Chapter 4

Glycolysis Fermentative By-Products and Secondary Metabolites Involved in Plant Adaptation under Hypoxia during Pre- and Postharvest

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Additional information is available at the end of the chapter

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Abstract

Floods inducing hypoxia (reduction of available O_2) in the plants are current major constrains for agricultural production. Oxygen deficiency in the plant cells induces the secondary response of anatomical and physiological modifications. Hypoxia triggers glycolysis fermentative pathway and other alternative pathways, when the plant lacks energy. During cultivation, some submerged plants can adapt themselves to survive by modifying some parenchyma cells in the roots to be aerenchyma cells to detain available oxygen for oxidative phosphorylation. Furthermore, carbon sources in the cells will be accumulated in N store that recovers back to a C source at the end of hypoxia. In postharvest, long period in modified atmosphere storage could activate hypoxia in the plant parts that produce off-flavor perception. However, in some fruits at a particular maturity, ethanol, a hypoxic product, can be modified into ethyl ester compounds as the detoxification.

Keywords: oxygen deficiency, fermentative by-products, adaptation, aerenchyma, off-flavor

1. Introduction

Recently, climate changes on Earth have frequently been fluctuated due to unbalances of natural resources used. The consumption of fossil fuel and industrial activities produce a lot of gas pollutions endangering the atmospheric conditions. Cosmic radiation from outside directly passes through the Earth surface and cannot be reflected out to the space, resulting in increasing temperature of the troposphere. Subsequently, ice packs from mountain tops or icebergs from the North and South poles are gradually melting, causing higher sea mean level, in particular near the equator areas. Furthermore, when heavy rains come, mass of water may sometimes not be completely absorbed by the soil and slowly runoff into the sea. Lands close to the sea are risk to encounter flash flooding. As a result, flooding is frequently invaded over some agricultural areas where the crops are forced to be damaged and then perhaps dead depending on the level of submerging and the duration of flooding. Although floods are crucial disasters to agricultural crops across the world, the evidence occurs repetitiously in low lands of the tropics such as South America, Africa, and South Asia.

Floods are typically classified into two types including water logging and flooding. Water logging conducts low levels of water flooded and slowly runoff only over the plant root system in which an anaerobic condition is alternately taken place. On the other hand, water flooding, showing excessive water, could be subcategorized to partial and complete submergences. For the partial submergence, some plant parts are immersed in stand-still water, whereas the whole plant is being under the water level for the complete submergence. The respiration of plant parts under water is forced to be switched to an anaerobic pathway due to slower O_2 diffusion from the air. Thus, hypoxia (low O_2 concentration) is mostly generated in partial submerged plants, when metabolisms of upper water parts are under normoxia, but those of submerged parts are switched to an anaerobic condition. Flooded soil leads the decrease of O_2 concentration surrounding the plant roots. The severity of cell damage is relied on the responses of the plants. Water depth and turbidity are important factors defining this scenario.

Although water is important for agricultural cultivation in particular for industrial propose, excessive water supply may cause changes in the anatomy and physiology of cultivated plants. Flooding is caused by heavy raining under poor draining of soil, which could result in the losses of field crop production. The degrees of losses are due to types of plants, stages of development, and duration of water flooding. Horticultural crops need time of recovery when the levels of flood tolerance are different. For example, durian, pummelo, and jackfruit trees are very sensitive to flooding. In contrast, some evergreen trees containing big canopy such as mango will not be damaged by flash flooding as the root system receives some metabolites from the normoxic leaves for surviving. As a result, it is crucial to understand the response and adaptation of plants to hypoxic conditions that would be beneficial for proper management of agricultural supply chains.

1.1. Evidences involved in hypoxia

Water logging and partial submerging could cause hypoxia in agricultural crops during the growth and development. Referred to a whole plant under water logging, the shoots are in normoxia, when the roots turn to be under an anoxic condition. Although hypoxia could be a major abiotic stress, inhibiting the growth and development in many higher plants, hypoxic tolerant plants can generate some metabolites and modify cell structure for recovery to survive. The level of damage from flooding is apparently relied on soil structure. Soil with high porous in the structure contains high O_2 concentration. Nevertheless, when rain falls, soil is then saturated with water which is the trigger mechanism generating the plant response or adaptation. Most triggers include the by-product substances surrounding the plant roots such as soil redox, pH, and decreasing O_2 level. Soil potential (Eh) is used as a key indicator for chemical changes throughout flooding. The Eh is generally reduced when soil is flooded. Under anaerobic conditions, Eh comprises approximately 350 mv which leads to a high competitive demand for O_2. However, the changes in soil Eh result that Fe^{++}, Mn^{++}, and other cations will be dissolved out and changed into ferrous ions. Furthermore, in contrast to Eh, soil pH has a trend to be increased when flooded. The increasing pH can be indicated from dissolving carbonate and bicarbonate at the initial stages of flooding. Soil pH affects turn-over of soil organic matters and nitrification [1].

Floods induce the decrease of available O_2 in the plant parts submerged in water. Gas diffusion in the air is 10,000 times faster than in water when O_2 diffuses at the rate of 0.201 cm^{-2} s^{-1} in air, compared to 2.1×10^{-5} cm^{-2} s^{-1} in water [2]. The O_2 available level will affect plant cellular metabolisms in three different levels (**Table 1**, [3]):

1. Normoxia comprises aerobic respiration and the ATP production is mainly derived from oxidative phosphorylation.

2. Hypoxia generates when the available O_2 reduces until reaching the limiting factor for oxidative phosphorylation.

3. Anoxia starts when the ATPs are generated only from fermentative glycolysis. This indicates that there is no longer O_2 available. An anaerobic condition in plant during flooding enhances the plants to produce fermentative by-products that accumulate in the roots. In this situation, available energy has dramatic consequence on cellular processes, resulting the generation of unbalances between water and minerals. The plants will be then suffered to other stresses especially disease infection contaminated from flooding.

Apart from plant cultivation, postharvest treatments can either induce hypoxia in stored fresh produce. Fresh fruits and vegetables comprise high rates of respiration after harvest. Thus, some storage conditions such as controlled or modified atmosphere (CA/MA) for long period can generate hypoxia in some parts of the fresh produce. Oxygen cannot diffuse through all tissues causing partial normoxia, hypoxia, and anoxia in the fruit that are responsible for different metabolisms and energy supplies in the fruit tissues.

1.2. Metabolic adaptation plants under hypoxia

Under hypoxia with O_2 deficiency, an inter-conversion of free amino acids is sharply increased. Among the amino acids, alanine is increasingly predominant [4–6]. The production of alanine is come from the inter-conversion of free amino acids derived from proteolysis. Alanine production is related to anaerobic assimilation of NH_4^+ which, in this case, indicates the detoxified of NH_4^+ in the cells [7].

	Normoxia	Hypoxia	Anoxia
Metobolism	Aerobic	Increasing anaerobic	Anaerobic
NAD^+ regenration	Oxidative phosphorylation	Alcoholic and lactic fermentation pathways	Alcoholic and lactic fermentation pathways
ATP production	36-38 mol ATP·mol^{-1} glucose	Dependent on species	~4 mol ATP·mol^{-1} glucose
ATP/ADP content	Normal	Low ATP	Low ATP and High ADP
O_2 content	8-8.5 mg O_2·L^{-1}	1.5-6.0 mg O_2·L^{-1}	0 mg O_2·L^{-1}

Noted: 8.5 mg O_2·L^{-1} = 21% O_2 = 270 μM O_2 =100% O_2 saturation

Table 1. Different levels of available O_2 deficiency of plant cells (modified from [3]).

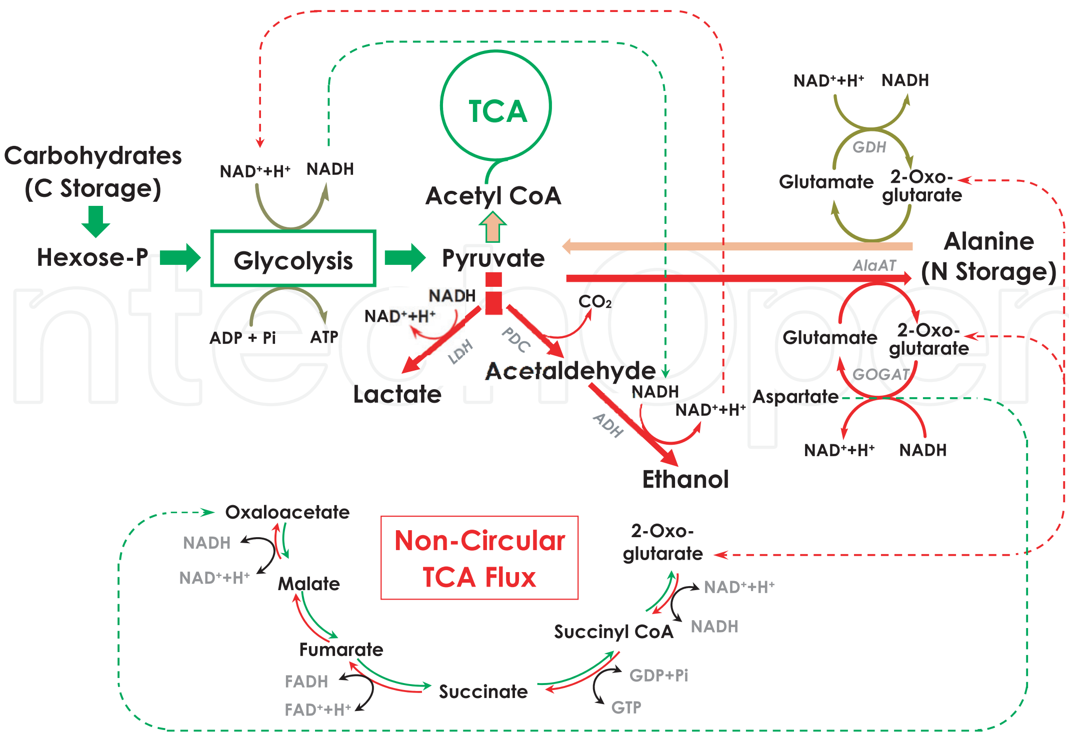

Figure 1. Schematic pathway of carbohydrate and nitrogen metabolisms in plant cells under hypoxic conditions (modified from [1, 8, 9]). LDH: lactate dehydrogenase; PDC: pyruvate decarboxylase; ADH: alcohol dehydrogenase; GDH: glutamate dehydrogenase; AlaAT: alanine aminotransferase; GOGAT: glutamine oxo-glutarate aminotransferase.

The adaptation of plants to survive under O_2 lacking atmosphere includes various evidences in the anatomical, physical, and biochemical changes. Adjustment of N metabolisms in the plants conducts two key enzymes including alanine aminotransferase (AlaAT) and glutamate dehydrogenase (GDH) [8]. Under O_2 lacking conditions, pyruvate derived from glycolysis is alternatively modified to be alanine by AlaAT in coupling with GDH to NAD^+ production. All carbon sources will not be lost through ethanolic fermentative pathway of anaerobic respiration, but some are temporarily accumulated in the form of available N sources instead. When being at the post-stage of hypoxia, alanine is mobilized back to be used as a carbon source by the AlaAT/GDH route. Pyruvate is re-produced again by the reverse reaction of AlaAT in simultaneous with deaminating of GDH to produce NADH and 2-oxoglutarate. Finally, the re-produced pyruvate can go through TCA cycle in mitochondria for oxidative phosphorylation process (**Figure 1**).

2. Plant growth and flowering induced by hypoxia

Hypoxia could affect plant metabolisms throughout the growth and development. This present chapter exemplifies the interesting responses of plant parts to hypoxic conditions that would occur during plant cultivation through the postharvest period.

2.1. Case study: elongation of rice stem during flash flooding

Many rice (*Oryza sativa* L.) varieties can be adapted well with low oxygen conditions to flooding. The crucial adaptations include rapid stem elongation and growth of adventitious roots, and metabolic changes. Rice plants will resume aerobic metabolisms and photosynthesis by raising their shoots and leaves above the water surface. Young rice stem at the vegetative growth stage can be elongating when encounters flooding, but after flowering, the mature plant, however, lose the ability. Low O_2 and high CO_2 of hypoxia during flooding promote the ethylene biosynthesis and then enhance the growth-promoting effect of ethylene [10]. *Sub1* family genes (*Sub-1A*, *Sub-1B*, and *Sub-1C*), transcriptional factors involved in ethylene response domains increase in submergence-tolerant rice cultivars. *Sub-1A* interferes with the normal ethylene-response pathway leading to faster extension growth [11]. Thus, rapid internodal growth of rice under flash flooding results in an increase of ethylene mediates. Endogenous ethylene then alternately induces a reduction of abscisic acid (ABA) concentrations and an increase of gibberellic acid (GA) production in the internodes that promote the stem elongation.

Furthermore, the fast-elongating shoot dramatically retrieves non-structural carbohydrates (NSCs) from other developed parts to avoid complete submergence [12]. New developing shoot and leaves of the submerged rice are supplied by NSCs from the developed leaves under flooding, increased in the carbohydrate consumption for cell division and elongation.

2.2. Case study: flowering induction of wax apple by water logging

Success of fruit production during off-season can be done in commercial orchards of wax apple induced by abiotic stresses. The induction of flowering is related to the management of the root system. In general, farmers either prune the root system or apply a short-term flood that is a famous procedure in Taiwan. Water logging induces hypoxic soil environment when the roots respond to the stimulus. In anatomical study, wax apple roots, being changed, acquire a special type of protective tissues called "**polyderm**" that consists of suberized and non-suberized alternating layers. The change in the cell wall by accumulating lignins and suberins, secondary cell wall components, is a developmental program when the surrounding environments are changed. In addition, in the root system, some parenchyma cells dramatically adapted and transformed to be "**aerenchyma**" cells in the cortical areas under water logging due to hypoxia [13]. Modified to be an aerenchyma cell, several parenchyma cells are fused together and the cell then produces and accumulates lignins and suberins in the secondary cell wall, resulting detaining available O_2 in the cell. Consequently, the development of air space allows the diffusion of O_2 from aerial portions of plants into the roots. An increase of lignification and modification of anatomical structure of aerenchyma are recently reported in carrot tap root grown under hydroponic cultivation [14].

Under water logging stress, wax apple tree, a flood tolerant plant, undergoes the different balances of C and N metabolisms in the shoots and roots. The oxidative response in hypoxic root could be via H_2O_2 signaling [15]. In the leaves, starch content increases and the total N decreases 14 days after flooding (DAF), whereas the total soluble sugars increase in the roots. After flooding, a C accumulation in the leaves and an increase of sugars in the roots are responsible for a reduction of the growth and metabolisms in the roots in which reduces the sink demand of carbohydrates. Furthermore, the roots reveal a reduction of soluble protein content 7 DAF, directly related to an increase of free amino acid content. Glutamate dehydrogenase activity in the leaves reduces 7 DAF, but it is higher in the roots that is responsible for high N compound accumulation (**Figure 1**). As a result of high C:N ratio in the leaves/stems, formation of floral buds is activated after a short period of water logging to the wax apple trees [16].

3. Fruit development and ripening under hypoxia

During fruit maturation on the tree, many kinds of fruits in particular climacteric fruits acquire high biological changes including high respiration and ethylene production rates. The physiological changes during environmental changes could affect the quality of the fruit.

3.1. Case study: mangosteen translucent flesh induced by rain fall during on-tree fruit maturation

Fruit flesh is typically developed from the ovary wall of fertilized flowers, but the edible parts of some tropical fruit are developed from other else. For example, durian flesh so anatomically called "aril" is developed from the seed funiculus, while mangosteen aril is developed from integument of the seed coat. Mangosteen fruit contains 4–5 aril segments developed from apomictic seeds. Each big fruit segment typically contains a complete seed [17]. Mangosteen takes 11–12 weeks after anthesis for fruit development. Rain falls above 20 mm/day for 2–3 consecutive days during mangosteen fruit maturation on the tree induces translucent flesh for 30–60% which is specifically progressed only in ripe fruit. Translucent flesh is an internal disorder which the white opaque flesh turns to translucency and the texture changes from soft to crispy firm. Translucent flesh is usually found in the big segment containing a complete seed which behaves high vitality. The pericarp (peel) of fruit containing translucent flesh absorbs high water matter from rain, when water content in the arils is non-significantly different between translucent and normal fruit. Lignins highly accumulate in the cell wall of translucent aril. During ripening of fruit, solubility of pectin increases due to high demethylation and de-esterification by pectin methylesterase (PME) and polygalacturonse (PG), respectively. Healthy aril behaving white flesh contains an increase of water soluble pectin (WSP), whereas the EDTA-SP and Na_2CO_3-SP are reduced, resulting in rapid reduction of the firmness. On the other hand, translucent aril contains a mild increase of WSP, but the Na_2CO_3-SP significantly increases in parallel of high lignin accumulation in the aril cell wall, especially in the segment containing a complete seed. Thus, the aril firmness of translucent flesh is higher than healthy aril, exhibiting stiffness flesh [18].

The actual cause of translucent flesh in mangosteen fruit during raining is due to water absorption into intercellular space of the pericarp by capillary force. The capillary water functions as a barrier of air movement and circulation in the pericarp resulting generating a hypoxic condition of O_2 deficiency in the fruit. The evident induces higher respiration of the aril, but low energy and high free radicals are released. Thus, this suggests that the high respiration of fruit could be caused partial anaerobic respiration. A high accumulation of CO_2 is found in the intercellular space that could suppress succinate dehydrogenase activity [19, 20], causing noncircular TCA flux (**Figure 1**). The level of reactive oxygen species (ROS) in aril increases during O_2 deficiency [18]. However, adaptability of mangosteen fruit under hypoxia is detected by inducing lignification in the aril flesh, which is related to the phenylpropanoid pathway, including phenylalanine amonialyase (PAL), cinnamyl alcohol dehydrogenase (CAD), and peroxidase (POD) activities. For a hypoxic tolerant mechanism, the ROS production in particular O_2^- that damages cellular membrane and macromolecules is detoxified by modifying

O_2^- to H_2O_2 by superoxide dismutase (SOD). The H_2O_2 is then served as a co-substrate for lignification by POD. In mangosteen fruit ripening on-tree during rain fall, the aril pectin structure is re-esterified, responsible for more covalent crossed-link network and forming building boxes for cell-to-cell adhesion. Consequently, the cell wall structure generates stiff texture of insoluble jelly-like translucent pectin in the middle lamella (**Figure 2**, [18]).

To prevent the internal disorder of translucent flesh in mangosteen fruit, cultivation management would be applied. Firstly, protection of fruit during on-tree maturation from rain fall would be a great deal, but it is difficult for the practice in out fields. Thus, fruits are usually harvested at early stages of maturation to avoid the risk from rain fall that is a typical procedure of Thai farmers for commercial practice. The harvested fruit will turn to full ripening quickly at room temperature. The other recommendation for the preharvest treatment is to spray some waxes covering the fruit at onset of maturation. The thin covering wax will protect the pericarp from the force of capillary water from runoff during rain fall.

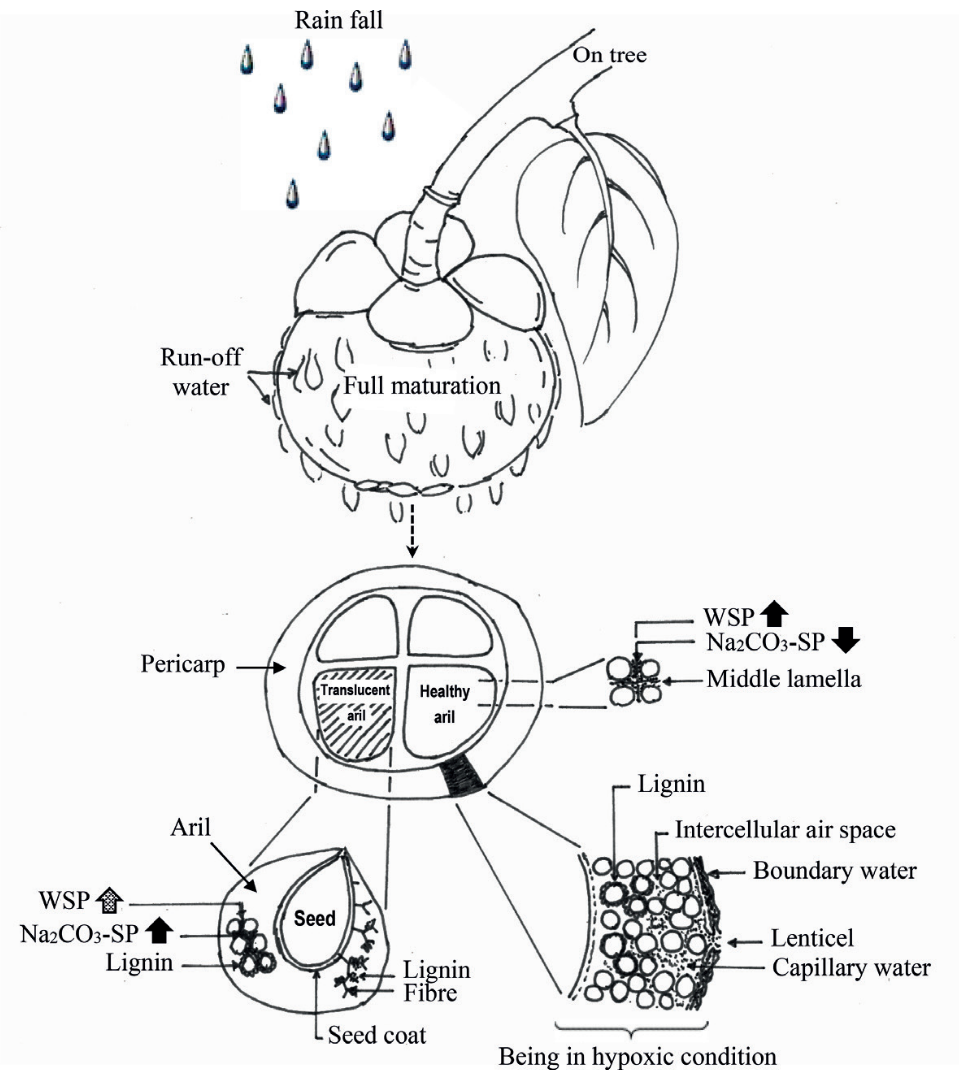

Figure 2. The proposed mechanisms of generation of translucent aril in mangosteen fruit during fruit maturation on tree. WSP: water soluble pectin; Na_2CO_3-SP: sodium carbonate soluble pectin.

Figure 3. Internal disorder of ripe tissues surrounding the seed of "Num Dokmai" (Left) and "Oak Rong" mango fruit (Right) during on-tree ripening.

3.2. Case study: internal tissue disorder of mango during on-tree fruit ripening

The off-flavor of ripe tissue around seed of mango is an internal disorder of some mango fruit cultivars including "Num Dokmai" and "Oak Rong" of Thailand during on-tree ripening (**Figure 3**). The ripe yellow tissue turns to jelly-like translucent tissues with a bid fizzy taste, often generating during on-tree ripening. The disorder would be induced from hypoxia in the fruit upon the physiological changes. Mango among pear and apple produces thick cuticle covering the fruit [21] and the cuticle is even thicker during fruit maturation [21, 22]. Furthermore, as a climacteric fruit, mango shows a climacteric respiration and a peak of ethylene during fruit ripening. Some mango ripening-related genes including alcohol dehydrogenase (*MiADH1*) are sharply expressed at onset of the process [23]. As a result, fruit ripening on the tree could undergo the metabolisms under a hypoxic condition of low O_2 in tissue near the seed. The cuticle thickness of mature mango is a good barrier for O_2 to diffuse into the fruit. The deep tissue could be in a hypoxic condition and start to accumulate acetaldehyde and ethanol compounds, resulting mild off-flavor. Moreover, some modifications in the cell wall of tissue adjacent to the seed are expected to be similarly related to the changes in translucent flesh of mangosteen. In commercial practice, mango fruit are harvested at around 80–90% of maturity and an artificial ripening by applying some ethylene-related compounds is used to the fruit for accelerating the complete ripening to prevent the internal disorder.

4. Hypoxia affecting fruit quality during storage

After harvest, fresh produce lost their quality attributes quickly. Postharvest techniques have been developed and applied to preserve the fruit quality and extend the storage life. However, some evidences of storage treatments could induce hypoxia that is responsible for the quality changes.

4.1. Case study: MA storage of fresh produce

Controlled and modified atmosphere (CA/MA) conditions have been used for extending storage life of agricultural commodities for many decades. Under low O_2 and high CO_2 for long storage, the biological metabolisms of horticultural crops are stimulated by hypoxia to retard the ripening and ethylene response. Hypoxic conditions enhance the synthetic of anoxic proteins as well as the cell ability to survive to the subsequent of anoxia [24, 25]. Hypoxia can reduce the respiration and the ATP biosynthesis by reducing the flow of glycolysis and TCA cycle [8, 9]. This condition retards ripening due to the disruption of ethylene biosynthesis by inhibition of ACC-oxidase. The ripening of stored fruits would be delayed. As a result, the quality of horticultural crops especially climacteric fruits can be preserved, and the storage life is extended. Furthermore, for long period under CA/MA storage, some tissues of the fresh produce may be involved in anoxia that induces an enhancement of the activity of alcohol dehydrogenase (ADH) [14, 24]. This increases the accumulations of acetaldehyde and ethanol in the cells that lead to the perception of off-odor.

4.2. Case study: flavor changes in ripe mango fruits under artificial hypoxia

Our current study (unpublished data) revealed that mango fruit at different maturities showed different response to hypoxic conditions. In this case, the maturities of mango affected different responses to hypoxia. Mature green and ripe "Num Dokmai" mangoes were incubated under artificial hypoxic conditions of continuous air flowed through water and through 10% ethanol solution at 25°C. Under air flowed through 10% ethanol, mature mango fruit behaved severe off-flavor after 3 days of storage, whereas ripe mango obviously released additional volatiles in particular ethyl esters on day 7, compared to the control under normal air flowed (**Figure 4**; **Table 2**).

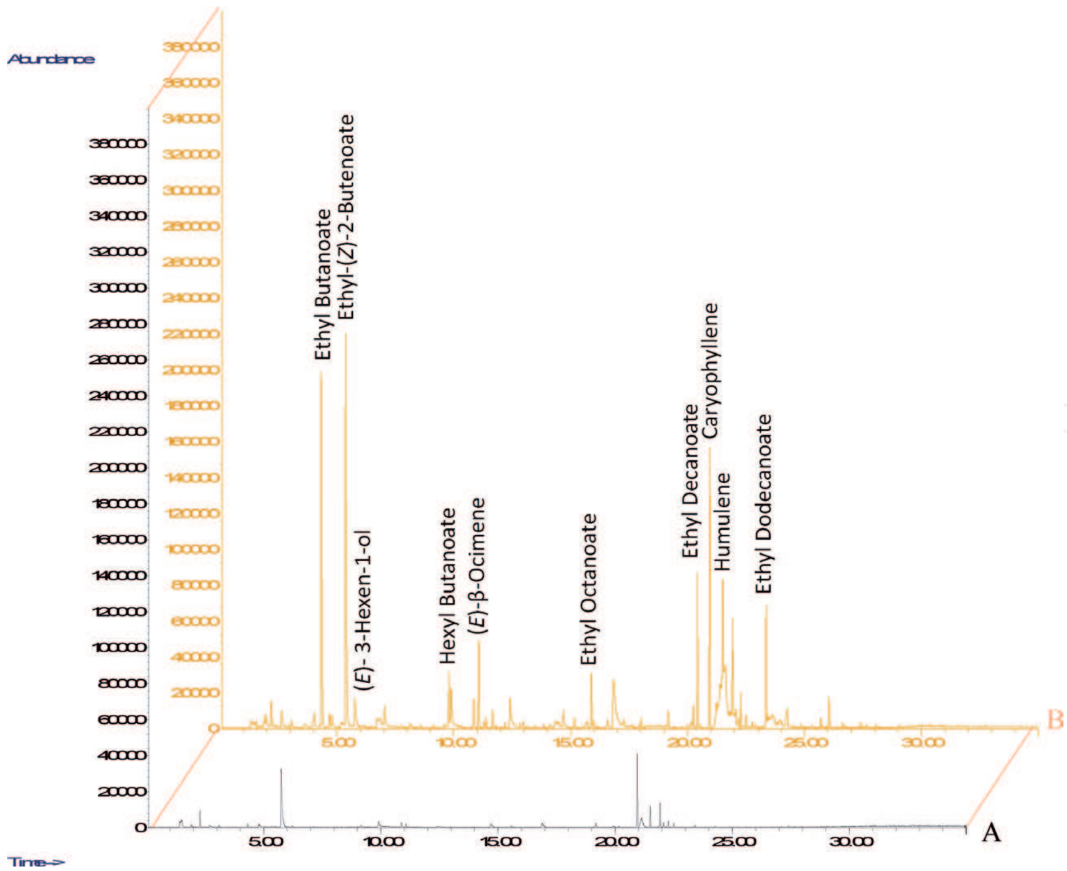

Figure 4. GC-MS volatile profile patterns of ripe flesh of "Num Dokmai" mango fruit incubated with continuous air flowed through water (A) and continuous air flowed through 10% ethanol (B) for 7 days at 25°C.

RT (min)	Normal air	10% Ethanol solution
4.329	-	Ethyl Butanoate
5.369	-	Ethyl-2-Butenoate
5.740	(*E*)- 3-Hexen-1-ol	(*E*)- 3-Hexen-1-ol
9.804	-	Hexyl Butanoate
11.067	-	(*E*)-β-Ocimene
15.879	-	Ethyl Octanoate
20.423	-	Ethyl Decanoate
20.949	Caryophyllene	Caryophyllene
21.503	Humulene	Humulene
23.360	-	Ethyl Dodecanoate

Table 2. Lists of key volatiles released from ripe "Num Dokmai" mango fruit incubated with continuous air flowed through water and continuous air flowed through 10% ethanol for 7 days at 25°C, accorded to **Figure 4**.

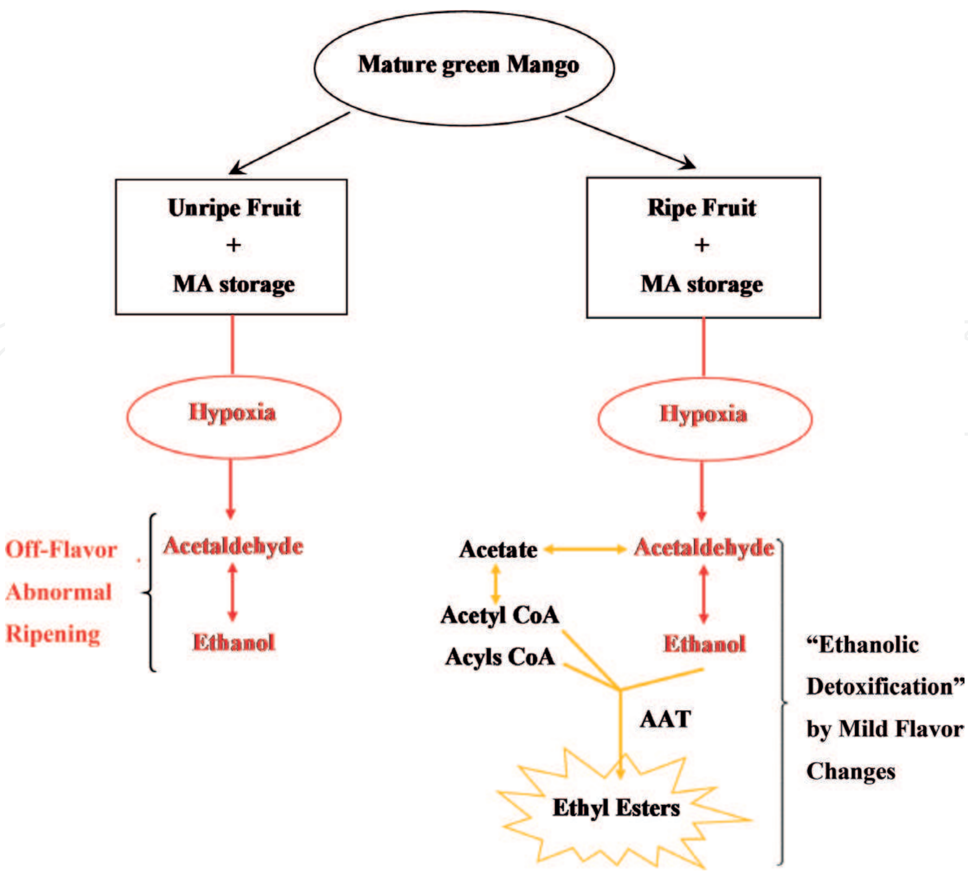

Figure 5. Putative schematic pathway of ripe and unripe mango fruit stored for long period under hypoxic condition.

There is no report about alcohol acyltransferase (AAT), an enzyme producing ester compounds, isolated from mango. Ripe "Num Dokmai" belonging to the Indo-Chinese mango exhibits yellow peel and pulp and high turpentine flavor, whereas many ripe mangoes in the Indian type mango comprising intense peel color, strong aroma and fragrance, and high nutrition value [26] such as "Tommy Atkins" produces a variety of ester volatiles [27]. Alcohol acyltransferase (AAT) has been reported in many fruit as a fruit ripening specific enzyme and is the rate-limiting step for ester biosynthesis regulated by ethylene [28, 29]. It implies that mature green "Num Dokmai" mango fruit contains no AAT activity. When incubated under artificial hypoxia of saturated ethanol vapor, the mango tissues absorbed high amounts of ethanol and released strong off-flavor in a short period. On the other hand, there could be some AAT activities expressed in the ripe fruit, even though typically there is a trace of esters in ripe "Num Dokmai" mango. This is suggested by the conversion of ethanol absorbed in the tissue to be a series of ethyl ester compounds at the late storage as shown in **Figure 4** and **Table 2**. It is in consistent with the report of Jin et al. [30] that ethanol vapor improved aroma profiles especially ester compounds during sweet melon storage. Consequently, some ripe fruits at particular maturity stages can adapt themselves and detoxify the glycolysis fermentative metabolite under hypoxic storage condition by converting ethanol accumulated in the cell to ester compounds by AAT (**Figure 5**).

5. Conclusions

Hypoxia in plants is generated whenever the plants are under conditions of available O_2 reduced such as under water logging/flooding, physiological changes during fruit maturation, and MA/CA storage of plant parts at postharvest. Oxygen deficiency in the cells induces the secondary response of anatomical and physiological modifications. Normoxia, hypoxia, and anoxia can be simultaneously generated in different tissues of the same plant part. Hypoxia triggers glycolysis fermentative pathway and other alternative pathways. Hypoxic tolerant plants are depended on types of plants, maturities, and degrees of hypoxia. Plant defensive mechanisms under hypoxia are signaled mainly by increasing endogenous H_2O_2 and/or ethylene, which are responsible for cascade controls of further endogenous hormones. The responses include an increase in cell wall lignification, different changes in cell wall component, and the production of hypoxic by-products such as fermentative mediate, N-store, and ethyl ester compounds.

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