Paper No. 6

Paper Title: Technology of Fruits and Vegetables

Module No. 6

Module Title: Respiration of Fruits and Vegetables

6.1 RESPIRATION

A continuous supply of energy is essential for each and every living organism. The energy is required to perform various necessary biochemical functions like maintenance of cellular organization, membrane permeability, transportation of metabolites etc. Respiration is defined as the process by which cells release energy from organic compounds to generate ATP through a series of chemical reactions involving the transfer of electrons and these ATP molecules act as energy resource for cell. A reduction in respiration cause decreased ATP production and consequently lesser energy is available for carrying biochemical processes associated with ripening, resulting in quality changes. Respiration is considered as a programmed continuation of both catabolic and anabolic processes. This process occurs in mitochondrion of the cell.

There are mainly two types of respiration: aerobic respiration and anaerobic respiration, depending upon availability of oxygen. In aerobic respiration, oxygen (O_2) is the final electron acceptor while in anaerobic respiration, or fermentation, some other compound is the final electron acceptor.

6.1.1 Aerobic respiration

Aerobic respiration is the major biochemical process supplying energy. It involves the oxidation of certain organic compounds i.e. glucose, maleic acid, stored in the tissues. The compounds that are oxidised during this process are known as respiratory substrates. Aerobic respiration occurs in three phases: glycolysis or Embden-Meyerhoff-Parnas (EMP) pathway, the Krebs or Tri-carboxylic acid cycle and the electron transport chain.

In glycolysis, a molecule of the six-carbon sugar glucose is oxidized to two molecules of the three-carbon pyruvate. The Krebs cycle completes the oxidation of pyruvate to produce carbon dioxide (CO_2) and reduced electron carriers. In the electron transport chain, a proton (H^+) gradient drives the production of even more ATP and is coupled with the transfer of electrons to oxygen (O_2), producing water (H_2O). After the entire process of respiration is complete, much of the energy released from the glucose is recaptured in the production of ATP.

Glucose is the most favoured substrate for respiration. All carbohydrates other than glucose are converted into glucose first before they are used for respiration. Fats are metabolized into glycerol and fatty acids first and then to acetyl CoA and glyceraldehyde-3-phosphate, respectively. Proteins are degraded individual amino acids (after deamination) and then enter the respiratory pathway. The respiration is exothermic in nature and theoretically, 60% of the bond energy is lost as heat. However, calorimetry studies have shown that respiration in postharvest tissues often results in even more dissipation of energy as heat loss (90% or more) and less ATP synthesis. This heat contributes to an increase in the temperature of the commodity and is known as vital heat or heat of respiration. Heat of respiration is a primary consideration in designing the storage for horticultural crops.

6.1.2 Anaerobic respiration

Aerobic respiration is preferred energy producing pathway in fruits and vegetables. But under the limiting O_2 conditions, fermentation becomes increasingly important. During this process, pyruvic acid produced during the glycolysis is converted to lactic acid, ethanol or acetaldehyde. Increase in fermentation helps the cell meet its ATP requirement under anaerobic conditions. Anaerobic respiration produces much less energy than aerobic pathway and elevated CO_2 concentrations have deteriorative effect on the product quality. Anoxia results in injuries to tissue. High concentrations of fermentative metabolites are also associated with various physiological disorders like necrosis, discoloured tissues, off-flavours, off-odours etc. The oxygen concentration at which anaerobic respiration starts is called extinction point. It varies between the tissue types and also species, cultivar, development stage, maturity etc.

Apart from aforementioned respiratory processes, two other forms, alternative and residual respiratory processes are also found.

Alternative respiration is an alternative pathway to cytochrome pathway of electron transport to O_2. This exothermic process results in production of CO_2 but no ATP generation occurs because the electron transfer chain is not coupled to oxidative phosphorylation. This respiration occurs when cytoplasm respiration is blocked. It is also known as thermogenic respiration or cyanide insensitive respiration. Alternative respiration causes a rapid loss of storage polysaccharides, electron transport without the loss of reducing power, and generation of heat. The alternate system has been found responsible for increasing the internal temperature of ripening mangoes by 10°C.

Another type of respiration is residual respiration. The enzymes like polyphenol oxidase and peroxidase are involved in wound repair and in defence mechanism against intruding micro-organisms. The consumption of O_2 by these processes, results in what is called as residual respiration. The browning of fruits and vegetable is essentially a wound repairing phenomenon carried out by PPO. In damaged/peeled/cut/sliced produce, level of tissue metabolic activity and the respiration rate is higher than whole produce due to residual respiration. Residual respiration does not produce ATP but contributes consistently to O_2 consumption.

6.2 SIGNIFICANCE OF RESPIRATION

Respiration plays a major role in the postharvest life of fresh fruits and vegetables. Respiration continues even after harvest. After harvest the produce is dependent entirely upon its own food reserves as no replenishment is there. Therefore, losses of respiratory substrates and moisture are not made up and deterioration has started. Produce' possessing a high respiration rate can be stored for longer time duration. An enhanced respiration rate is associated with perishability of food. The significant effects of respiration are:

- **Loss of substrates:** The process of respiration utilizes various substrates and thus results in loss of food reserves in the tissue, loss of taste quality and food value. During the extended storage the loss in weight can be highly significant.
- **Oxygen consumption**: For aerobic respiration, presence of oxygen is must. Reduction of oxygen concentration is a useful tool for controlling respiration rate and slowing down the senescence.
- **Carbon Dioxide production:** Accumulation of CO_2 produced due to respiratory metabolism can be beneficial or harmful. Carbon dioxide concentration is also used as an effective measure for delaying senescence.
- **Release of Heat:** As respiration is exothermic in nature, heat generated raises the temperature of produce. The heat generated is one of the prime consideration for designing the packaging/ storage of horticultural crops.
- **Shelf life indicator:** Respiratory process indicates the metabolic activity of living produce and determines the post-harvest physiology and deteriorative ability of plant produce. Respiration rate is well correlated with rate of deterioration, and thus is a

good measure of the storage potential of the plant produce. Generally, a higher respiration rate indicates shorter shelf life.

- **Classification:** Respiration rate is an important criterion to compare perishability of fruits and vegetables. Depending upon respiratory rate Kader and Barrett classified fruits and vegetables into five different classes, rate as shown below:

Table1. Classification of fruits and vegetables on the basis of respiration rate.

Class	Respiration rate ($mg\ CO_2$/ kg/ h) 10 °C	20°C	Examples
Very Low	<10	<40	Nuts, dates, dry fruits
Low	10	40	Potatoes, onion cucumber, apple pear kiwifruit, pomegranate
Moderate	10-20	40-80	Pepper, carrot, tomato, eggplant, citrus fruit, banana
High	20-40	80-120	Peas, radish, apricot, fig, ripe avocado, papaya
Very High	>40	>120	Mushrooms, green onion, cauliflower, dill, parsley, melons, okra, strawberry , blackberry, raspberry

Source: Data from I. Burzo. Acta Hortic. 116:61, 1980; A. A. Kader and D. M. Barrett. In Processing Fruits: Science and Technology, Vol. 1, Biology, Principles, and Applications. (L. P. Somogyi, H. S. Ramaswamy, and Y. H. Hui, Eds), Technomic Publishing Co., Pennsylvania, 2003, p. 1.

- **Change in quality:** The physiological processes leading to enhanced quality (e.g., color development, softening, astringency loss, and aroma production) are deeply influenced respiration. Extremes in respiration rate results in the development of specific physiological disorders, resulting in loss of quality. Respiration is beneficial in providing carbon skeleton intermediates for pigment synthesis, flavor development, formation of ripening enzymes, fats, sterols etc.

6.3 RESPIRATORY QUOTIENT

The ratio of moles of CO_2 Produced per mole of O_2 consumed is called the respiratory quotient. RQ is as an indication of which substrates are being used in the respiratory pathway. RQ is 1 for glucose catabolism. When substrates other than glucose are respired, the RQ is different than 1. The complete oxidation of malate by the TCA cycle results in a RQ of 1.6 (oxidation of respiratory malic acid, leads to the production of additional CO_2). The RQ can also exceed 1 when O_2 is not involved, such as in fermentation. RQ values below 1 are expected when lipids or proteins, molecules often containing less oxygen than carbohydrates, are respired.

6.4 RESPIRATION MEASUREMENT

The rate of any reaction can be determined by measuring the rate at which the substrates disappear or the products appear. The measurement of respiration is very important because of its association with cell metabolism because the energy derived from respiration drives all other reactions within a cell. Measurement of Respiratory gas exchange is often used as a general measure of the metabolic rate of tissues. It was first conducted by Kidd on pea and mustard seeds in 1917. The respiration rate can be determined by measuring the O_2 and CO_2 concentrations in the atmosphere surrounding the product, determination of loss of dry weight, heat production and loss of energy content. Determination of gas exchange is the most convenient means of measuring respiration rate. It is known, however, that gas concentrations inside the product differ from the concentrations outside due to diffusion barriers exiting between the external atmosphere and respiration seat (mitochondria). Therefore, bulky intact fruits respond to O_2 concentrations differently than cell cultures. There are various ways of measuring gas exchange, but the three methods used most often are:

- **Static method**: In the closed system, a gas-tight container of known volume is filled with product and the container, containing ambient air as the initial atmosphere, is closed. The changes in concentration of O_2 and CO_2 over time duration are measured to estimate the respiration rates.
- **Flow through method**: In the flow through system, the product is enclosed in an impermeable container through which a gas mixture flows at a constant rate. The respiration rates are calculated from the absolute differences in gas concentrations between the outlet and the inlet when the system reaches steady state.
- **Permeable method**: In the permeable system, a package of known dimensions and film permeability is filled with product. The steady-state concentrations of O_2 and CO_2 are determined and a mass balance is performed on the system in order to estimate the respiration rates.

6.5 FACTORS AFFECTING RESPIRATION

6.5.1 Temperature

The temperature dependence of respiratory rate varies among and within commodities. Generally, the respiration increases significantly as the storage temperature increases. Within the physiological range of temperature (0°C–30°C), the rate of respiration increases exponentially, and a large amount of heat is produced as heat of respiration. The relationship between temperature and respiration rate can be expressed in the form of Q values, which allows the estimation of the rate of respiration at an unknown temperature provided the rate of respiration at a known temperature is available. The rate of respiration is described mathematically by use of temperature quotient (Q_{10}),

$$Q_{10} = \left(\frac{q_2}{q_1}\right)^{\left(\frac{10}{T_2 - T_1}\right)}$$

where, q_2 and q_1 are rate of respiration at temperature T_2 and T_1 respectively.
The plant metabolism is drastically affected, not only when temperature is increased but also under reduced temperature conditions. Thus the system undergoes heat stress or chilling stress.

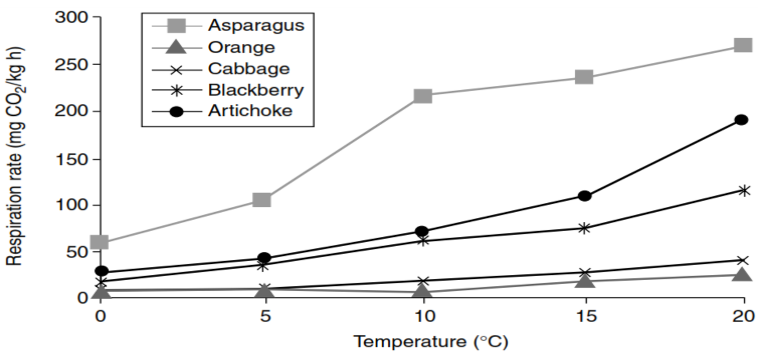

Figure 2.3 Effect of temperature on the respiration rates of some fruits and vegetables. (Data taken from K. C. Gross et al. The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks—A Draft Version of the Revision to USDA Agricultural Handbook Number 66 (2002) (revised in 2004) (www.ba.ars.usda.gov/hb66/index.html.)

Heat Stress: If the temperature rises beyond the physiological range, respiration rate falls. When tissue reaches its thermal death point, metabolism is disordered as enzyme proteins are denatured. Continued exposure to high temperatures causes phytotoxic symptoms and tissue collapse.

Chilling stress: Although respiration is normally reduced at low, but non-freezing temperatures, certain commodities particularly those originating in the tropics and subtropics, exhibit abnormal respiration when their temperature falls below 10 to 12 °C. Respiration may increase dramatically at the chilling temperatures or when the commodity is returned to non-chilling temperatures. Enhanced respiration rate is cells' effort to detoxify metabolic intermediates that accumulated during chilling, as well as to repair damage to membranes and other sub-cellular structures. Enhanced respiration is one of the symptoms that signal the onset of chilling injury.

6.5.2 Oxygen and carbon dioxide

A slight reduction of oxygen concentration does not have any adverse effect on produce, but a comparable increase in carbon dioxide may cause deteriorative changes in fruits and vegetables. The injury threshold of various fruits and vegetables to O_2 varies considerably. Reduction of O_2 concentration below 2%–3% gives beneficial reduction in rates of respiration and other metabolic processes for most produce. Increasing the CO_2 level around some commodities reduces respiration, delays senescence and retards fungal growth. However, complete removal of O_2 is not recommended as anaerobic environment is detrimental to the quality of the produce as it leads to fermentation, decay and development of off flavor, and change in color and texture.

6.5.3 Stage of development

Respiration rates vary due to stage of the development. 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. The 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.

Table 2. Classification of tissue on basis of respiration rate

Respiration rate	Type of tissue
High	Young tissues, meristematic tissues, growing plants (asparagus), partly developed flower buds (broccoli), developing seeds (green bean, peas), immature fruit (sweet corn, okra)
Intermediate	leafy vegetables (spinach, cabbage), unripe fruit (cucumbers)
Low	storage organs roots (sweet potato), tubers (potatoes), bulbs (onions), mature fruits (pumpkins)

After harvest, the respiration rate typically declines; slowly in non-climacteric fruits and storage organs, rapidly in vegetative tissues and immature fruits. The rapid decline presumably reflects depletion of respirable substrates that are typically low in such tissues.

6.5.4 Stress/ Injury

Physical stress during cultivation, harvesting, and postharvest handling influences respiratory behaviour significantly. Tissue injury increases the rate of respiration and induces ethylene production, which may further catalyse an increase in respiration with consequent loss of quality. However, in some tissues wounding stimulates developmental changes, e. g., promote ripening, that result in a prolonged increase in respiration. The extent of increase in respiration rate is usually proportional to the severity of bruising. Water stress which is induced by lower than optimal relative humidity in air surrounding the commodity can stimulate its respiration rate. However when water loss exceeds about 5% the respiration rate is reduced, but at the same time wilting and shrivelling becomes noticeable. Biological stress like disease also increases the respiration rate. Other stresses that stimulate the respiration rate of vegetables including exposure to ionizing radiation and to various chemicals such as methyl bromide (fumigant) etc.

6.5.5 Other

- Surface of tissue: Thickness of surface dermal system, wax composition and arrangement, number and distribution of stomata on the tissue influences the respiration rate. More the thickness of coating, less is the respiration rate.
- Surface to volume ratio: Smaller fruits large surface area, high respiration rate.
- Growing conditions: Cultural practices, irrigation, fertilizer also influence the respiration rate. High nitrogen fertilizer increases respiration while high calcium fertilizer decreases respiration.
- Application of chemicals: Certain chemicals like malic hydrazide, methyl cyclo-propene (MCP), polyamines like putrescine, spermidine and spermine slows down the rate of the ethylene production and respiration while application of ethylene, acetylene, propylene, ethephon has a positive effect on respiration rate due to positive impact on ethylene (ripening hormone) generation.

6.6 CONTROLLING RESPIRATION

Respiration rate is most affected by altered atmospheres and the temperature. The alteration in these two factors forms the basis of storage of horticultural produce upon harvest. The low temperature storage with right relative humidity is particularly beneficial for produce and helps in increasing the shelf life to an acceptable level. Similar effects are achieved by modified storage methods. Decreased O_2 and increased CO_2 levels also influence ethylene production and action and suppress microbial growth. Nowadays, low O_2 and high CO_2 concentrations are involved in several storage or packaging methodologies. The most common being, controlled atmosphere (CA) and modified atmosphere (MA) storage. CA storage generally refers to decreased O_2 and increased CO_2 with monitoring and active adjustment of the gas composition, whereas MA refers to a difference in gas composition as compared with ambient air, without any active control of the gas composition. The anti-senescence agents like polyamines, maleic hydrazide, MCP are also applied to control respiratory rates.

6.7 Conclusion

Respiration is breakdown of complex substrates with concurrent production of energy and useful carbon skeletons. Respiration rate is an excellent indicator of metabolic activity of the tissue and a very useful guide for potential storage life of the horticultural produce. The process is affected by numerous external and internal factors particularly important being temperature and concentration of oxygen and carbon dioxide. The primary aim of any fruit and vegetable technologist is to alter these external factors for maximizing the shelf life of the produce with minimal of biochemical changes, thus preserving the food value in itself.

Measurement and modelling of respiration rate for any particular crop provides an efficient means for the optimal storage conditions for that particular crop.

References:

De Ell, J. R., Prange, R. K. and Peppelenbos, H. W. (2003). Postharvest Physiology of Fresh Fruits and Vegetables, In Chakraverty, A., Mujumdar, A. S., VIjaya Raghavan, G. S. and Ramaswamy, H. S. (eds.) Handbook of Postharvest Technology: Cereals, Fruits, Vegetables, Tea, and Spices, 1st edition, Marcell Dekker, Inc., New York

Fonseca, S. C., Oliveira, F. A. R. and Brecht, J. K. (2002). Modelling respiration rate of fresh fruits and vegetables for modified atmosphere packages: a review. *Journal of Food Engineering*, **52**:99–119

Haard, N. F. and Chism, G. W. (1996). Characteristics of Edible Plant Tissues, In Fennemma, O. R. (ed.), 3rd edition, Marcell Dekker, Inc., New York

Kader, A. A. (1987). Respiration and gas exchange of vegetables, In Weichmann, J. (ed.), Postharvest Physiology of Vegetables, Marcel Dekker, Inc., New York

Mishra, V. K. and Gamage, T. V. (2007). Postharvest physiology of fruits and vegetables, In Rehamn, M. S. (ed.) Handbook of Food Preservation, 2nd edition, CRC Press, Boca Raton

Saltveit, M. E. (2004). Respiratory metabolism, In Gross, K. C., Wang, C. Y. and Saltveit, M. (eds.) The Commercial Storage of Fruits, Vegetables, and Florist and Nursery Stocks, Agricultural Handbook Number 66, A Draft Version of the Revision to USDA, ARS (available at http://www.ba.ars.usda.gov/hb66/019respiration.pdf, accessed on 11 October, 2013)

Wills, R. B. H., McGlasson, W. B., Graham, D., Lee, T. H. and Hall, E. G. (1996). Postharvest: An introduction to the physiology and handling of fruit and vegetables, 1st Indian Edition, CBS Publishers & Distributors, New Delhi