MEMBRANE LIPID DYNAMICS AS MOLECULAR BASIS FOR FRESHNESS ASSESSMENT IN FRESH PRODUCE STORAGE USING A TARGETED LIPIDOMICS APPROACH

DISERTATION

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SUMMARY

Freshness is one of the key quality attributes that determine quality, market value and consumer acceptance of fresh produce. In the context of postharvest studies, the evaluation of freshness typically relied upon external quality attributes and physicochemical indicators, such as colour, texture, respiration rate, organic acids, soluble sugars, antioxidants and microbial quality. Although all the mentioned parameters are commonly applied in postharvest research, they represent visual or physiological manifestations of freshness deterioration and provide limited information about the associated biological processes. The recent studies suggest that biological membranes play a key role in postharvest deterioration since they are prone to damage under environmental stress, ripening, mechanical injury, and senescence. However, the associations among physiological responses, metabolic changes and membrane lipid remodeling during postharvest storage are not sufficiently clarified.

This dissertation was designed to present the integrative approach to the study of freshness deterioration in fresh produce by evaluating physiological responses, metabolic manifestations and membrane lipid remodeling during postharvest storage. Instead of the comparison of different horticultural products, tomato fruit, mango fruit, and fresh-cut cabbage were chosen as the representative biological models of different manifestations of freshness deterioration. Tomato fruit was used for the study of physiological responses to the low-temperature storage, mango fruit was chosen for the characterization of metabolic changes associated with freshness deterioration, whereas fresh-cut cabbage was selected for the investigation of membrane lipid remodeling. By means of these complementary biological models, this dissertation combines physiological, metabolic and molecular approaches to the investigation of freshness deterioration.

The first part of the study characterizes the physiological responses of tomato fruit to the low-temperature storage based on respiration rate, colour development and ion leakage changes. The low-temperature storage suppresses the respiratory activity and delays the colour development, whereas ion leakage significantly increases after the prolonged storage at 2 °C. However, the statistical analysis does not show significant differences in ion leakage among temperature-storage time combinations. These results imply that respiration rate, colour development, and ion leakage are the complementary physiological indicators that provide the information about the physiological status of chilling sensitive tomato fruit during storage, but

none of these parameters sufficiently represents freshness deterioration under the studied conditions.

The second part of the study investigates metabolic changes associated with freshness deterioration during mango storage. The high-performance liquid chromatography analysis has shown that citric acid decreases progressively, whereas sucrose accumulates. On the contrary, the concentrations of glucose and fructose demonstrate relatively small changes. These metabolic changes are the manifestation of the ripening process and the progression of freshness deterioration and prove the importance of the metabolic approach to the characterization of biochemical changes during postharvest storage.

To further investigate freshness deterioration at the molecular level, the targeted lipidomic analysis was performed using fresh-cut cabbage as the model of accelerated senescence. The freshness deterioration was characterized through the integration of cumulative CO_2 production, colour, ascorbic acid content, microbial growth, sensory evaluation, and membrane lipid remodeling. Among 1,308 monitored multiple reaction monitoring (MRM) transitions, 133 putatively annotated lipid species were selected for the multivariate analysis. The heatmap analysis has shown that the coordinated changes take place in the membrane lipid composition, whereas the Partial Least Squares Regression has revealed a strong association between the lipidomic profiles and cumulative CO_2 production, which indicates the importance of the lipidomic analysis for the characterization of physiological deterioration during postharvest storage.

The further biomarker screening has revealed five triacylglycerol (TAG) molecular species, namely TAG 52:5_FA16:1, TAG 52:4_FA16:0, TAG 54:7_FA18:3, TAG 52:4_FA18:1, and TAG 52:6_FA16:0, as the candidates for the lipid markers associated with freshness deterioration. These lipid species have the greatest contributions to the predictive model and demonstrate strong statistical associations with the cumulative CO_2 production. Comparing with the author's previous lipidomic study of the whole cabbage, it has been found out that although the direction of TAG abundance differs between intact and fresh-cut tissues, the TAG consistently represents the lipid class most closely associated with freshness deterioration. This implies that the membrane lipid dynamics depend on tissue integrity and physiological state and highlights the importance of TAG metabolism as the informative molecular response associated with postharvest deterioration.

In conclusion, this dissertation shows that the freshness deterioration involves the interrelated physiological, metabolic and molecular changes which are not fully reflected by the single conventional quality attribute. By means of the evidence gained from the tomato fruit, mango fruit and fresh-cut cabbage as the representative biological models, this study provides the unified approach for the examination of freshness deterioration at different biological levels. In addition, the targeted lipidomic analysis proves the potential of the membrane lipid dynamics as the molecular aspect of the freshness assessment and reveals five TAG species as the candidates for the molecular markers associated with freshness deterioration in fresh-cut cabbage. These candidate markers should currently be considered as specific for the studied fresh-cut cabbage system, not the universal markers for fresh produce. Nonetheless, the identification of these candidates provides the scientific basis for their further validation in different fruits and vegetables, cultivars, and storage conditions. Such validation may lead to the future development of the lipid-based molecular markers and their integration with the physiological, metabolic and non-destructive measurements for the comprehensive freshness assessment in fresh produce systems.