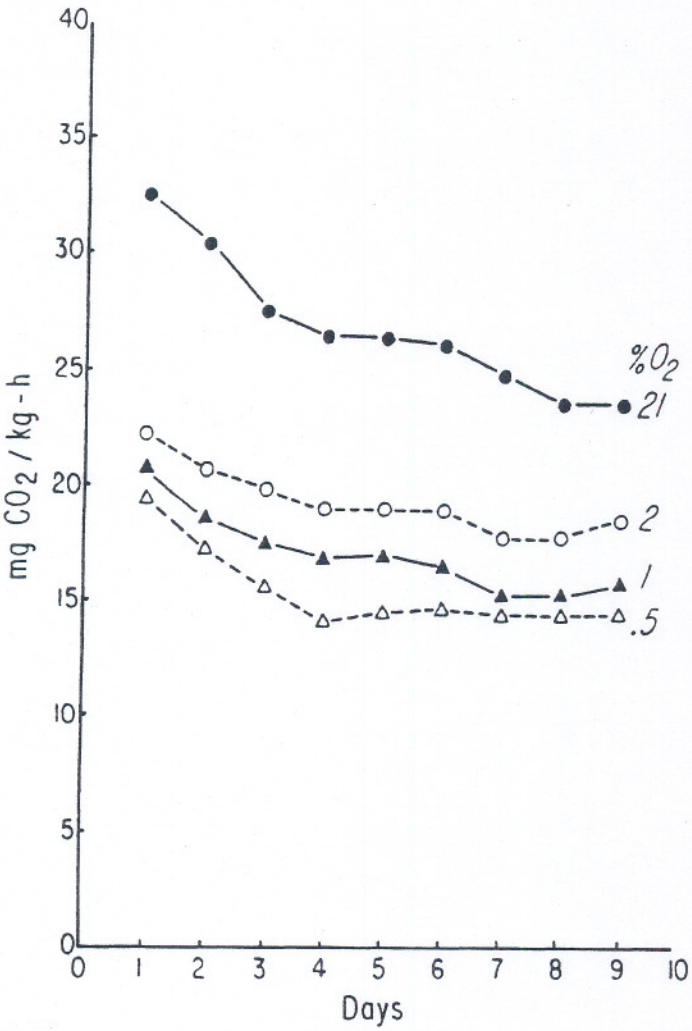

Figure 6 Effect of O_2 concentration on the respiration rate of broccoli kept at 2.5°C. (From Kasmire et al., 1974.)

A more general schematic representation of the effects of O_2 concentration on the respiration rate of fresh vegetables is shown in Figure 7. As the O_2 concentration is reduced below that in air (20.9%) and especially below 10%, a significant reduction in respiration rate is observed (Gorny, 1997; Toledo et al., 1969). However, when O_2 concentration drops to less than about 2% (the exact concentration depends on the commodity, temperature, and duration), anaerobic respiration rate becomes predominant and CO_2 production increases. Also, a substantial accumulation of fermentative metabolites—e.g., ethanol and acetaldehyde—could be toxic to plant cells.

Superatmospheric O_2 concentrations (i.e., above 20.9%) slightly stimulate the respiration rate of fresh vegetables. Above 80% O_2, the respiration rate of some commodities may increase significantly (Fig. 7) because of O_2 toxicity to their tissues.

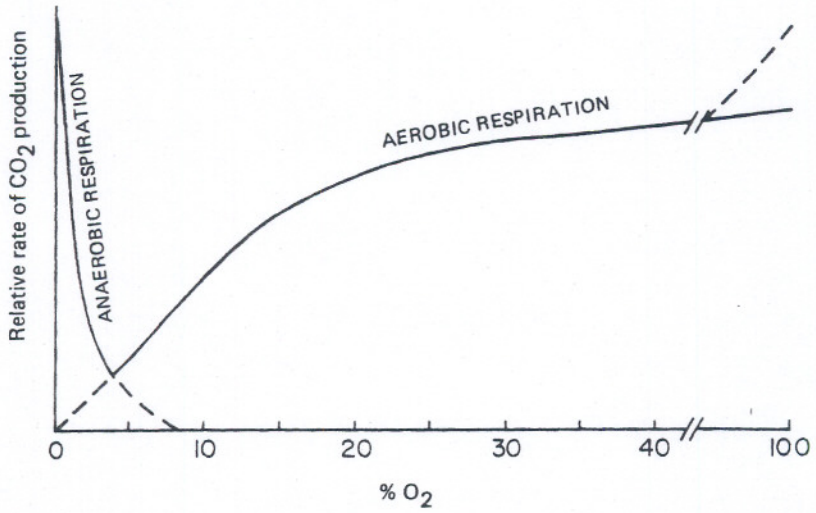

Figure 7 A schematic representation of the effects of O_2 concentration on aerobic and anaerobic respiration rates of fresh vegetables.

3. Carbon Dioxide Concentration

Elevated CO_2 levels reduce aerobic respiration (O_2 consumption) (Fig. 8). However, at CO_2 concentrations of about 20%, a significant increase in anaerobic respiration (i.e., ethanol and acetaldehyde accumulation) occurs and can irreversibly damage the tissue. Increased CO_2 levels, as in the case of O_2 depletion, inhibit the decarboxylation reactions

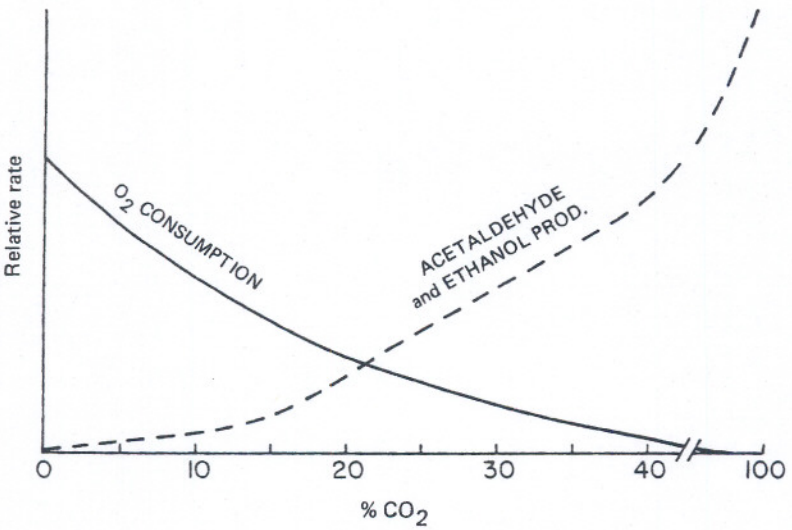

Figure 8 Schematic representation of the effects of CO_2 concentration on aerobic respiration (O_2 consumption) and anaerobic respiration (acetaldehyde and ethanol production) rates of fresh vegetables.

of normal respiration. Thus, the TCA cycle slows down, the demand for a continued supply of ATP stimulates glycolysis, pyruvate accumulates, and the NAD^+ supply is maintained by the anaerobic reduction of pyruvate to ethanol. The extent of damage depends upon CO_2 and O_2 concentrations around the commodity, temperature, and duration of exposure to these conditions. Carbon dioxide–induced physiological disorders can result in tissue injury and increased respiration rate.

4. Carbon Monoxide Concentration

Carbon monoxide at 1% to 10% added to air or controlled atmospheres reduces the respiration rate of vegetative tissues. However, as an ethylene analog, CO added to air stimulates the respiration rate of climacteric fruits, such as tomatoes. This stimulatory effect is minimized when CO is added to atmospheres in which the O_2 level is below 5%.

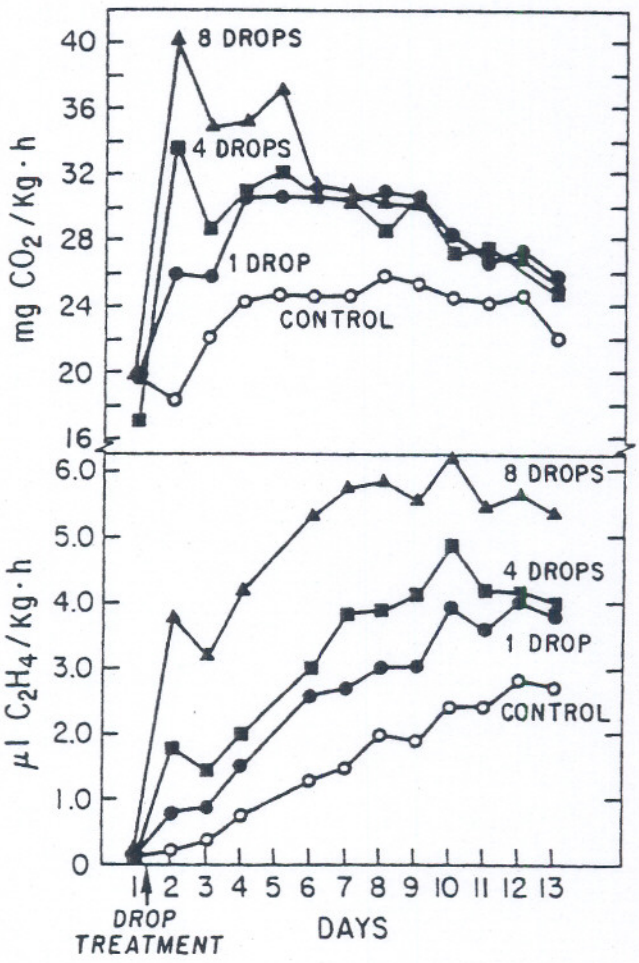

Figure 9 Effect of impact bruising on respiration and ethylene production rates of tomatoes damaged at the mature green stage and ripened at 20°C. (From MacLeod et al., 1976.)

5. Ethylene Concentration

Exposure of climacteric tissues during their preclimacteric stage to C_2H_4 shortens the time to the start of the climacteric rise in respiration. Once the respiratory rise has begun, the tissue's endogenous rate of C_2H_4 production increases and the internal C_2H_4 concentration also increases, reaching levels that saturate its biological activity. Thus, the rate of endogenous C_2H_4 production, not the added C_2H_4, controls the height of the final respiratory rise in climacteric tissues. In contrast, C_2H_4 treatment of nonclimacteric tissues, in which endogenous C_2H_4 levels are very low, induces a climacteric-like rise in respiration that is proportional to C_2H_4 concentrations. However, unlike the case in climacteric tissues, endogenous C_2H_4 production remains unaffected. Removal of C_2H_4 results in a return of the respiration rate to its pretreatment level. The respiratory response of nonclimacteric tissues to C_2H_4 can be repeatedly induced throughout their postharvest life.

6. Other Hydrocarbons

Several other hydrocarbons, such as propylene and acetylene, mimic ethylene's effects on the respiration rate, ripening, and senescence of harvested vegetables.

7. Stresses

Physical stress stimulates the respiration rate of fresh vegetables. For example, impact bruising of mature-green tomatoes increases their rates of respiration and ethylene production during subsequent ripening at 20°C (Fig. 9). The extent of this increase in respiration rate is usually proportional to the severity of bruising; however, extensive injury can actually depress respiration.

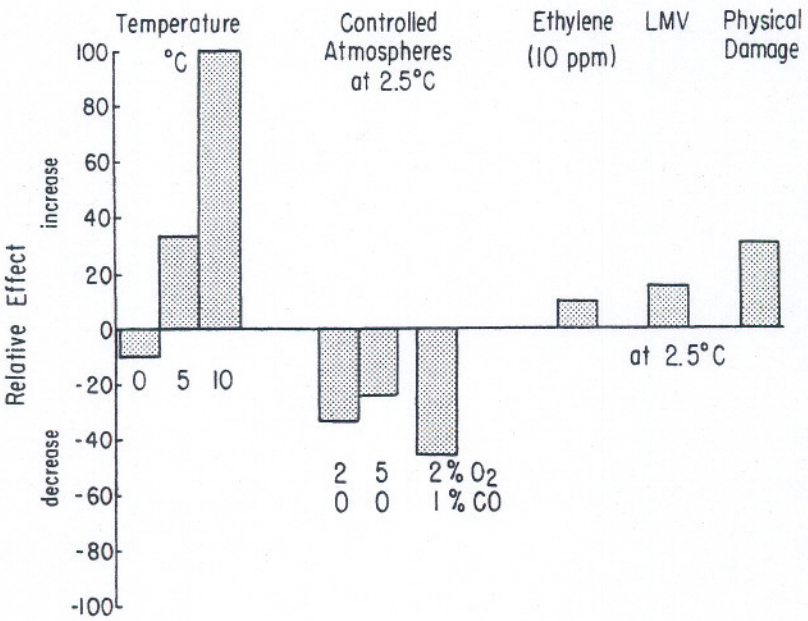

Figure 10 Relative effects of temperature, reduced O_2, added CO, ethylene, lettuce mosaic virus (LMV), and physical damage on respiration rate of crisphead lettuce in comparison with its respiration rate in air at 2.5°C.

Any mechanical injury—such as cutting, abrading, slicing, and shredding of vegetables during harvesting, handling, or processing into fresh-cut or minimally processed, value-added products—increases their respiration rates. The magnitudes of CO_2 production and O_2 consumption increase with the degree of wounding (Saltveit, 1997). For example, the respiration rate of grated or shredded carrots (*Daucus carota* L.) is higher than that of sliced carrots, whose respiration rate is higher than that of whole-peeled carrot sections (Gorny, 1997).

Water stress, which is induced by lower than optimum relative humidity in the air surrounding the commodity, can stimulate the rate of respiration. When water loss exceeds about 5%, the respiration rate may be reduced, but at the same time wilting and shriveling become noticeable, resulting in an unmarketable product.

Biological stress, such as the incidence of disease, also increases respiration rate, as shown in Figure 10 for lettuce mosaic virus (LMV)–affected lettuce.

Other stresses that stimulate the respiration rate of vegetables include exposure to ionizing radiation and to various chemicals, such as methyl bromide and other fumigants.

8. A Comparison Among Environmental Factors

The relative effects of temperature, reduced O_2, added CO, C_2H_4, LMV, and physical damage on the respiration rate of lettuce are summarized in Figure 10. It is clear that temperature is the most important factor, followed by O_2 concentration. Physical damage has a greater effect than C_2H_4 or LMV on stimulating respiration rate of lettuce kept at 2.5°C.

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