Respiratory Metabolism

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Introduction

All of the commodities covered in this handbook are alive and carry on processes characteristic of all living things. One of the most important of these is respiratory metabolism. The process of respiration involves combining O_2 in the air with organic molecules in the tissue (usually a sugar) to form various intermediate compounds and eventually CO_2 and water. Energy produced by the series of reactions making up respiration can be captured as high-energy bonds in compounds used by the cell in subsequent reactions, or it can be lost as heat. The energy and organic molecules produced during respiration are used by other metabolic processes to maintain the health of the commodity. Heat produced during respiration is called "vital heat," and it contributes to the refrigeration load that must be considered in designing storage rooms.

There is little the postharvest physiologist can do to alter the internal factors affecting respiration of harvested commodities, because they are largely a function of the commodity itself once harvested. However, a major part of postharvest technology is devoted to reducing respiration and other metabolic reactions associated with quality retention by manipulating the external environment.

In general, the storage life of commodities varies inversely with the rate of respiration. This is because respiration supplies compounds that determine the rate of metabolic processes directly related to quality parameters such as firmness, sugar content, aroma, and flavor. Commodities and cultivars with higher rates of respiration tend to have shorter storage life than those with lower rates of respiration. Storage life of broccoli, lettuce, peas, spinach, and sweet corn, all of which have high respiration rates, is short in comparison to that of apples, cranberries, limes, onions, and potatoes, all of which have low respiration rates (table 1).

Table 1. Respiration rates of various perishable commodities

Class	Range at 5 °C	Commodities
	mg CO_2 kg^{-1} h^{-1}	
Very Low	<5	Nuts, dates
Low	5 to 10	Apple, citrus, grape, kiwifruit, onion, potato
Moderate	10 to 20	Apricot, banana, cherry, peach, nectarine, pear, plum, fig, cabbage, carrot, lettuce, pepper, tomato
High	20 to 40	Strawberry, blackberry, raspberry, cauliflower, lima bean, avocado
Very High	40 to 60	Artichoke, snap bean, Brussels sprouts, cut flowers
Extremely High	>60	Asparagus, broccoli, mushroom, pea, spinach, sweet corn

Factors Affecting Respiration

Respiration is affected by a wide range of environmental factors that include light, chemical stress (for example, fumigants), radiation stress, water stress, growth regulators, and pathogen attack. The most important postharvest factors are temperature, atmospheric composition, and physical stress.

Temperature. The most important factor affecting postharvest life is temperature, because temperature has a profound effect on the rates of biological reactions; for example, metabolism and respiration. Over the physiological range of most crops, 0 to 30 °C (32 to 86 °F), increased temperatures cause an exponential rise in respiration. The Van't Hoff Rule states that the velocity of a biological reaction increases 2 to 3-fold for every 10 °C (18 °F) rise in temperature.

The temperature quotient for a 10 °C (18 °F) interval is called the Q_{10}. The Q_{10} can be calculated by dividing the reaction rate at a higher temperature by the rate at a 10 °C (18 °F) lower temperature: $Q_{10} = R_2/R_1$. The temperature quotient is useful because it allows us to calculate the respiration rates at one temperature from a known rate at another temperature. However, the respiration rate does not follow ideal behavior, and the Q_{10} can vary considerably with temperature. At higher temperatures, the Q_{10} is usually smaller than that at lower temperatures.

Following are typical values for Q_{10} at various temperature ranges:

Temperature	Q_{10}
0 to 10 °C	2.5 to 4.0
10 to 20 °C	2.0 to 2.5
20 to 30 °C	1.5 to 2.0
30 to 40 °C	1.0 to 1.5

These typical Q_{10} values allow us to construct a table showing the effect of different temperatures on the rates of respiration or deterioration and relative shelf-life of a typical perishable commodity (table 2). This table shows that, if a commodity has a mean shelf-life of 13 days at 20 °C (68 °F), it can be stored for as long as 100 days at 0 °C (32 °F) but will last no more than 4 days at 40 °C (104 °F).

Table 2. Effect of temperature on rate of deterioration

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

Chilling stress. Although respiration is normally reduced at low but nonfreezing temperatures, certain commodities, chiefly those originating in the tropics and subtropics, exhibit abnormal respiration when their temperature falls below 10 to 12 °C (50 to 54 ºF). Typically, the Q_{10} is much higher at those low temperatures for chilling-sensitive crops than it would be for chilling-tolerant ones. Respiration may increase dramatically at the chilling temperatures or when the commodity is returned to nonchilling temperatures. This enhanced respiration presumably reflects the cells' efforts to detoxify metabolic intermediates that accumulated during chilling, as well as to repair damage to membranes and other subcellular structures. Enhanced respiration is only one of many symptoms that signal the onset of chilling injury.

Heat stress. As the temperature rises beyond the physiological range, the rate of increase in respiration falls. It becomes negative as the tissue nears its thermal death point, when metabolism is disorderly and enzyme proteins are denatured. Many tissues can tolerate high temperatures for short periods of time (for example, minutes), and this property is used to advantage in killing surface fungi on some fruits. Continued exposure to high temperature results in phytotoxic symptoms and then complete tissue collapse. However, conditioning and heat shocks—that is, short exposure to potentially injurious temperatures—can modify the tissue's responses to subsequent harmful stresses.

Atmospheric composition. Adequate O_2 levels are required to maintain aerobic respiration. The exact level of O_2 that reduces respiration while still permitting aerobic respiration varies among commodities. In most crops, O_2 levels at around 2% to 3% produce a beneficial reduction in the rate of respiration and other metabolic reactions. Levels as low as 1% improve the storage life of some crops—for example, apples—but only when the storage temperature is optimal. At higher storage temperatures, the demand for adenosine triphosphate (ATP) may outstrip the supply and promote anaerobic respiration (see chapters "Controlled Atmosphere Storage" and "Modified Atmosphere Packaging"). The need for adequate O_2 should be considered in selecting the various postharvest handling procedures, such as waxing and other surface coatings, film wrapping, and packaging. Unintentional modification of the atmosphere, by packaging for example, can result in production of undesirable fermentative products and development of foul odors.

Increasing the CO_2 level around some commodities reduces respiration, delays senescence, and retards fungal growth. In low O_2 environments, however, increased CO_2 levels can promote fermentative metabolism. Some commodities tolerate brief storage in a pure N_2 atmosphere (for example, a few days at low temperatures) or in very high concentrations of CO_2**.** The biochemical basis for this ability to withstand these atmospheres is unknown.

Physical stress. Even mild physical stress can perturb respiration, while physical abuse can cause a substantial rise in respiration that is often associated with increased ethylene evolution. The signal produced by physical stress migrates from the site of injury and induces a wide range of physiological changes in adjacent, non-wounded tissue. Some of the more important changes include enhanced respiration, ethylene production, phenolic metabolism, and wound healing. Wound-induced respiration is often transitory, lasting a few hours or days. However, in some tissues, wounding stimulates developmental changes, such as promotion of ripening, that result in a prolonged increase in respiration. Ethylene stimulates respiration and stress-induced ethylene may have many physiological effects on commodities besides stimulating respiration.

Stage of development. Respiration rates vary among and within commodities. Storage organs such as nuts and tubers have low respiration rates. Tissues with vegetative or floral meristems such as asparagus and broccoli have very high respiration rates. As plant organs mature, their rate of respiration typically declines. This means that commodities harvested during active growth, such as many vegetables and immature fruits, have high respiration rates. Mature fruits, dormant buds, and storage organs have relatively low rates.

After harvest, the respiration rate typically declines—slowly in nonclimacteric fruits and storage organs and rapidly in vegetative tissues and immature fruits. The rapid decline presumably reflects depletion of respirable substrates, which are typically low in such tissues. An important exception to the general decline in respiration following harvest is the rapid and sometimes dramatic rise in respiration during the ripening of climacteric fruit (figure 1). This rise, which has been the subject of intense study for many years, normally consists of four distinct phases: (1) preclimacteric minimum, (2) climacteric rise, (3) climacteric peak, and (4) postclimacteric decline.

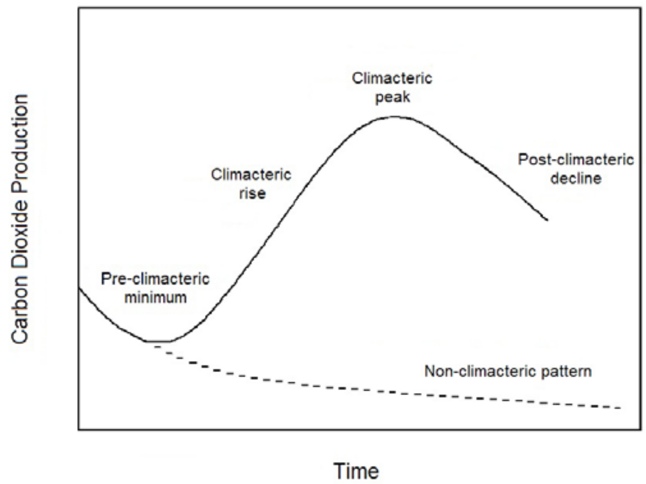

Figure 1. The climacteric pattern of respiration in ripening fruit.

The division of fruits into climacteric and nonclimacteric types has been very useful for postharvest physiologists. However, some fruits, kiwifruit and cucumber for example, appear to blur the distinction between the groups. Respiratory rises also occur during stress and other developmental stages, but a true climacteric only occurs coincident with fruit ripening. The following is a general classification of fruits according to their respiratory behavior during ripening:

Climacteric Fruits		Nonclimacteric Fruits
Apple	Papaya	Blueberry
Apricot	Passion fruit	Cacao
Avocado	Peach	Caju
Banana	Pear	Cherry
Biriba	Persimmon	Cucumber
Breadfruit	Plum	Grape
Cherimoya	Sapote	Grapefruit
Feijoa	Soursop	Lemon
Fig	Tomato	Lime
Guava	Watermelon	Olive
Jackfruit		Orange
Kiwifruit		Pepper
Mango		Pineapple
Muskmelon		Strawberry
Nectarine		Tamarillo

Significance of Respiration

Shelf-life and respiration rate. In general, there is an inverse relationship between respiration rates and postharvest life of fresh commodities. The higher the respiration rate, the more perishable the commodity usually is; that is, the shorter postharvest life it has. Respiration plays a major role in the postharvest life of fresh commodities because it reflects the metabolic activity of the tissue that also includes the loss of substrate, the synthesis of new compounds, and the release of heat energy. See the section "Summary of Respiration and Ethylene Production Rates" in the Introduction of this Handbook.

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 used for dehydrated product, the loss of dry weight due to respiration can be significant. When a hexose sugar (for example, glucose) is the substrate, 180 g of sugar is lost for each 264 g of CO_2 produced by the commodity. The rate of dry weight loss can be estimated as follows:

$$\text{Dry weight loss (g kg}^{-1}\text{ h}^{-1}) = \text{Respiration (mg CO}_2\text{ kg}^{-1}\text{ h}^{-1}) \times 0.068$$

or

$$\text{\% dry weight loss (g 100 g}^{-1}\text{ h}^{-1}) = \text{Respiration (mg CO}_2\text{ kg}^{-1}\text{ h}^{-1}) \times 68 \times 10^{-6}$$

For example, onions held at 30 °C (86 °F) will respire at about 35 mg CO_2 kg^{-1} h^{-1}. The

percentage dry weight loss per hour would be 35 x 0.68/10,000 = 0.0024%, while the percentage dry weight loss per month would be 0.0024 x 24 x 30 = 1.73%.

Synthesis of new compounds. Postharvest storage can be used either to prevent any reduction in quality or to promote changes that increase quality. The quality of most vegetables (for example, cucumbers and lettuce) and nonclimacteric fruit (for example, strawberries) is maximal at harvest, and storage conditions are optimized to prevent quality loss. In contrast, many flowers (for example, carnations and roses), nonclimacteric fruit (for example, lemons and oranges), and climacteric fruit (for example, bananas and tomatoes) are harvested before they reach their best quality, and storage conditions are optimized to permit development of optimum quality. In the first case, the synthesis of new compounds is unnecessary because they lead to reduced quality (for example, enzymes that destroy chlorophyll in lettuce or promote lignification in asparagus). In the second case, synthesis of pigments and volatiles (for example, lycopene in tomatoes and amyl esters in banana), loss of chlorophyll (for example, chlorophyll-degrading enzymes in banana and lemons), and the conversion of starch to sugar (for example, sweetening of apples and bananas) is necessary for development of maximum quality. These synthetic reactions require energy and organic molecules derived from respiration.

Release of heat energy. The heat produced by respiration (vital heat), about 673 kcal for each mole of sugar (180 g) used, 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, and stacking of packages, as well as selection of refrigerated storage facilities (that is, refrigeration capacity, air circulation, and ventilation). The approximate rates of heat production by various crops at different storage temperatures can be calculated from the respiration rates for many fruits and vegetables given in section "Summary of Respiration and Ethylene Production Rates" in the Introduction of this Handbook.

Calculation of heat production from the respiration equation shows that production of 1 mg of CO_2 yields 2.55 cal. In the language of the refrigeration engineer, a respiration rate of 1 mg CO_2 kg^{-1} h^{-1} indicates heat production of 61.2 kcal $tonne^{-1}$ day^{-1} (220 BTU ton^{-1} day^{-1}). The British thermal unit (BTU) is the heat required to raise 1 lb of water by 1 °F.

Some commodities have high respiration rates and require considerably more refrigeration than more slowly respiring produce to keep them at a specified temperature. For example, asparagus, broccoli, mushrooms, and peas respire about 10 times faster than apples, cabbage, lemons, and tomatoes.

Meaning of the respiratory quotient (RQ). The composition of a commodity frequently determines which substrates are used in respiration and consequently the value of the respiratory quotient (RQ). RQ is defined as the ratio of CO_2 produced to O_2 consumed; CO_2 and O_2 can be measured in moles or volumes. Depending on the substrate being oxidized, RQ values for fresh commodities range from 0.7 to 1.3 for aerobic respiration. When carbohydrates are being aerobically respired, 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 RQ can be used as an indication of the shift from aerobic to anaerobic respiration.

Measuring the Rate of Respiration

The rate of any reaction can be determined by measuring the rate at which the substrates disappear or the products appear. Apart from the water produced by respiration, which is relatively trivial compared with the very high water content of most harvested commodities, all the substrates and products of respiration have been used to determine the rate of respiration. They are loss of substrate (for example, glucose) loss of O_2, increase in CO_2, and production of heat. The most commonly used method is to measure production of CO_2 with either a static or a dynamic system.

In a static system, the commodity is enclosed in an airtight container and gas samples are taken after sufficient CO_2 has accumulated to be accurately detected by any one of a number of commercially available instruments (for example, gas chromatograph and infrared CO_2 analyzer). If the container is properly sealed, CO_2 should increase linearly with time. Multiplying the change in concentration times the container volume and dividing by weight of the commodity and duration of time between samples gives the production rate.

In the dynamic system a flow of air (or other gas mixture) is passed through the container at a known rate. The system will come into equilibrium (>99.3%) in about the same time it takes for 5 times the volume to flow through the container. The difference in CO_2 concentration between the inlet and outlet is measured after the system has reached equilibrium by taking gas samples at both points and analyzing them. Multiplying the difference in concentration by the flow rate and dividing by the weight of the commodity calculates the production rate.

Biochemistry of Respiration

Respiration is the oxidative breakdown of complex substrate molecules normally present in plant cells, such as starches, 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 metabolic reactions essential for the maintenance of cellular organization and membrane integrity of living cells. Since respiration rate is so tightly coupled to the rate of metabolism, measurements of respiration provide an easy, nondestructive means of monitoring the metabolic and physiological state of tissues. For example, events of senescence and ripening are often signaled by abrupt changes in respiration.

Maintaining a supply of high-energy compounds like adenosine triphosphate (ATP), nicotinamide adenine dinucleotide (NADH), and pyrophosphate (PPi) is a primary function of respiration. The overall process of aerobic respiration involves regeneration of ATP from ADP (adenosine diphosphate) and P_i (inorganic phosphate) with release of CO_2 and H_2O. If glucose is used as substrate, the overall equation for respiration can be written as follows:

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O + 686\ \text{kcal mole}^{-1}$$

The components of this reaction have various sources and destinations. The one mole of glucose (180 g) can come from stored simple sugars like glucose and sucrose or complex polysaccharides

like starch. Fats and proteins can also provide substrates for respiration, but their derivatives (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 × 32 g mol^{-1}) 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 H_2O (108 g) that are produced are simply incorporated into the aqueous solution of the cell.

There are three fates for the energy (686 kcal mol^{-1}) released by aerobic respiration. Around 13 kcal is lost due to the increase in entropy (disorder) when the complex glucose molecule is broken down into simpler molecules. Of the remaining 673 kcal that are capable of doing work, around 281 kcal (about 41% of the total energy) is used to produce 38 ATP molecules (38 ATP × 7.4 kcal ATP^{-1}). The remaining 392 kcal (57%) is lost as heat. In actuality, most energy is lost as heat since energy is lost to heat every time energy is transferred during a metabolic reaction.

Aerobic respiration involves a series of three complex reactions, each of which is catalyzed by a number of specific enzymes that perform one of the following actions: add an energy-containing phosphate group to the substrate molecule, rearrange the molecule, and break down the molecule to a simpler one. The three interconnected metabolic pathways are glycolysis, tricarboxylic acid (TCA) cycle, and electron transport system.

Glycolysis, the breakdown, or lysing, of glucose, occurs in the cytoplasm of the cell. It 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. Two key enzymes in glycolysis are phosphofructokinase (PFK) and pyruvate kinase (PK). Cells can control their rate of energy production by altering the rate of glycolysis, primarily through controlling PFK and PK activity. One of the products of respiration, ATP, is used as a negative feedback inhibitor to control the activity of PFK. Glycolysis produces two molecules of ATP and two molecules of NADH from the breakdown of each molecule of glucose.

Tricarboxylic acid (TCA) cycle, which occurs in the mitochondrial matrix, involves the breakdown of pyruvate into CO_2 in nine sequential, enzymatic reactions. Pyruvate is decarboxylated (removal of CO_2) to form acetate, which condenses with a co-enzyme 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 that is then ready to accept another acetyl CoA molecule. In addition to producing the many small molecules that are used in the synthetic reactions of the cell, the TCA cycle also produces one molecule of flavin adenine dinucleotide ($FADH_2$) and four molecules of NADH for each molecule of pyruvate metabolized.

Electron transport system, which occurs on membranes in 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 molecule produces two ATP molecules. The production of ATP depends not only on the energy contained in NADH and $FADH_2$ but also on the chemical environment (pH and ion

concentrations) within the cell and mitochondria.

In the absence of O_2, NADH and $FADH_2$ accumulate in the reduced form. As the 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 and takes place during the reductive decarboxylation of pyruvate to ethanol in fermentative metabolism.

Fermentation, or anaerobic respiration, involves the conversion of hexose sugars into alcohol and CO_2 in the absence of O_2. Pyruvate produced through glycolysis via a series of reactions that do not require O_2 can be converted to lactic acid, malic acid, acetyl CoA, or acetaldehyde. The pathway chosen depends on cellular pH, prior stresses, and the current metabolic needs of the cell. Acidification of the cytoplasm enhances the activity of pyruvic decarboxylase that then shunts pyruvate to form CO_2 and acetaldehyde. The acetaldehyde is converted by the enzyme alcohol dehydrogenase to ethanol with the regeneration of NAD^+. Two molecules of ATP and 21 kcal of heat energy are produced in anaerobic respiration (alcoholic fermentation) from each molecule of glucose. To maintain the supply of ATP at the aerobic rate, 19 times as many glucose molecules would be needed, and glycolysis would increase 19-fold. However, since only two molecules of CO_2 are produced during glycolysis, instead of six during aerobic respiration, the rate of CO_2 production would not increase by 19-fold but only by 6.3-fold (that is, $19 \div 3$). Concomitantly, there would be substantial accumulation of ethanol and smaller amounts of acetaldehyde. However, glycolysis usually increases only 3- to 6-fold.

The O_2 concentration at which a shift from predominantly aerobic to predominantly anaerobic respiration occurs varies among tissues and is known as the extinction point, the anaerobic compensation point, and the fermentative threshold. Since O_2 concentration at any point in a fruit or vegetable varies with rates of gas diffusion and respiration, some parts of the commodity may become anaerobic while others remain aerobic.

Further Reading

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