I.INTRODUCTION

1.1 Background

Fresh produce, including fruits and vegetables, is one of the most perishable products within postharvest systems because of the biological and metabolic processes that persist after harvesting. In contrast to inert agricultural products, harvested horticultural tissues retain their physiological activity and continuously go through respiration, transpiration, enzymatic and biochemical processes, which lead to senescence and reduction in quality (Kader, 2002; Saltveit, 2002). Thus, maintaining freshness during storage and transport represents one of the main challenges of modern horticultural systems, since freshness has a direct effect on the quality, consumer preference, economic value, and storability of the product (Péneau et al., 2009; Valenzuela, 2023).

Postharvest deterioration of product freshness involves many factors that interact with each other such as the temperature of storage, the time of storage, the level of respiratory and metabolic processes, enzyme activity, oxidative stress, and in the case of fresh-cut produce mechanical injury due to disruption of tissues (Ferdousi et al., 2024; Iakimova et al., 2024; Umeohia & Olapade, 2024). All those factors can disrupt cell homeostasis and affect membrane stability, metabolic balance, pigment synthesis, and other quality-related processes. The scale and nature of responses vary for different products under different conditions of storage leading to different manifestations of deterioration at physiological, metabolic and molecular levels (Belay & James Caleb, 2022; Iakimova et al., 2024). Moreover, in fresh-cut products, disruption of tissues creates additional factors that cause exposure of internal tissues and reduce cellular compartmentalization, contributing to oxidative and metabolic changes and accelerating product quality deterioration (Yuan et al., 2025).

In this context, traditional methods for evaluating the freshness of fresh produce have been based on quality indicators and physiochemical/physiological parameters such as color, texture, aroma, weight loss, respiration, biochemical components (Barrett et al., 2010; Paull & Jung Chen, 2000). This type of indicator will be important because it is fairly easy to obtain and has strong associations with the consumers' perceptions of produce quality. However, each traditional quality indicator often indicates one aspect or expression of freshness. In this way, changes in visible quality attributes do not necessarily correlate with physiological and cellular deterioration, especially in cases where stress is induced. The inability of conventional indicators alone to fully characterize the development of freshness deterioration is evident from this point of view (Palumbo et al., 2022; Toivonen & Brummell, 2008).

Chilling injury (CI) is an example of the cellular deterioration of produce under stress. Chilling injury is a physiological phenomenon that occurs in several agricultural products when subjected to low temperature which is above the freezing level and lower than the optimal physiological temperature for the organism (Paull & Jung Chen, 2000; Wang, 2019). The process is associated with destabilization of the cellular membranes, enhanced cell membrane permeability, oxidative stress, and metabolic dysfunction. It is important to note that the changes to cellular membranes due to chilling injury may happen even before visible manifestations appear. Previous researches indicate that an increase in the leakage of ions as a measure of loss of cell membrane permeability control is possible even if respiration rate and visual quality of products remain fairly unchanged (Saltveit, 2002). Thus, chilling injury may be considered a good physiological model for studying the association between cellular changes and visual deterioration.

Membrane integrity at cellular level is an essential factor for the maintenance of homeostasis and quality during postharvest storage. The plant membrane is composed primarily of phospholipids, glycolipids, sterols, and membrane proteins that together influence the fluidity, permeability, compartmentation, and metabolic stability of the membranes (Li-Beisson et al., 2013; Uemura & Steponkus, 1997). During postharvest storage, membrane systems are highly susceptible to environmental stresses, senescence, and oxidative stress. The alteration in membrane permeability leads to the loss of compartmentalization, ions and metabolites leakage, enzyme malfunction, and continuous deterioration of freshness (Hodges et al., 2004; Marangoni et al., 1996). Moreover, recent studies have shown that the remodeling of membrane lipids is strongly correlated with senescence and oxidative stress response in postharvest horticultural products (S. Liu et al., 2026; Whitaker, 2012).

In the postharvest storage, as the deterioration progresses, the latent membrane dysfunction starts to be manifested with the involvement of other physiological and metabolic symptoms of ripening and senescence. In climacteric fruit, the postharvest deterioration process is usually accompanied by the changes in the rate of respiration, organic acids degradation, soluble sugar accumulation, and the change in the coloration during ripening (Wills & Golding, 2016a). The organic acids such as citric acid are gradually used as respiratory substrates, and the sugars like sucrose, glucose, and fructose accumulate as a result of the carbohydrate metabolism and ripening (Batista-Silva et al., 2018; Prasanna et al., 2007;

Xiao et al., 2024). At the same time, the pigments and physicochemical properties are altered, and this reflects the fact of progressing deterioration stage. This metabolic shift is caused by the downstream effect of cellular dysfunction and the reduced freshness. Consequently, the metabolic changes could act as a useful indicator of connecting latent cellular deterioration and visual quality loss.

Another postharvest system worth considering is the fresh-cut produce system since tissue dissection causes the mechanical injury, oxidative stress, the increase of respiration, and rapid senescence (Fonseca et al., 2002; Marangoni et al., 1996; Ragaert et al., 2007; Whitaker, 2012). Mechanical dissection results in the exposure of internal tissues to oxygen and cell compartmentation disruption, which leads to the acceleration of reactive oxygen species (ROS) accumulation, membrane degradation, and metabolic imbalance (Hodges et al., 2004; Toivonen & Brummell, 2008).

Whereas antioxidant components like ascorbic acid are often used to assess freshness loss in fresh-cut products, these markers merely indicate the overall oxidation state of a product and do not provide an adequate insight into the molecular mechanisms involved in the freshness loss process (Giannakourou & Taoukis, 2021). Fresh-cut loss involves complex physiological, biochemical, and membrane-related mechanisms, which cannot be addressed via traditional physicochemical markers. In this connection, it is necessary to obtain additional information on the molecular mechanisms at the membrane level to understand the mechanisms involved in the postharvest deterioration of fresh-cut products.

With the recent advancements in the field of lipidomics, it became possible to study the postharvest deterioration process at the molecular level. Lipidomics makes it possible to characterize the membrane lipid composition and lipid remodeling in response to storage, senescence, and stress exposure (Higashi & Saito, 2019; Narayanan et al., 2020; Sutliff et al., 2021; Zainal et al., 2023). The changes in membrane lipid profile have been correlated with membrane stability, oxidation resistance, and freshness loss and thus may be considered as a source of additional molecular markers complementary to the physiological and biochemical indicators. Moreover, lipidomics proved to be a promising method of biomarker discovery in biological systems (Züllig, Trötzmüller, & Köfeler, 2020). Some earlier studies also revealed that the dynamic changes in membrane lipids are related to the loss of freshness in cabbage, thereby indicating that membrane lipids could serve as biomarkers of freshness loss (Zainal Putri et al., 2023). However, even though the lipidomics is gaining popularity in plant and food science, the combined approach of membrane degradation, metabolic manifestation, and lipid remodeling still lacks.

In this dissertation, an integrated perspective is adopted for the study of freshness deterioration in terms of physiological, metabolic, and molecular properties. In particular, tomato fruit is used as the biological model for studying physiological effects of cold storage, mango fruit is used to study metabolic consequences of postharvest deterioration, and fresh-cut cabbage is studied for membrane lipid remodeling at different temperatures by lipidomics. The three biological models discussed above are not intended to be compared in terms of being commodity equivalents but, instead, are selected in order to demonstrate complementary aspects of freshness deterioration at different biological levels. In this respect, this dissertation aims to provide evidence that membrane lipids can be recognized as a significant molecular aspect of freshness determination and certain specific lipid molecules can be identified as molecular markers of freshness deterioration. The lipid markers identified in the context of the fresh-cut cabbage case can only be recognized as potential candidates which need to be verified with respect to other fresh produce commodities.

1.2 Problem Formulation

Postharvest freshness deterioration in fresh produce is manifested through a series of complicated physiological, biochemical, and molecular membrane changes during postharvest storage. The previous studies on postharvest deterioration have generally focused on individual fresh produce and particular quality traits such as chilling injury, ion leakage, sugars, organic acids, antioxidants, and lipids. This has led to fragmented investigations into postharvest deterioration of commodities either by commodity or by particular quality trait.

Even though tomato, mango, and fresh-cut cabbage have unique physiological properties and postharvest responses, the current dissertation does not intend to make direct comparison between these commodities and the quality traits. On the contrary, these commodities have been purposely selected as biological systems that demonstrate different phases and forms of postharvest freshness deterioration progression. Likewise, the chosen quality traits are also relevant to biological mechanisms of freshness deterioration in the particular biological systems. Fresh tomato fruit represents early, hidden membrane changes in the form of chilling injury progression, fresh mango fruit shows the metabolic changes associated with deterioration progression, while fresh-cut cabbage represents the rapid senescence-associated lipid remodeling of the membranes. Thus, the main focus of the dissertation is to understand the mechanisms of freshness deterioration progression from initial membrane changes to downstream metabolic and lipid changes of the membranes.

Although there has been considerable scientific investigation into physiological deterioration, metabolic changes, oxidative stress response, and membrane lipids dynamics, there is still very limited information on the relationships between these stages of deterioration. The research questions for this dissertation can therefore be phrased as follows:

1. How does concealed membrane deterioration occur before any quality deterioration becomes visible, using the development of chilling injury in tomato fruit as an example?
2. How do the metabolic changes in relation to the deterioration of freshness occur during postharvest storage, as seen in the changes in citric acid and soluble sugars in mango fruit?
3. How are the membrane lipids profiles changing during postharvest freshness deterioration and senescence development, using fresh-cut cabbage as an example?
4. Can membrane lipid components associated with freshness deterioration progression serve as potential molecular biomarkers for freshness assessment in fresh produce?

1.3 Research Objective

The goal of this thesis is to create a mechanistic model for evaluating freshness deterioration in fresh produce and to understand how membrane-associated deterioration proceeds, from its physiological manifestation to molecular changes in membrane lipids during postharvest storage. Another aim is to study the possible use of membrane lipid dynamics as a molecular indicator for freshness evaluation in fresh produce. Objectives of the research are:

1. To study the occurrence of the hidden deterioration process associated with the membranes that takes place before the visible quality decline, using the example of chilling injury development in tomato fruits.
2. To study metabolism changes related to the freshness deterioration process during postharvest storage, as shown by the changes in citric acid and soluble sugars content in mango fruits.
3. To study dynamic lipid changes in membrane lipids associated with freshness deterioration and senescence development during postharvest storage, using fresh-cut cabbage as an example.
4. To identify membrane lipid components related to the freshness deterioration progression and that could be used as molecular markers for freshness assessment in fresh produce.

I.4 Research Benefit

The present dissertation is intended to make scientific contributions towards the understanding of the deterioration mechanisms in postharvest freshness of fresh produce systems. With an inclusion of the physiological response, metabolic manifestation, and membrane lipid modification, it seeks to develop a mechanistic basis for assessing the postharvest deterioration in freshness. The expected contributions include:

1. A contribution towards improving the scientific understanding of membrane-related mechanisms of deterioration in postharvest freshness of fresh produce systems.
2. Scientific understanding of the connection between early membrane damage, metabolic changes, and membrane lipid modification at the course of deterioration.
3. An improvement in existing knowledge of postharvest deterioration mechanisms in different physiological states such as chilling injury, ripening-related deterioration, and senescence-related deterioration in fresh-cut produce systems.
4. To contribute to the advancement of agricultural sciences by improving the understanding of physiological and molecular mechanisms underlying senescence stress in horticultural commodities.
5. The application of the lipidomic approach towards understanding membrane lipids dynamics in relation to freshness deterioration and senescence development in horticultural products.
6. Provision of scientific information on the membrane lipids involved in the freshness deterioration as the foundation for future freshness assessment studies in fresh produce systems.
7. The development of a mechanistic basis which can be used as the scientific platform for future studies on postharvest deterioration mechanisms and molecular freshness in horticultural crops.

I.5 Novelty

This dissertation provides an alternate view regarding postharvest quality by shifting the traditional postharvest quality assessment paradigm from a descriptive and symptom-based one to a mechanistic and molecular understanding of postharvest deterioration. **According to agricultural science principles, horticultural produce that is postharvest is a live organism that constantly interacts with its surroundings and experiences physiological and biochemical changes during storage**. Therefore, the concept of postharvest quality should not be considered a mere description of quality attributes; instead, it should be viewed as a biological condition that results from the gradual physiological responses to storage.

In the past, postharvest quality evaluation in fresh produce was mainly based on visual, physiological, and physicochemical traits such as color, texture, respiration rate, sugars, organic acids, antioxidants, and many others. Although these traits are important for postharvest assessment of quality, many of them are the signs of deterioration, rather than the processes involved in the deterioration itself. As a result, conventional postharvest quality assessment can provide limited information about the cellular mechanisms that occur prior to visible deterioration.

The scientific contribution of this dissertation to agricultural science is the reconsideration of freshness deterioration as a progressive biological process rather than an alteration of the quality attributes that can be observed. In this study, the preservation of postharvest quality is associated with the maintenance of cell integrity and the regulation of physiological, metabolic, and molecular processes that take place in the cells after harvesting. Under this concept, the membrane integrity and membrane lipid remodeling will contribute to the molecular mechanism of postharvest deterioration.

The novelty of this research is not in the individual assessment of some quality parameters such as ion leakage, organic acids, soluble sugars, antioxidants, or lipids, which have been studied before separately for various horticultural products. The novelty of this study is in the mechanistic interpretation of physiological response, metabolic manifestation, and molecular membrane lipid remodeling in the context of postharvest deterioration as a progressive biological process.

In addition, this dissertation asserts that freshness deterioration follows a process that starts with an initial, hidden disturbance of membrane integrity, progresses to the metabolic stage, and further moves to membrane lipid remodeling at an advanced senescent state. Using tomato fruit, mango fruit, and fresh-cut cabbage as models for illustrating different stages and aspects of freshness deterioration, rather than using them as commercial items for comparison, is the key point in this framework.

Therefore, originality of this dissertation is illustrated in the following points:

1. Showing that freshness deterioration can start from an early, hidden disturbance of membrane associated with freshness deterioration, which is demonstrated by the

development of chilling injury and ion leakage in tomato fruit under cold storage conditions.

2. Showing that freshness deterioration continues with metabolic manifestation of freshness deterioration in terms of changes in citric acid and soluble sugars in mango fruit.
3. Illustrating dynamic membrane lipid remodeling during advanced senescence, which is shown through the change in lipidomic profiles of fresh-cut cabbage stored over time, analyzed using targeted lipidomics along with multivariate statistical analysis.
4. Formulating an integrated mechanism of physiological deterioration, metabolic manifestation, and membrane lipid remodeling in terms of freshness deterioration in fresh produce systems.
5. Providing a membrane-centered theory for postharvest freshness deterioration, in which membrane integrity and membrane lipids provide crucial molecular aspects in addition to observable quality attributes in relation to freshness deterioration.
6. Membrane lipid molecules as possible indicators for assessing freshness of produce in the future.