CHAPTER 4

Respiratory Metabolism

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4.1 INTRODUCTION

The postharvest life and quality of a commodity are influenced by the rate at which the many interrelated metabolic reactions of a cell occur. Respiratory metabolism furnishes not only the energy required to drive all these metabolic reactions, but it also produces the raw material used as substrates by these reactions. In its simplest form, respiration reacts with stored substrates (usually a carbohydrate) and with O_2 to produce high-energy compounds (e.g., ATP) and CO_2. However, the production of carbon fragments used in subsequent synthetic reactions is also of major importance.

The rate of respiration changes throughout the life of the commodity. Metabolic activity is especially high during the initial growth of the commodity, during ripening of climacteric fruit, and during periods of wound healing. After an initial surge to repair wounds encountered during harvest, respiration usually declines in vegetative tissues and nonclimacteric fruit (Fig. 4.1). In contrast, the ripening of climacteric fruit is accompanied by a rapid rise in ethylene production and respiration. It is thought that the added energy derived from this rise in respiration is necessary to power the many metabolic processes (e.g., tissue softening, and synthesis of pigments and volatiles) that accompany ripening. However, nonclimacteric fruit undergo similar ripening changes without a concomitant rise in respiration, and climacteric fruit left attached to the parent plant ripen with a pronounced lower rate of respiration than detached fruit, even though the rise in ethylene production is similar in both cases. The climacteric rise in respiration during ripening may be more an artifact of harvested fruit ripening than an integral function of fruit ripening.

The four major pathways of respiratory metabolism are (1) glycolysis, (2) the citric acid cycle, (3) electron transport, and (4) the pentose–phosphate shunt.

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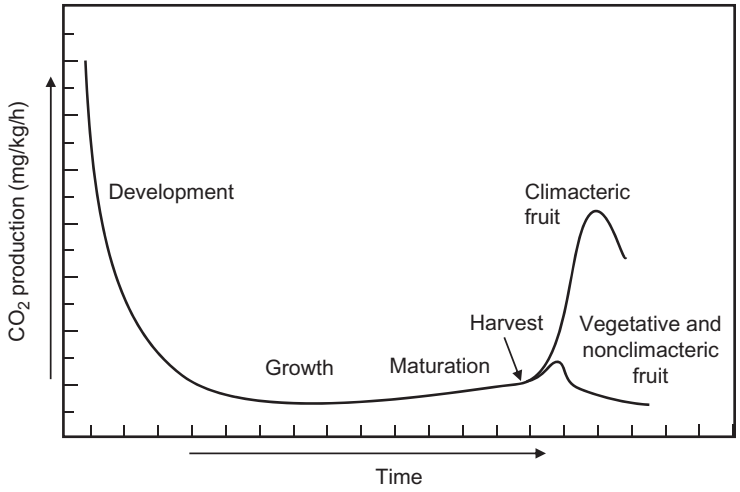

FIGURE 4.1

Change in respiration (CO_2 production) during the development, growth, maturation, and harvest of vegetative commodities and of climacteric and nonclimacteric fruit.

The pentose–phosphate shunt diverts material from glycolysis to the production of intermediates for specific synthetic reactions (Fig. 4.2). Unlike the many diverse and often unique reactions comprising secondary metabolism, these four pathways contain sets of reactions that are common to all vascular plants. The primary objective of postharvest research has been to maintain quality while extending the shelf-life of harvested horticultural commodities. These objectives have been best realized through control of the rate of respiratory metabolism.

4.2 CONTROL OF RESPIRATORY METABOLISM

Respiratory metabolism can be reduced and the shelf-life extended by lowering the temperature of harvested horticultural commodities. This has been the primary method used to retain quality since antiquity. Temperature is a measure of kinetic energy. Rapidly moving (or vibrating) molecules have higher energy; that is they are warmer than slower-moving molecules. Heat moves from warm to cold environments by conduction, convection, and radiation. Conduction moves heat from the interior of a warm commodity to its surface through intervening tissue. At the surface of the commodity, moving cold liquids (i.e., air or water) remove the heat by convection.

All bodies radiate energy. You can feel the energy being radiated by an extremely hot body like the sun or a fire. The rate of heat movement by radiation is proportional to the fourth power of the temperature difference between the radiating surface and a nearby cold surface. Therefore, the amount of energy lost by radiation approaches zero as the temperature of a commodity

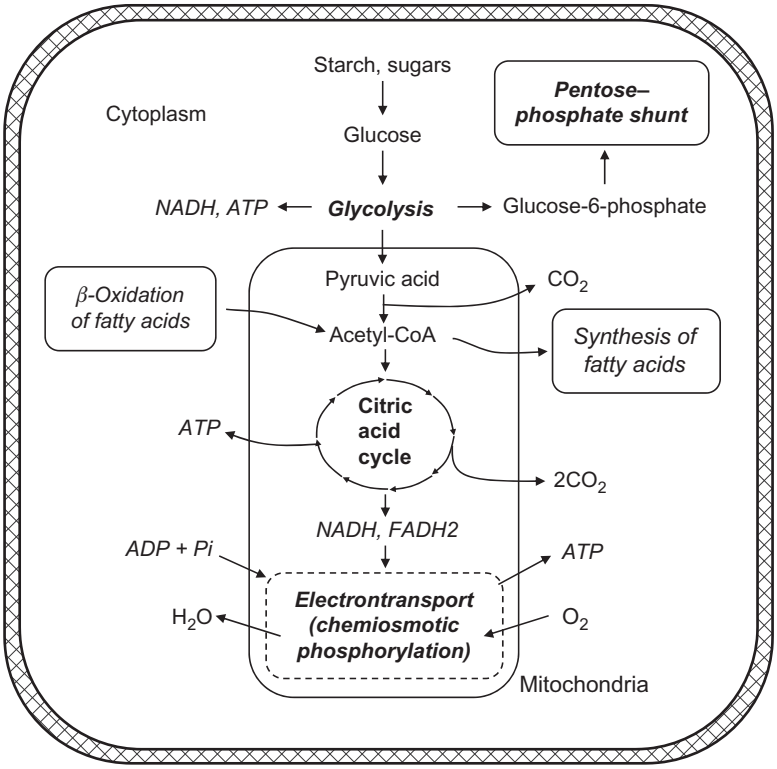

FIGURE 4.2

Overview of respiratory metabolism showing the relationship among the four major pathways (glycolysis, citric acid cycle, electron transport, and the pentose–phosphate shunt), where the reactions comprising these pathways are located in the cell, and some of the products derived from these pathways. The cellular components are not drawn to scale.

decreases to common storage temperatures. An exception is the heating of harvested commodities exposed to direct sunlight.

4.2.1 Temperature

The energy that must be removed from a harvested commodity is comprised of field heat (because of its warm temperature when harvested) and vital heat (due to ongoing respiration). This energy can be removed from the commodity by a cold medium circulating around the commodity (e.g., air in room or forced-air cooling, and water in hydrocooling), or by ice in contact with the commodity. Energy can also be removed by the evaporation of water from the surface of the commodity during vacuum cooling.

Each of these methods is particularly useful for specific crops. For example, forced air is used with commodities that do not tolerate being wetted (e.g., flowers, berries), hydrocooling is used with commodities that tolerate being wetted (e.g., sweetcorn, melons), and vacuum cooling is appropriate for

commodities with a large surface area (e.g., lettuce). Because of its ease of operation, forced air is the most commonly used method to remove field heat. To realize the maximum benefit of cooling, it should be done as soon as possible after harvest. Delaying cooling for even a few hours can drastically shorten the shelf-life of rapidly respiring crops like asparagus, broccoli, and strawberries.

As a rule of thumb, the rate of respiration is halved and the shelf-life doubled for every 10°C reduction in temperature. This level of reduction is referred to as having a respiratory quotient (RQ) (Q_{10}) of 2. However, Q_{10} values vary among specific commodities, during their development and storage, and for different temperature intervals. For example, harvested broccoli has a respiratory rate of about 20 mg CO_2/kg/h at 0°C and 80 at 10°C giving it a Q_{10} of 4 (80/20) between 0°C and 10°C, while the rate at 20°C is 300 mg/kg/h giving it a Q_{10} of 3.8 (300/80) between 10°C and 20°C. In contrast, harvested turnips have a Q_{10} of 2.1 between 0°C and 10°C and 1.5 between 10°C and 20°C. Most commodities that are natural storage organs (e.g., carrots, potatoes, turnips) have lower rates of respiration and lower Q_{10} values, while commodities that are rapidly developing after harvest (e.g., asparagus, broccoli) have higher rates of respiration and higher Q_{10} values. In both cases, lowering the commodity's temperature will maintain its quality and prolong its shelf-life.

4.2.2 Altered Gaseous Atmospheres

Respiratory metabolism can also be reduced by restricting the availability of oxygen to the tissue. Air contains 78% N_2, 21% O_2, 0.9% Ar, 0.04% CO_2, and 0.06% of an assortment of other gases. Water vapor is also part of the atmosphere. Most harvested commodities should be stored under high ($>90\%$) relative humidity (RH). A cubic meter (10^6 L) of 90% RH air at 0°C contains 4.52 g of water. That amount of water as a vapor occupies a volume of 5.6 L or 0.00056% of the total volume. Although water vapor is not a major component of the atmosphere, it exerts a significant effect on the rate of water loss from harvested commodities.

4.2.2.1 CONTROLLED AND MODIFIED ATMOSPHERE STORAGE

If the composition of the atmosphere around a commodity is actively regulated it is called controlled atmosphere (CA) storage, whereas if the concentration of the atmosphere is not actively controlled it is called modified atmosphere (MA) storage. If the package holding the commodity is designed to reduce gas exchange between the commodity and the surrounding atmosphere it is called modified atmosphere packaging (MAP). MAP relies on a delicate balance between the respiratory rate of the enclosed commodity and the diffusive properties of the package. Each must be within a narrow range for the desired atmosphere to be created and maintained within the package. The inherent variability in the respiratory rate of the commodity (e.g., resulting from differences in cultivar, growing conditions, maturity, ripening, and prior stress), and

the inability to maintain the temperature within a narrow range during transport and marketing have limited the use of MAP to a few products. Various attempts to design "smart" packages that overcome these problems have met with limited success because of their complexity and expense.

The biological activity of a gas is dependent on its concentration in the cytoplasm. Gas solubility is affected by the cell's temperature and solute concentration. The amount of gas in the cytoplasm is also governed by the partial pressure of the gas in contact with the liquid portion of the cell. For example, at atmospheric pressure (i.e., 101 kPa) the 21% concentration of O_2 in air has a partial pressure of 0.21 atmospheres or 21 kPa. The storage life of many commodities is lengthened when they are stored in an atmosphere containing 2% O_2 (i.e., O_2 at a partial pressure of 2 kPa). This atmosphere can be made by diluting 1 volume of air (21% O_2) with 9 volumes of nitrogen to create 10 volumes of 2% O_2.

4.2.2.2 LOWER PRESSURE STORAGE

A partial pressure of 2 kPa O_2 can also be created by lowering the pressure of the air surrounding the commodity to 0.1 atmospheres (10 kPa). This is termed low-pressure (LP) storage. An added advantage of LP storage is that the diffusion of gases is much higher at low pressures so the movement of gas into and out of the commodity is much faster and the likelihood of developing anaerobic conditions within the center of a bulky commodity in a low O_2 environment is greatly reduced. However, problems with excessive water loss may occur during LP storage since the diffusion of water vapor from the commodity would also be enhanced. The main impediment limiting the use of LP storage has been the expense of constructing containers that can withstand the vacuum necessary for LP to be effective. While vacuum chambers are commonly used for the vacuum cooling of lettuce, their cost is spread over the many loads they cool per day, and they can be moved to different production areas during the season, further amortizing their initial cost.

Increasing the concentration of CO_2 within the commodity also prolongs the storage life of some commodities by a combination of reducing respiration, reducing the biological activity of ethylene, and inhibiting microbial growth. However, not all commodities tolerate the often high CO_2 concentrations needed for these benefits to be realized.

4.3 MEASURING RESPIRATION

The many and varied reactions taking place in respiratory metabolism are often difficult to measure individually. However, since the rate of respiratory metabolism is tightly coupled to the rate of respiration (i.e., the production of high-energy intermediates like ATP and NADH from the oxidation of glucose) measuring the production of CO_2 or the consumption O_2 by the commodity will give a good approximation of the rate of other metabolic processes. Under the aerobic respiration of glucose, one molecule of O_2 is consumed for

every molecule of CO_2 produced. So either O_2 consumption or CO_2 production could be used to measure the rate of respiration. Since the background concentration of O_2 is around 21%, while that of CO_2 is around 0.04%, a small percentage error involved in measuring a change in O_2 concentration would be much greater than it would be in measuring a change in CO_2 concentration. For example, the rate of respiration of a commodity in a closed container that would lower the O_2 concentration from 21.0% to 20.9% (around a 0.5% reduction), would increase the concentration of CO_2 from 0.04% to 0.14% (around a 350% increase). Given the inherent variability in biological material, and errors associated with these types of measurements, a change of 0.1% against a background of 21% (a 0.5% change) would be much harder to detect as significant, than would a change of 0.1% against a background of 0.04% (a 350% change).

Measuring changes in dry weight (e.g., loss of substrate, like starch) or energy production (heat loss during the production of ATP) is appropriate in some situations. Dry weight loss can be found by periodically drying a sample of the commodity and calculating the rate of loss. Energy production can be found by enclosing a portion of tissue in a calorimeter and measuring the heat produced. However, unlike measuring gases evolved (CO_2, C_2H_4) or consumed (O_2) by the commodity, neither of these are nondestructive measurements. Dry weight loss can be calculated by multiplying the weight of CO_2 lost from the commodity by 0.68 (the ratio of the molecular weight of glucose (180) divided by the weight of the lost CO_2 ($6 \times 44 = 264$)).

The cost, robustness, ease of use, and rapidity of different measurement techniques often have a pronounced influence on which method is used. The predominant instrument used to measure respiration is the infrared CO_2 analyzer. Such analyzers range in cost from $400 for commercial units designed to control gas levels in greenhouses and office buildings to tens of thousands of dollars for laboratory dual-beam analytical instruments.

4.3.1 The Static System

The rate of CO_2 production and/or O_2 consumption can be measured using a static or flow-through setup (Fig. 4.3). In the static setup, a commodity of known weight is enclosed in a rigid container of known volume for a specified time. Respiration will consume O_2 in the container lowering its concentration, and will produce CO_2, raising its concentration. Samples of the gas in the container are taken at the beginning and end of the sampling period and analyzed. The change in concentration is multiplied by the volume of the container and the result is divided by the length of the sampling period and the fresh weight of the sample. It is important to know the exact volume of the container and to seal the container so no leaks occur.

For CO_2 the formula is: ((Final minus initial CO_2 level) $\times$ (0.01 if concentration is in percent) $\times$ (volume of the container))/((fresh weight of the commodity) $\times$ (length of the sampling period)) = mL CO_2/kg/h. For example,

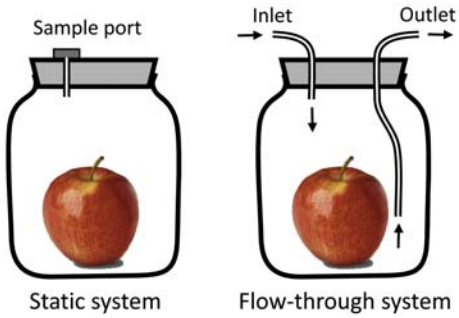

FIGURE 4.3

Setup for measuring respiration using a static or a flow-through system. In the static system, the commodity (e.g., apple) is sealed within a rigid container. Gas samples are periodically taken through the sample port. In the flow-through system there is a flow of gas at a known rate through the rigid container. Gas samples are taken from the inlet and outlet streams of gas after the system has come to equilibrium.

a 100 g apple at 20°C increased the CO_2 concentration in a closed 400 mL jar from 0.04% to 0.14% in 18 min. The CO_2 concentration increased 0.10%, so 0.4 mL of CO_2 was produced by the apple in the 400 mL container ($0.1 \times 0.01 \times 400 = 0.4$). The apple weighed 0.1 kg and the sampling period was 0.3 h, so dividing 0.04 mL by 0.1 kg and 0.3 h gives a production rate of 13.3 mL/kg/h at 20°C.

Since gases expand and contract with an increase or decrease in temperature, rates of respiration are often given in mg of CO_2 instead of in mL; as was done in the example given above for Q_{10} values. At 0°C, 10°C, and 20°C, 1 mL of CO_2 weighs 1.96, 1.89, and 1.83 mg, respectively. Applying this factor changes the rate of respiration from the apple at 20°C from 13.3 to 24.3 mg/kg/h. Similar changes would be needed to convert the consumption of O_2 in this closed system from milliliter to milligram. What would the O_2 concentration be in the gas sample? It would be 20.9%.

In both setups, the concentration of CO_2 should not be allowed to rise high enough, nor the concentration of O_2 to fall low enough, to significantly alter the rate of respiration. This is more of a concern with levels of CO_2, since CO_2 levels above 0.2% can alter rates of respiration, while levels of O_2 must fall below around 7% to have a significant effect on respiration (Fig. 4.4). In the static system, CO_2 accumulates linearly until it reaches a concentration that affects the rate of respiration ($\sim$ 0.2% in Fig. 4.4). At this point the rate of accumulation starts to deviate from linearity; it usually declines, but in some tissues the high CO_2 concentration causes damage that increases the rate of respiration.

In the previous calculation, no correction was made for the volume of the container taken up by the commodity. Apples have a density of about 0.9 g/mL, so the 100 g apple would have an external volume of 111 mL (100/0.9) and

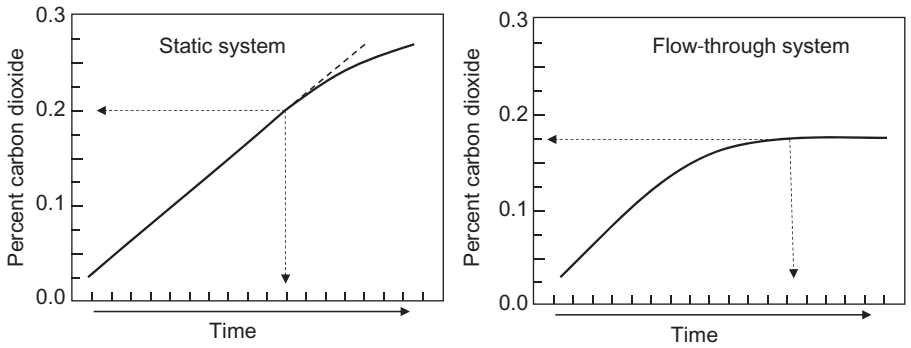

FIGURE 4.4

Graphs showing the concentration of CO_2 within a static and a flow-through system over time. The concentration increases linearly in the static system until it reaches a level that affects the rate of respiration. In a flow-through system, the concentration increases until equilibrium is reached between the amount of CO_2 produced by the commodity and the amount removed from the container in the flow of gas.

the void volume of the container would be 289 mL instead of 400 mL (400 − 111). Far less CO_2 (only 0.29 mL) would have been required to raise its concentration from 0.04% to 0.14% in the 289 mL volume compared to the 400 mL volume. The newly calculated production rate would be 9.6 mL CO_2/kg/h (17.6 mg/kg/h). However, this correction is not necessary because the very high solubility of CO_2 in water (1713, 1194, and 878 mL/L at 0, 10, and 20°C, respectively) means that the aqueous volume of the commodity can be ignored in these calculations. At 10°C 1194 mL of CO_2 will dissolve in water, above which is an atmosphere of pure CO_2 (100% CO_2 at 101 kPa). The amount of CO_2 dissolved will be proportional to the partial pressure of CO_2 over the liquid. That means that a molecule of CO_2 is equally likely to dissolve in the cell solution as to be partitioned into the surrounding gases. Only a minor error (<5%) will be introduced into the calculations by using the whole container volume; it is not necessary to subtract the volume of the commodity from the container volume.

The same caveat does not apply to O_2 because of its much lower solubility in water (49, 38, and 31 mL/L at 0, 10, and 20°C, respectively). When measuring O_2 consumption using the static system, the volume of the commodity should be subtracted from the container volume to get the appropriate void volume.

4.3.2 The Flow-Through System

While simple to set up and use, the static system does not lend itself for use in long-term studies or studies employing atmospheres of modified composition. In those cases, a flow-through system is preferred. The commodity is again enclosed in a rigid container, but instead of it being sealed shut, a flow of gas is directed through the container. The flow leaving the container will be diminished in O_2 and increased in CO_2 (and in other gases like ethylene produced by the commodity). After a period of time greater than three times the

volume of the container divided by the flow rate, an equilibrium will be reached in which the amount of CO_2 exiting the container will equal the amount of CO_2 produced by the commodity (this assumes complete mixing of gases within the container). For example, a 900 mL container holding 400 g of apples will have a void volume of 456 mL (900−(400/0.9)). At a flow rate of 100 mL/min, it would take 14 min for this setup to come to equilibrium ((456 mL × 3)/100 mL/min). At that time, samples could be taken from the inlet and outlet and analyzed. The difference in concentration times the flow rate will give the amount of CO_2 produced in the time interval. For example, there would be a 0.1% difference if the inlet and outlet levels were 0.04% and 0.14% CO_2, respectively. In an hour, the 100 mL/min flow would have carried away 6.0 mL of CO_2 ($0.1 \times 0.01 \times 100 \times 60$), and dividing that by 0.4 kg gives a production of 15 mL CO_2/kg/h.

Unlike in the static system, it is not critical to know the exact volume of the container or to seal it to prevent all leaks. A larger container will just take a longer time to come to equilibrium, and small leaks are not important if the internal gas is thoroughly mixed. However, it is crucial to know the exact flow rate.

4.4 RESPIRATORY QUOTIENT

The three main substrates for respiration in harvested horticultural commodities are glucose, organic acids, and fatty acids. Proteins (i.e., amino acids) are usually a very minor component of respiration in harvested horticultural commodities. In aerobic respiration, O_2 is the terminal electron acceptor and the substrates are oxidized to CO_2 and water. However, as pointed out earlier, respiration also produces many simple carbon compounds that participate in the subsequent reaction. RQ is the ratio of CO_2 produced to O_2 consumed. Carbohydrates have an RQ of 1.0, fatty acids have an RQ of 0.7, and organic acids have an RQ of 1.3. In anaerobic respiration CO_2 is produced but no O_2 is consumed so the RQ approaches infinity.

The complete oxidation of glucose ($C_6H_{12}O_6$) has an RQ of 1.0 ($6CO_2$ produced divided by $6O_2$ consumed).

$$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$$

Organic acids are synthesized by the partial oxidization of glucose (i.e., more O_2 was consumed than CO_2 produced during their synthesis), so they contain more O_2 than carbohydrates and therefore more CO_2 is produced per O_2 consumed during their subsequent complete oxidation (RQ > 1.0). The incomplete oxidation of glucose to form the organic acid malic acid ($C_4H_6O_5$) has an RQ of 0.66 ($2CO_2/3O_2$).

$$C_6H_{12}O_6 + 3\ O_2 \rightarrow C_4H_6O_5 + 2\ CO_2 + 3\ H_2O$$

The complete oxidation of malic acid ($C_4H_6O_5$) has an RQ of 1.33 ($4CO_2/3O_2$).

$C_4H_6O_5 + 3\ O_2 \rightarrow 4\ CO_2 + 3\ H_2O$

Therefore, the RQ for the complete oxidation of glucose through the intermediate of malic acid is still 1.0 ((0.66 + 1.33)/2).

In contrast, fatty acids are partially reduced during their synthesis, so they contain far less O_2 per carbon than carbohydrates, and therefore more CO_2 will be produced per O_2 consumed during their subsequent oxidation (RQ < 1.0). A stoichiometric balanced equation for the synthesis of the fatty acid palmitic acid ($C_{16}H_{32}O_2$) from glucose shows how little O_2 would have been consumed during its synthesis.

$4\ C_6H_{12}O_6 + O_2 \rightarrow C_{16}H_{32}O_2 + 8\ CO_2 + 8\ H_2O$

But this is an illusion since the synthesis of the 16-carbon palmitic acid requires 16 NADPH and eight 2-carbon acetate groups. The synthesis of fatty acids is very energy-intensive, and O_2 is consumed in other reactions that are needed to produce the energy needed to create the reducing power and transport intermediate substrates (e.g., pyruvate, acetyl-CoA) within the cell, and to drive subsequent synthetic reactions. The route from glucose through palmitic acid is very circuitous.

The complete oxidation of palmitic acid ($C_{16}H_{32}O_2$) has an RQ of 0.70 ($16CO_2/23O_2$).

$C_{16}H_{32}O_2 + 23\ O_2 \rightarrow 16\ CO_2 + 16\ H_2O$

Because of their diverse structures and biosynthetic origin, no single RQ can be given for the oxidation of all proteins. However, the amino acids comprising proteins have the general structure $NH_2COOHCH_2$. Excluding the nitrogen, the formula becomes $C_2H_5O_2$ and the complete oxidation of a simple amino acid such as alanine has an RQ of 0.89 ($8CO_2/9O_2$).

$4\ C_2H_5O_2 + 9\ O_2 \rightarrow 8\ CO_2 + 10\ H_2O$

4.5 PRIMARY REACTION OF RESPIRATORY METABOLISM

The four primary pathways in respiratory metabolism are: (1) glycolysis, (2) citric acid cycle, (3) electron transport, and (4) pentose–phosphate shunt (Fig. 4.4).

4.5.1 Glycolysis

As its name implies, the set of 10 enzyme-coupled reactions in glycolysis splits or lyses the 6-carbon glucose molecule into two 3-carbon fragments. Glucose is a monosaccharide or simple sugar that usually occurs in small amounts in a cell. It occurs in much higher levels as a disaccharide such as sucrose, or in

polymerized forms such as starch. Glycolysis starts with an isomerase reaction rearranging the 6-carbon glucose molecule into a 6-carbon fructose molecule. Next, two reactions add phosphate from two ATPs to form 1,6-fructose diphosphate. This energized molecule is then split into two 3-carbon molecules that undergo an isomerization to form two 3-carbon molecules of pyruvate after five additional enzyme-coupled reactions. This essentially anaerobic series of reactions initially consumes two ATPs, but produces 4 ATPs and 2 NADHs. About 20% of the energy in glucose is captured in glycolysis. This pathway, which occurs in the cytoplasm, consumes no O_2 and produces no CO_2, but they do rely on NAD^+ being available.

4.5.2 Pentose–Phosphate Shunt

Most carbohydrates have an even number of carbons (the majority of carbohydrates are hexoses: 6-carbon sugars). However, both RNA and DNA have backbones made of pentoses (i.e., the 5-carbon sugars, ribose and deoxyribose). These 5-carbon sugars are synthesized from glucose-6-phosphate in the pentose–phosphate pathway. The many interrelated reactions also produce 4-carbon sugars, 7-carbon sugars, and NADPH. In the initial oxidative part of the pathway, two molecules of NADPH and one molecule of CO_2 are produced during the synthesis of ribulose-5-phosphate. In the final anabolic part of the pathway, other compounds are produced during rearrangements of the molecules. Most steps in these reactions take place in plastids.

4.5.3 Acetyl-Coenzyme A

Before entering the citric acid cycle, the product of glycolysis, the 3-carbon pyruvate, is decarboxylated (a CO_2 is removed and a NADH is produced) resulting in a 2-carbon acetate fragment (CH_3CO-) that is coupled to coenzyme A forming acetyl-CoA. This compound is composed of the nucleotide adenine linked to a ribose, which is linked through two phosphate atoms to pantothenic acid (vitamin B_5) which links to the acetyl fragment through a thioester bond. Because of the thioester bond and the two phosphate atoms, the resulting electron configuration in coenzyme A makes it relatively easy to add or remove the acetyl unit. Acetyl-CoA is used to transfer the 2-carbon acetate group in many reactions. It is used in the synthesis of fatty acids and is produced during β-oxidation of fatty acids. It is involved in the synthesis of the 5-carbon isoprenoid unit that is important in the synthesis of many molecules of secondary metabolism. It is produced during the oxidation of ethanol, ketones, amino acids, and of course pyruvate.

4.5.4 Synthesis of Fatty Acids

Lipids are composed of one or more fatty acids. Diglycerides are composed of two fatty acids linked by a molecule of glycerol, whereas triglycerides contain three fatty acids linked by glycerol. If all the bonds between the carbon atoms making up the fatty acid's backbone are single, the fatty acid is saturated,

whereas if some of them are double bonds the fatty acid is mono-, di-, or polyunsaturated. Saturated fatty acids have a higher melting point than unsaturated fatty acids and are linear in structure (Fig. 4.5). In contrast, double bonds produce a bend in the molecule so that it occupies a greater volume.

When incorporated in the diglyceride phospholipids that make up the bulk of cellular membranes, the degree of saturation of the component fatty acids exerts a significant effect on the membrane's fluidity. The fatty acid composition of the cell's membranes is modified during daily temperature fluctuations to maintain a constant fluidity in cellular membranes. Such changes can affect the tissue's chilling sensitivity by altering the membrane's phase transition temperature.

Fatty acids are synthesized in the cytosol and organelles by the sequential addition of the 2-carbon acetyl group from acetyl-CoA, so almost all fatty acids contain an even number of carbon atoms; 16 and 18 being the most common. Because of their chemical reactivity, most fatty acids are quickly processed after their synthesis to form monoglycerides, diglycerides, triglycerides, phospholipids (a significant component of cellular membranes), fats, and waxes (found in the cuticle). Because of their reduced state, fatty acids are an excellent store of energy. They are found as triglycerides (oils) in many seeds (e.g., cotton, rapeseed, safflower, and sunflower).

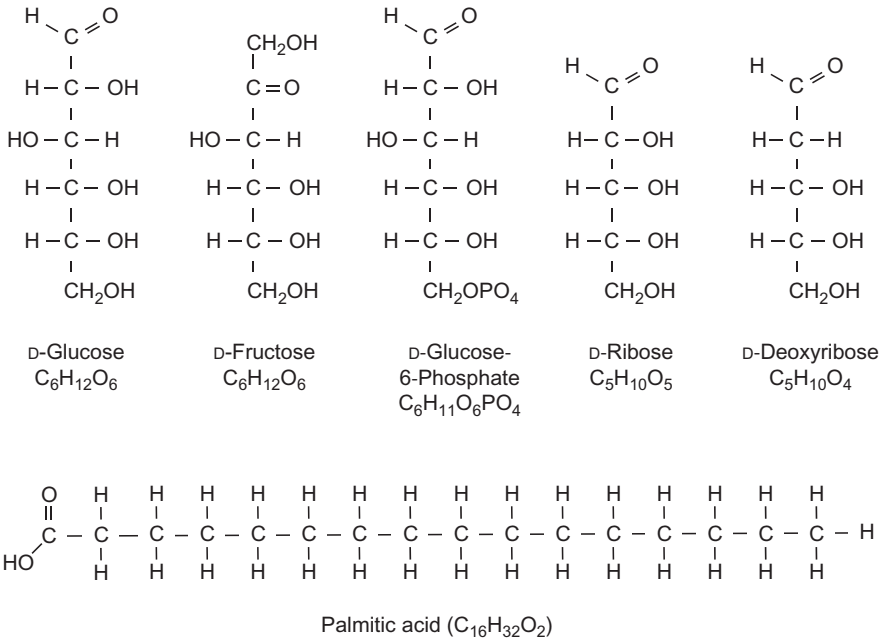

FIGURE 4.5
Linear structures of some important molecules found in respiratory metabolism.

Unlike the synthesis or organic acids (e.g., citric, malate) which are products of oxidative pathways (e.g., glycolysis and the citric acid cycle) fatty acids are synthesized by a reductive pathway. The reducing agent during fatty synthesis is NADPH (a reductase enzyme adds an H^+ from NADPH to a molecule forming $NADP^+$), while NAD^+ is the oxidizing agent (a dehydrogenase enzyme removes an H^+ from the molecule and adds it to NAD^+ forming NADH) in β-oxidation. In general, NADPH is consumed during biosynthetic reactions, whereas NADH is generated by energy-yielding reactions (e.g., the citric acid cycle).

4.5.5 Anaerobic Respiration

Without the regeneration of NAD^+ from NADH in electron transport (i.e., chemiosmotic phosphorylation) glycolysis would cease and there would be insufficient energy (e.g., ATP) to maintain the cell. This problem is overcome in anaerobic cells by the production of NAD^+ during the synthesis of lactic acid and/or ethanol from pyruvic acid (Fig. 4.6). In a nonacidic cell, lactate dehydrogenase uses NADH to convert pyruvic acid to lactic acid with the production of NAD^+. The accumulation of lactic acid acidifies the cell. In acidic cells, pyruvic decarboxylase is active and removes a CO_2 from pyruvic acid,

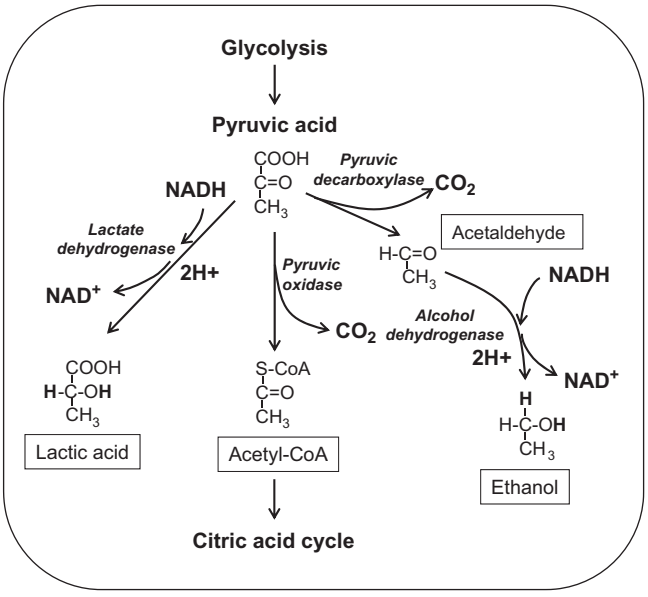

FIGURE 4.6

Fate of pyruvic acid under aerobic (acetyl-CoA produced) and anaerobic (lactic acid, and acetaldehyde and ethanol produced) respiration. Molecular structure and the regeneration of NAD^+ from NADH during anaerobic respiration. *Redrawn from Saltveit, M.E., 2016a. Respiratory metabolism. In: Gross, K.C., Wang, C.Y., Saltveit, M. (Eds.), Agricultural Handbook 66—The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks. USDA Agricultural Research Service. Available from the National Technical Information Service, Springfield, VA, or at http://www.ba.ars.usda.gov/hb66/contents.html; Saltveit, M.E., 2016b. Respiratory metabolism. In: Sunil (Ed.), Postharvest Ripening Physiology of Crops. CRC Press. ISBN 9781498703802.*

converting it to acetaldehyde, which is quickly used by alcohol dehydrogenase to form ethanol with the production of NAD^+. These two anaerobic reactions allow glycolysis to proceed in the absence of O_2, however, glycolysis only captures about 20% of the energy in a glucose molecule and the accumulation of too much lactic acid or ethanol can be toxic to the cell.

4.5.6 Citric Acid Cycle

The starting point of the citric acid cycle (also called the Krebs cycle, or the tricarboxylic acid cycle) is the addition of the 2-carbon acetyl group from acetyl-CoA to the 4-carbon oxaloacetate to form the 6-carbon, 3-carboxyl molecule citric acid. This completes the cycle by regenerating citric acid from oxaloacetate, which is at the end of the cycle. The two carbons in the transferred acetate are eliminated as two CO_2 during the cycle's regeneration of oxaloacetate. During this cycle, one ATP, one FADH2, and three NADH are produced from each pyruvate. The diversion of cycle intermediates provides the substrates for many other synthetic reactions.

For example, acetyl-CoA is used in the synthesis of fatty acids, cuticular compounds, isoprenoids, carotenoids, sterols, terpenes, and aromatic amino acids. α-Ketoglutaric acid is used in the synthesis of glutamic acid, other amino acids, chlorophyll, cytochromes, and phytochrome. Oxaloacetic acid is used in the synthesis of aspartic acid, alkaloids, and nucleic acids. The organic acids citric, succinate, and malic are also byproducts of the cycle. Citric acid can accumulate in citrus fruit (e.g., orange, lemon) and can easily enter the cycle. Malic acid can accumulate (as in apple, i.e., *Malus domestica*) and can readily enter the cycle, or it can be converted to pyruvate. So far, all the carbons from glucose have been released as CO_2, but no O_2 has yet been consumed.

4.5.7 Chemiosmotic Phosphorylation (Aka Electron Transport)

The high-energy compounds produced by the citric acid cycle (NADH, FADH2) contain more energy than is needed for most cellular functions. Partitioning that energy into smaller packets (i.e., ATP) occurs in electron transport via chemiosmotic phosphorylation. The terminal acceptor of the high-energy electron is O_2, which accepts electrons and protons to become H_2O. While many enzymes are involved in this process, these enzymatic reactions do not produce ATP, but rather establish a proton gradient across the inner mitochondrial membrane. High-energy electrons from NADH and FADH2 flow through the electron transport chain and furnish the power to pump protons (H^+) from the matrix into the intermembrane space, establishing a proton gradient as evidenced by the development of an electrical potential across the membrane, and a difference in pH (around seven in the matrix and eight in the intermembrane space). ATP is produced from ADP and inorganic phosphate as the protons flow from the intermembrane space back to

the matrix through an ATP-ase complex. This chemiosmotic process is very similar to that used to produce ATP in the chloroplast during photosynthesis.

An alternate oxidase is induced in some plants during certain phases of development or during periods of stress. This cyanide-resistant respiratory pathway diverts electrons from the main electron transport chain, thereby reducing ATP formation and increasing the tissue's temperature because the energy that would have been captured in the formation of ATP is released as heat. While useful in assisting pollination in some thermogenic flowers, this seemingly wasteful process also helps regulate the rate of carbon flow through the citric acid cycle and limits the production of reactive oxygen species during stress.

4.6 FACTORS AFFECTING RESPIRATORY METABOLISM

Since respiratory metabolism furnishes both the energy and the substrates for a myriad of subsequent reactions, it is not surprising that a number of morphological and physiological changes are associated with changes in the rate and direction of respiratory metabolism. We have already discussed how external factors, such as temperature and atmospheric composition, can alter respiration. Internal factors, such as the specific commodity, the cultivar of the commodity, its stage of growth and development (e.g., cell division, cellular expansion, maturation, ripening, and senescence), and metabolic responses to stresses and injury can also have a pronounced effect on the rate of respiratory metabolism.

4.6.1 Maturity and Fruit Ripening

Young, rapidly growing tissues have a much higher rate of respiration than more mature tissues. Asparagus and broccoli are examples of commodities that have rapid rates of respiration and short shelf-lives. In contrast, commodities that are natural storage organs (e.g., bulb onions, carrots, potato, and winter squash) have lower rates of respiration and longer shelf-lives.

Fruits harvested before full maturity (e.g., cucumbers, string beans, and zucchini) usually have relatively high rates of respiration that slowly decline in storage. Mature fruit can exhibit two dissimilar patterns of respiration following harvest (Table 4.1). Some, called climacteric fruit (e.g., apples, avocados, bananas, some melons, pears, and tomatoes), have a pronounced increase in respiration during ripening after harvest, while others, called nonclimacteric fruit (e.g., citrus, strawberries), lack this rise and rather show a slow decline after recovering from the trauma of harvesting. However, the distinction between climacteric and nonclimacteric is not hard and fast, with varying intensities of the climacteric rise apparent in cultivars within a species.

Table 4.1 A List of Some Fruits That Are Classified as Having a Respiratory Climacteric Coincident With Ripening. Fruits That Do Not Exhibit a Rise in Respiration Coincident With Ripening Are Termed Nonclimacteric

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

Climacteric fruit usually have extensive food reserves (e.g., starch) that are converted to sugars during ripening, while most nonclimacteric fruit lack theses extensive food reserves. Nonclimacteric fruit should be harvested at the peak of quality because very little improvement of quality is realized after harvest. In contrast, climacteric fruit can be harvested when mature, but far from acceptable levels of quality. For example, bananas and tomatoes can be harvested at the mature-green stage of development and ripened after transport to distant markets. The external application of ethylene is used in ripening rooms for both these fruit to hasten the color change, softening, and aroma development characteristic of high-quality ripe fruit.

Ethylene is a bioactive gas that is naturally produced by many plants to initiate and coordinate metabolic changes associated with responses to stress and developmental changes such as ripening. During ripening of climacteric fruit, a rapid rise in ethylene production and the resultant rise in internal ethylene concentrations within the commodity stimulates the many changes associated with ripening, including an increase in the rate of respiration. Exposure to ethylene also stimulates a rise in the respiration of vegetative tissue and nonclimacteric fruit, but the lack of a positive feedback of ethylene on ethylene synthesis in these commodities precludes the endogenous rise in ethylene that produces the rise in respiration in climacteric fruit. In fact, many nonclimacteric tissues exhibit a negative feedback wherein ethylene actually suppresses its further synthesis.

4.6.2 Biotic and Abiotic Stresses

Many biotic and abiotic stresses stimulate respiratory metabolism. Diseases (mainly caused by fungi) cause a rise in respiration as the plant mounts both a morphological and physiological response. Likewise, mechanical injury,

whether caused by chewing insects or the traumas of harvesting are met with both a morphological and physiological response. In lettuce, wounding stimulates respiratory metabolism that furnishes the energy and substrates necessary for the enhanced phenolic metabolism that is involved in wound repair and avoidance of further biotic injury. However, these same reactions foster the accumulation of phenolic compounds that can cause subsequent tissue browning.

While the plant has an impressive array of physiological and morphological responses to various stresses, the plant is often limited in what response is elicited by any specific stress. There appears to be a hierarchy in what response is elicited, with some responses taking precedence over others. This hierarchical response can be used to modify the plant's response to stress. For example, brief exposures to elevated temperatures elicit a heat-shock response in all living cells that entails the production of protective heat-shock proteins. When a heat shock is given before or soon after wounding (e.g., the production of fresh-cut lettuce) innocuous heat-shock proteins are produced instead of the proteins (i.e., enzymes) that contribute to tissue browning. The heat-shock treatment will be ineffective if the adverse effect (e.g., tissue browning) is the response of endogenous compounds (e.g., preformed phenolic compounds in apples and potatoes), and not the induced synthesis and accumulation of compounds. High-temperature treatments are used to disinfect harvested commodities of microorganism and insects by the lethal effect of the temperature on the pest.

4.6.3 Chilling and Freezing Temperatures

Temperature is the primary means to maintain quality after harvest, but not all harvest commodities respond similarly to low temperatures. Many commodities indigenous to tropical and subtropical regions (e.g., avocados, bananas, cucumbers, and tomatoes) suffer a physiological disorder called chilling injury when exposed to nonfreezing temperatures below about 10°C. The extent of injury depends on the temperature and duration of exposure. Some commodities are very sensitive (e.g., bananas) and suffer irreversible injury after a few hours, while other commodities can recover from a few days of chilling temperatures. This ability to recover is the basis of intermittent warming, where a chilling-sensitive commodity is held at a chilling temperature for a period of time shorter than that required to produce irreversible injury. The commodity is then warmed to a nonchilling temperature for a few days where it recovers before it is again returned to the chilling temperature. Cycling through these chilling and recovery phases can significantly extend the storage life. However, cooling and warming the commodity is expensive, and condensation of water on the cold commodity during warming can foster microbial growth.

Commodities are damaged if frozen. The solute concentration in plant cells lowers their freezing point (i.e., freezing-point depression) from a few tenths

of a degree (e.g., in tissue with few solutes like avocados and lettuce) to a few degrees (e.g., in tissue with high levels of solutes like artichokes, figs, and pomegranates). Formation of ice crystals in the low solute solution in the cell wall draws water out of the cell and causes damage by dehydrating the cell. Respiratory metabolism is disrupted as the solvent in which all the reactions occur (i.e., water) is removed from the cell and its contents become concentrated.

Just as too low a temperature can cause damage by chilling injury or freezing, too high a temperature beyond the physiological range can cause damage. Near the thermal death point, metabolism becomes disorderly and enzyme proteins are denatured. Many tissues can tolerate high temperatures for short periods of time (e.g., a few minutes), and this property is used to advantage in quarantine treatments that kill insects and surface fungi. Continued exposure to high temperature results in phytotoxic symptoms and then complete tissue collapse.

The deleterious responses to some stresses can be mitigated by prior conditioning treatments. While not as severe as a heat shock treatment, holding chilling-sensitive commodities near their chilling temperature can condition them to be more chilling tolerant. For example, holding grapefruit at 16°C for 7 days reduced their level of injury when exposed to chilling temperatures (i.e., temperatures below 10°C) during storage.

4.7 CONCLUSION

The quality and storage life of harvested horticultural commodities has been increased by the judicious use of temperature and altered atmospheres. Commodities developed by plant breeders with enhanced disease resistance, yield, and quality were the basic materials postharvest physiologists had to work with. Often the postharvest characteristics of a commodity were of minor interest in comparison to these other qualities. The commercial application of postharvest technology still relies heavily on maintaining the "cold chain" from harvest to the consumer, with CA, MA, and MAP being only significant for a few products. However, many postharvest problems could be reduced through plant breeding. In the past, naturally or induced mutations were incorporated in breeding programs to alter a deleterious postharvest characteristic. For example, sweetcorn has a naturally occurring recessive mutation that limits the conversion of sugar to starch. Yet this conversion still occurs after harvest and it was crucial to rapidly cool the corn and keep it on ice to limit starch formation during marketing. Additional mutations were incorporated in newer cultivars that essentially eliminated this problem. Now only normal-temperature precautions are needed to prevent sweetcorn from turning starchy during marketing. Advances in the ability to directly target genes involved in specific metabolic pathways now allow plant breeders to modify many traits that can adversely affect the postharvest life of many horticultural commodities.

REFERENCES

Saltveit, M.E., 2016a. Respiratory metabolism. In: Gross, K.C., Wang, C.Y., Saltveit, M. (Eds.), Agricultural Handbook 66—The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks. USDA Agricultural Research Service, Washington, DC. Available from the National Technical Information Service, Springfield, VA or at http://www.ba.ars.usda.gov/hb66/contents.html.

Saltveit, M.E., 2016b. Respiratory metabolism. In: Sunil (Ed.), Postharvest Ripening Physiology of Crops. CRC Press, Boca Raton, FL, ISBN 9781498703802.

FURTHER READING

Kader, A.A., Saltveit, M.E., 2003. Respiration and gas exchange. In: Bartz, J.A., Brecht, J.K. (Eds.), Postharvest Physiology and Pathology of Vegetables. Marcel Dekker, Inc, New Yrok, pp. 7–30. ISBN: 0-8247-0687-0.