



Role of integrated omics in unravelling fruit stress and defence responses during postharvest: A review

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ABSTRACT

Fruit are susceptible to quality loss and deterioration after harvest due to high metabolic and physiological activities. Over the last four decades various postharvest treatments have ensured maintenance of quality, control of diseases or decay by slowing down the postharvest ripening and senescence. The fruit quality change during postharvest however, has been mostly explored using physicochemical characteristics. Considering the complexity of fruit physiology and metabolism, the application of omics techniques could aid the in-depth analysis and understanding of fruit quality change during postharvest treatment. Therefore, this review presents recent information on the application of integrated omics (transcriptomics, proteomics, and metabolomics) in postharvest research, with an overview on fruit quality and safety. Trends in omics data analysis for fruit during postharvest handling was highlighted. The role of integrated omics in improving our understanding of fruit response during natural postharvest progression (towards decay) during storage, as well as in case of induced responses due to the application of biocontrols was discussed. The article concluded with the outlooks of future studies on the application of integrated omics as the catalyst for innovative postharvest solutions.

1. Introduction

After harvest fruit remain biologically active and continue the metabolic process (ripening and senescence) that leads to quality deterioration. The mechanism that leads to undesirable changes in fruit quality and the disruption of normal metabolism during stressful conditions are mostly linked to the interaction between phenols, oxidative process, enzymatic reaction, and decay incidences (Li, Ban, Limwachiranon, & Luo et al., 2017). Therefore, for many decades the use analytical approach and physical characterises has been the main markers of fruit quality change and deterioration (More, Ranadheera, Fang, Warner, & Ajlouni, 2020). Hence, little is known about the molecular events that lead to the physiological and quality disorders observed during postharvest (Lara et al., 2022). Molecular level information could serve as signalling molecules that alter the biochemical process and the consecutive quality changes in the fruit (Lara et al., 2022). Recent advances in high-throughput technologies such as, transcriptomic, proteomic and metabolomics enables qualitative monitoring

and understanding of biological processes and allow determination of their various states on genomic scales (Belay, Caleb, Vorster, Heerden, & Opara et al., 2020; Mathabe, Belay, Ndlovu, & Caleb et al., 2020; Tang, Chen, Hu, Deng, Chen, & Li et al., 2018; Sirangelo et al., 2022; Xie et al., 2022).

Transcriptomic approach deals with the complete set of RNA-transcripts that are produced by the genome, and proteomics focus on the study of protein abundance, protein expression and possible interactions within the organism or cell (Belay et al., 2020; Mathabe et al., 2020). On the other hand, metabolomics is the analysis of all metabolites present within the biological system at given condition (Pott et al., 2020). Genes and proteins are mostly involved in storing and unfolding information needed for actualization of cellular functional process, whereas metabolic analysis helps to understand the actual dynamic in a cell. Application of these omics tools has been reported to identify the molecular processes that could be responsible for various disorders occurs during postharvest handling.

Recently, various studies reported integrating two or more of the

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omics techniques for postharvest studies on fruit. In this regard, Duan et al. (2021) integrated metabolomics and transcriptomic data to reveal the quality between red and white ragaria pentaphylla (wild strawberries). Xie et al. (2022) applied a proteomic and transcriptomic analysis to elucidate the crown rot caused by *Fusarium proliferation* on banana (cv. Brazilian) fruit. Most recently, the key genes involved on the onset and formation of greasiness in different apple cultivars (cvs. Granny Smith, Golden Delicious, Fuji) during postharvest handling using co-expression network analysis on the differentially expressed genes was investigated by Yan et al. (2022). On another study, multi-omics approaches used to reveal the effect of room temperature storage for litchi fruit quality by Guo et al. (2022). Most of the above studies highlighted that the integrated omics approach provides a better knowledge to understand various molecular mechanisms at the whole genome level. In addition, the benefit of integrated omics analysis for postharvest research has been suggested by various studies (Chen, Chen, & Lei, 2017; Yun et al., 2019; Sirangelo et al., 2022).

Chen et al. (2017) reported a review, which summarized the meta-omics tools, specifically meta-transcriptomic, meta-proteomics, and meta-metabolomics to understand the microbial community dynamics and characteristics in fermented foods. On recent reviews, the food omics approach focusing on proteomics and metabolomics to highlight the methodologies and their application on food microbiology especially during fermentation was reported by Xu (2018). In another review, More et al. (2020) reported the biomarkers associated with quality and safety of fresh produce mainly focusing on the metabolomics analysis. Most recently, Sirangelo et al. (2022) reviewed different articles on the area of multi-omics approach for peach postharvest ripening process and suggested multi-omics studies with data integration could contribute to further knowledge about the complexity of peach postharvest physiology. However, these reviews lack information related to molecular responses of various fruit due to application of various postharvest treatments storage duration, the defence mechanisms of fruit against pathogens as well as the challenges and opportunities of omics data analysis during postharvest. Therefore, the aim of this review is to provide recent information focused on integrated omics (transcriptomic, proteomic and metabolomics) approach and analyse their roles in the identification of underlying mechanisms for fruit responses to postharvest treatment and storage conditions. The review included a discussion on the use of multi-omics approach on understanding of fruit defence responses based on the use of biocontrol technique.

2. Current application of omics in postharvest fruit quality and safety: An overview

Fruit quality and safety are the main factors determine the market competitiveness during postharvest time. Research on the molecular basis of these factors can be used for deciphering the mechanism of quality traits such as fruit colour, aroma, texture, aroma compounds, sugar, and acid content. Omics techniques such as, transcriptomics, proteomics, and metabolomics individually or as integrated has been used to study the influence of various postharvest treatments on the quality of fruit during storage. According to the Scopus library search, the application of these molecular techniques in fruit postharvest studies has been on the increase (Fig. 1).

Several studies have discussed the application of omics techniques, with their focus on available techniques, their respective targeted biological processes, and associated response to postharvest treatments, as well as changes in fruit quality. For instance, studies conducted on 'Dkue' blueberries (Zhang et al., 2020b), 'Ruiguang 1' nectarine fruit (Wang et al., 2022), and 'Cavandish' banana fruit (Si et al., 2022), all identified gene expression profiles of the fruit due to postharvest treatments using transcriptomics approach. On the other hand, studies reported for 'Akihime' strawberries (Ban et al., 2018), 'Xiahui 8' peaches (Jiang et al., 2020), and lane late naval orange (Li et al., 2022) reported that the use of proteomics was powerful to understand the protein function and interaction because of changes in fruit quality and physiology during storage. Similarly, Santin et al. (2021), used metabolomics approach to elucidate the mechanism of ripening, senescence, response to postharvest treatments and storage conditions for 'Fairtime' peaches. However, multi-omics and integrated approach is required for detailed mechanistic understanding of key aspects of postharvest treatments and storage on fruit. In this regard, the multi-omics approach was reported for 'Feizixiao' litchi fruit by Guo et al. (2022), for 'Jingtian No.3' green bell pepper by Fu et al. (2022), for 'Hayward' Kiwifruit fruit by Tian et al. (2021), and for apple by Yan et al. (2022). These studies provided detailed insight into the molecular mechanism related to the changes in fruit quality.

Considering the extremely vulnerable nature of fresh fruit during postharvest handling, storage and marketing to spoilage/pathogenic fungal infections, and the public health concern of chemical residues on ready-to-eat. The application of natural bioactive products and/or biocontrol agents has emerged as an alternative to chemical fungicides (Matrose et al., 2021). In this regard, the application of omics techniques

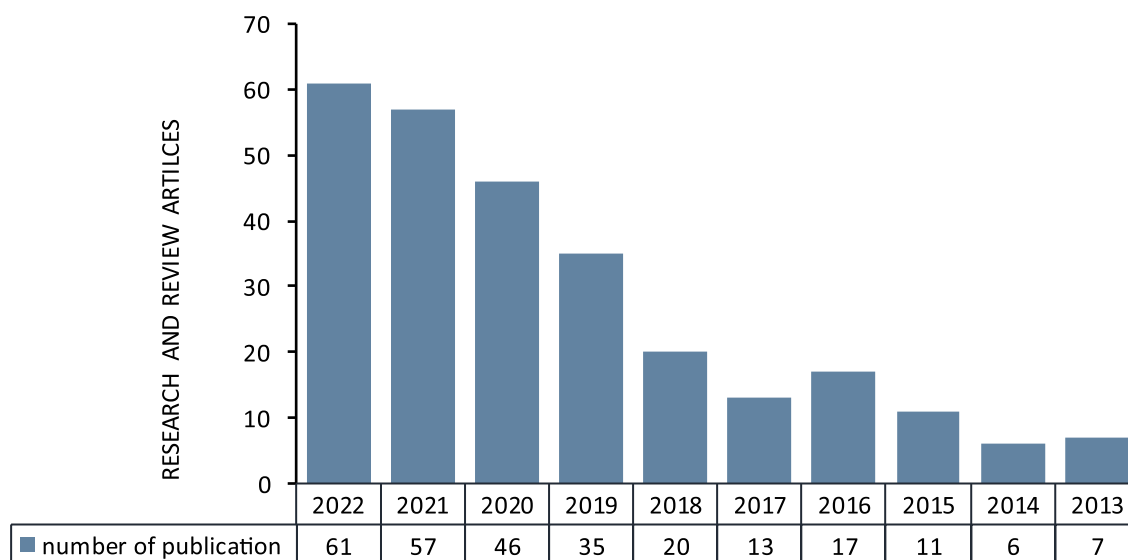


Fig. 1. Scopus search summary of the 232 review and research articles on the area of fruit postharvest omics.

would be important to study the molecular interaction between post-harvest fruit, natural bioactive compounds as treatments, and pathogen (Spadaro and Droby, 2016; Wang et al., 2021). Integration of transcriptomics and metabolomics techniques utilized to clarify the possible mechanism of how chitosan provide tolerance to *B. cinerea* on two grape varieties (cvs. Kyoho and Shine Muscat). The authors reported that the information from the differentially expressed genes and secondary metabolites from the two varieties differences and assisted to clarify the possible mechanism of how chitosan provide tolerance to *B. cinerea*. In addition, the study reported by Xu et al. (2022) identified 5037 differentially expressed transcripts of grapes (cv. Red globe) genes after infection by *T. rugulosu O1* were related to induction of Ca^{2+} influx, oxidative burst, protein kinases and synthesis of plant secondary metabolites. The study further stated that the corresponding expressed genes using RNA-Seq was modified by the *T. rugulosu O1* infection. The review by Zhang et al. (2021) presented the recent knowledge on microbial communities that have potential for biological control for post-harvest fruit pathology.

2.1. Trends in omics techniques

Analyses of transcriptomic, proteomic and metabolomics dataset has been reported to understand the important genes, proteins and metabolites participating in the continuous molecular processes that are responsible for quality changes during postharvest handling of fruit (Yun et al., 19). Combining these omics approaches is an important strategy since biological information flow is from genome to metabolome (Fig. 2) and could be advantageous to capture changes at all levels. Transcriptomic analysis can be done by hybridization or sequence-based approach, which involves incubating complementary DNA (cDNA) with microarray or direct sequencing of cDNA, respectively. Transcriptional levels are limited to a moderate predictor of protein expression, and they do not account for post-transcriptional processes such as translation regulation or protein stability. Other limitation of transcriptomic expression includes constraint of the array, i.e., not all the genes are present in the array, concealment of true expression

levels due to bias of the probe set (Li et al., 2020).

On the other hand, proteomics analysis use tools such as, electrophoresis and mass spectrometry (MS) (Mathabe et al., 2020). Compared to transcriptomic analysis, proteomics has additional merit for its potential to reveal post-translational modifications (PTMs). This is a requirement for evaluating the functional influence of protein modification (Mathabe et al., 2020). Like the other omics, metabolomics analysis also requires highly sensitive and selective techniques such as, mass spectrometry (MS) coupled with gas chromatography (GC), liquid chromatography (LC), and nuclear magnetic resonance spectroscopy (NMR) (Pott et al., 2020). The main challenges of metabolomics studies include diversity of metabolome and incomplete knowledge of metabolic pathways, as well as identification of molecules from structural detector signals with limited metabolite-specific libraries. Table 1 presents summary of the omics techniques, their benefits and limitations associated with fruit postharvest research.

2.2. Integrated omics data analysis and interpretation

Integrated omics data contributes to the elucidation of molecular mechanisms underpinning the processes leading to extended shelf life for fresh produce. The benefit of integrating transcriptomic and proteomic approaches provides more information during the complex fruit response process under stress. It is worth to mention that analysis of integrated omics data in fruit postharvest research is limited to the most obvious “union approach”, which considers a union of two different data sets from one sample to create a reference dataset (Fig. 3). Although an integrated omics approach has a great potential for fruit postharvest research, data analysis is a critical challenge because it needs a combination of high-throughput information from different sources. In addition, integration of omics data requires a development of user-friendly data analysis solution that is linked to the biological process.

For interpretation of integrated omics data for fruit postharvest research, several authors highlighted the potential use of different multivariate analysis such as, principal component analysis (PCA) and hierarchical clustering analysis (HCA), orthogonal partial least squares

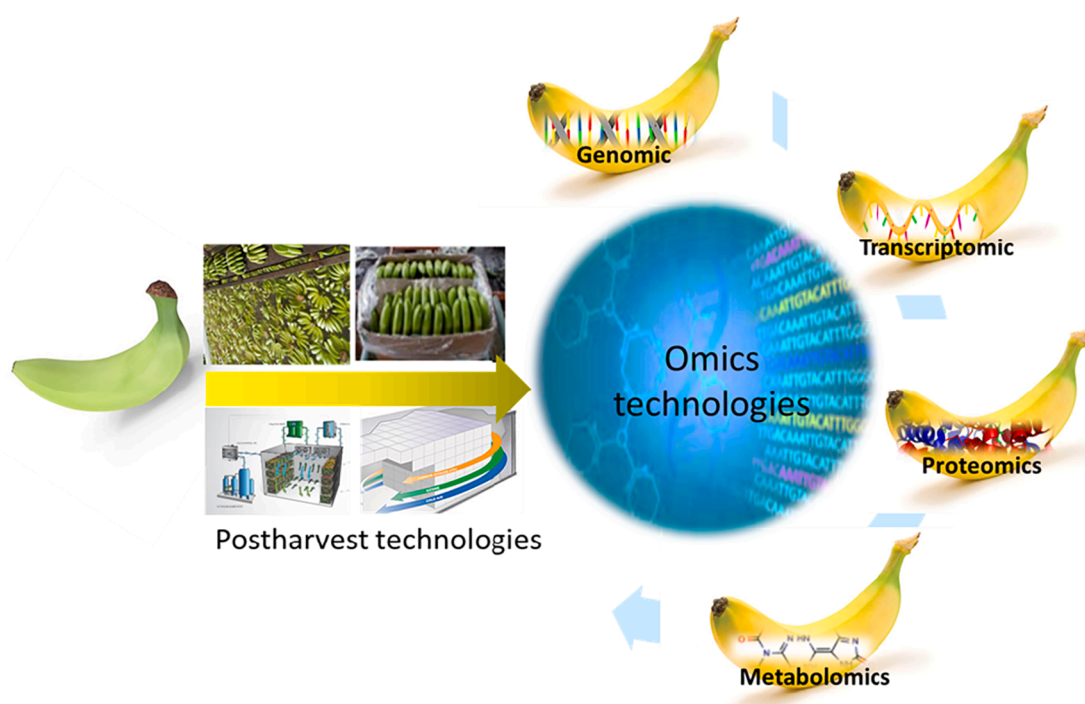


Fig. 2. Overview of postharvest technologies, different omics-techniques, and their biological information flow such as genomic, transcriptomic, proteomic and metabolomics.

Table 1
Techniques for transcriptomic, proteomic and metabolomics dataset generation.

Omics category	Methods	Benefit	Limitation
Transcriptomic	DNA microarray	Most widely used, relatively inexpensive, Availability of complete genome required for analysis, Knowledge of significant amount of transcript sequence	Detect only known genes, high background, limited dynamic range
	cDNA amplified fragment length polymorphism (cDNA-AFLP)	Allow detection of low abundance, can be used without sequencing information	High quality and quantity of DNA required, complicated methodology
	Expressed sequence tag (EST) sequencing	Highly reproducible, high degree of sequence	Required known sequencing database
	Serial analysis of gene expression (SAGE)	Used to analyse large number of transcripts, can help identifying new genes by using tag as a PCR primer	Cost and time required to perform PCR and sequencing reaction, multiple genes could have the same tag
	Massive parallel signature sequencing (MPSS)	Similar to SAGE except different sequencing approach to biochemical manipulation, producing more sequence	Requiring quantity of RNA, high cost
	RNA-seq	Most recent profiling method, Large amount of sequence coverage, and accuracy	High cost, require high power computing facilities, complex analysis of splice variants
Proteomics	2D gel electrophoresis (2D-GE) techniques	High sample size, analysis of all proteins in the sample Protein can be separated in pure form Semi-quantitative measurement of protein Possible post-translational analysis	Analysis of very low and very high is problematic, Limited reproducibility, Smaller dynamic range than other methods
	Matrix-assisted laser desorption/ionization (MALDI) imaging mass spectrometry	High sensitivity, relatively quick analysis	Require relatively pure sample, high homology protein sequence must present in database
Omics category	Methods	Benefit	Limitation
	Mass spectrometry liquid chromatography mass spectrometry (LC-MS)	Automation, multidimensional, high sensitivity	Time-consuming, sensitive towards interfering compounds, restricted mass range
	Liquid chromatography-tandem mass	High sensitivity (identifying peptides/proteins)	Potential peptides loss during clean-up High level of

Table 1 (continued)

Omics category	Methods	Benefit	Limitation
Metabolomics	spectrometry (LC-MS/MS) In-gel tryptic digestion liquid chromatography-tandem MS (geLC-MS/MS)	Suitable to examining complex biological samples Recent, more reproducible quantification, comprehensive	expertise required Identification of protein less direct, needs a robust data mining
	Gas chromatography mass spectrometry (GC-MS)	Able to measure small polar metabolites. Robustness and reproducibility that allow establishment of libraries and database facilitating identification of metabolites	Restricted to volatile and thermal stable molecules Requires derivatisation
	Liquid chromatography mass spectrometry (LC-MS)	Suitable for relatively polar compounds with low, moderate or high molecular weights Derivatisation is not required Thermos-unstable compounds can be analysed	No universal mass spectral library Fragmentation patterns are poorly reproducible Not all compounds in plant extract can be ionise under the same conditions Suitable for targeted analysis Uncommon
	Capillary chromatography mass spectrometry (CE-MS)	Allow detection of a wide range of highly polar or charged metabolites by separating then based on their mass-to-charge ratio	
	Nuclear magnetic resonance spectroscopy (NMR)	Low sensitivity compared to MS approaches Provides structural information, involving non-destructive sample preparation Provides rapid metabolites screening	High cost

Source(s): Kalia & Sharma (2019); Li et al. (2019); Pott et al. (2020).

discrimination analysis (OPLS-DA) and Partial least square discriminant analysis (PLS-DA) (Cigliano et al., 2022; Der Agopian et al., 2020; Duan et al., 2021; Fu et al., 2021; Li et al., 2019c; Li et al., 2019b; Li et al., 2019a; Li et al., 2022; Yun et al., 2019). In the metabolomic and proteomic integrated analysis for papaya (cv. Golden), metabolomic data was analysed by PCA determined that the polar metabolites of ethylene treated fruit for 6 hrs were significantly different from those treated for 12 hrs (Der Agopian et al., 2020). In another study, for proteomics/transcriptomics and transcriptomics/metabolomics data, Fu et al. (2021) performed Pearson correlation analysis, whereas metabolomics analysis was done using PCA, cluster analysis and OPLS-DA model, demonstrated MeJA treatment induced 2421 differentially expressed (DE) genes and 12 DE proteins and 93 DE metabolites. Integrating the DE gene with related protein and metabolites identified the mechanism of MeJA to alleviate chilling injury of green bell pepper fruit during storage at 4 °C for 6 days. Although linear model such as, PCA has been the major analysis reported in fruit postharvest omics studies,

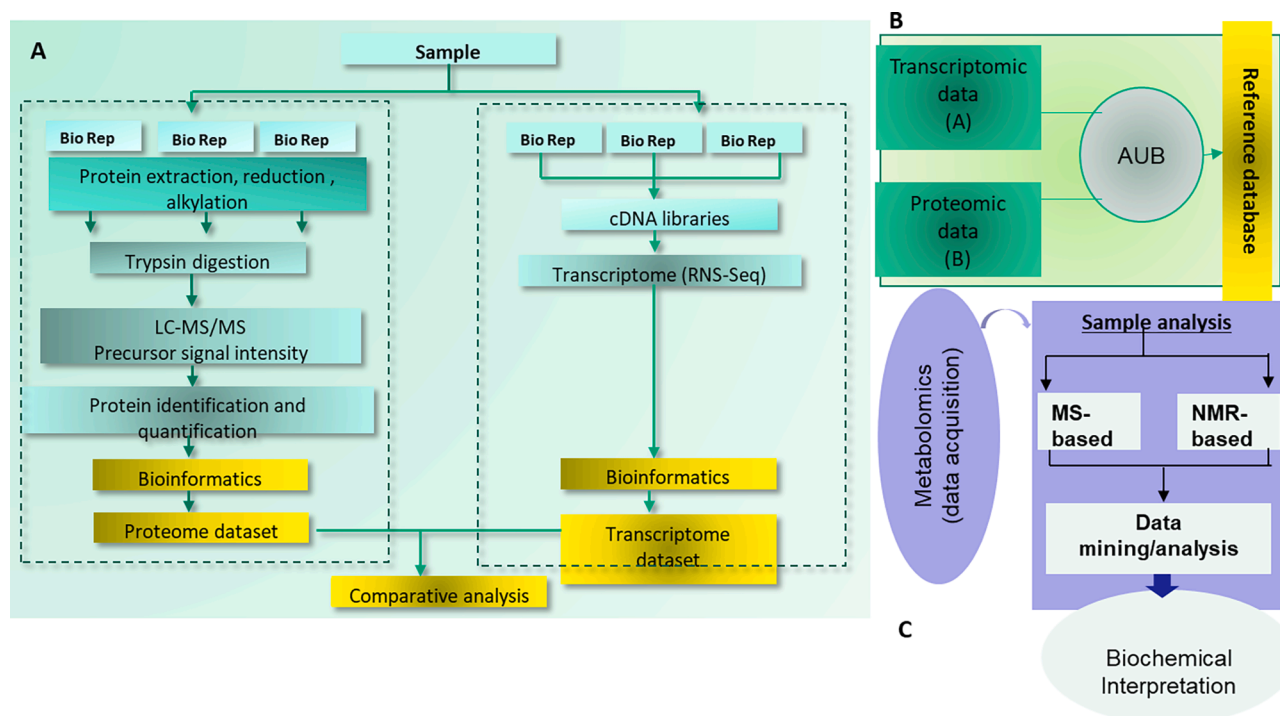


Fig. 3. Comparative analysis of transcriptomic and proteomic dataset (A) and Integration of transcriptomic and proteomic dataset by simple union method (B), the flow chart of metabolomics analysis (C) adapted and modified from Haider and Pal (2013).

multivariate analysis are more suitable than linear regression which could suffer from significant analysis derived from a poor ability to handle missing value in the data (Der Agopian et al., 2020).

As presented in Table 2, the omics data analysis for fruit postharvest commonly used DE, GO and dimension reductions tools to summarize linear combinations (PCA, PLS). According to Der Agopian et al. (2020) multivariate analysis are more suitable than linear regression which could suffer from significant analysis derived from a poor ability to handle missing value in the data. The limitation of these method includes only considering two group of variables and the tendency to consider every variable albeit with varying weight that could results in poor interpretability. For a more advanced analysis knowledge driven methods such as, co-expression network analysis based on pairwise correlation using observed data with external knowledge from data base has been proposed for fruit postharvest omics analysis (Yan et al., 2022; Zhang et al., 2021).

Co-expression network analysis and hierarchical clustering analysis (HCA) was employed by Li et al. (2022b) using weighted gene co-expression network analysis (WGCNA) and identified 197 key hypoxia responsive proteins from 1972 proteins of waxed Navel orange (cv. Lane Late) during storage at room temperature. In this study, topological overlap matrix (TOM) was used to conduct one-step network construction and module detection, resulted high correlation of protein expression, primary (fatty acid, amino acid, and organic acid) and secondary metabolites (ethanol and aldehyde) response to hypoxia stress. Even though the applicability is till limited, the potential of non-linear machine learning methods such as, random forest (RF), support vector machine (SVM) and artificial networks (ANN) for analysing metabolomic data was suggested by Mendez et al. (2019).

3. Fruit stress and induced defence response in – An omics prospective

Fruit quality is a combination of various attributes and characteristics that includes visual appearance, physiochemical properties, nutritional composition, and microbial safety. After harvest, fruit ripening

and senescence progresses depending on the internal flesh-to-rind transport of nutrient and water via transporter-mediated process. This phenomenon critically affects the quality of fruit. Harvested fruit exhibited various physiological and biochemical changes to cope with biotic and abiotic stress when stored at different storage conditions. These biotic and abiotic factors such as storage temperature, RH, oxidative stress, pathogen invasion and natural senescence. Quality attributes mostly affected during postharvest includes organic acids, soluble sugars, titratable acidity, colour, and texture. Therefore, the main aim of postharvest management strategies is to delay senescence, maintain quality attributes, delaying microbial growth, and prolong shelf-life by slowing down the fruit metabolism (Li et al., 2022; Pott et al., 2020).

Treatments such as cold storage, delayed cooling, chemicals, and heat treatments have been widely used prior to cold storage to control the postharvest senescence to maintain quality and have been shown to have beneficial effects on various fruit. In recent years, technologies such as edible coating, modified and control atmospheres, as well as non-thermal treatment such as, cold plasma, active water, light has been reported as a control strategy during fruit postharvest quality. In addition, the application of biocontrol has been introduced for fruit postharvest pathology research. In other instances, exposure of various fruit to various postharvest treatments could also lead to changes in molecular mechanisms. During these conditions, fruit acquires stress tolerance and defence mechanisms to prevent cellular damage and re-build cellular homeostasis (Zhang et al., 2017). However, fruit differ highly in their ability to acclimate and survive from postharvest stress (Pott et al., 2020).

3.1. Postharvest senescence

During postharvest handling and storage, dramatic increase in ethylene synthesis and respiration can be observed in climacteric fruit, unlike that of non-climacteric fruit (Oak et al., 2022). Senescence can be influenced by several factors such as the accumulation of sugar or by plant hormones including ethylene, abscisic acid, salicylic acid (SA) and

Table 2

Recent articles on integrated omics analysis of fruit, postharvest condition, type of omics integration and data analysis and major output.

Fruit type	Postharvest condition	Integrated omics	Method of data analysis*	Major outputs	Reference
Apple (cv. Red Fuji)	<i>Y. lipolytica</i> ($1 \times 10^{6-1} \times 10^8$ cells)	POs & TOs	GO, DEGs	<ul style="list-style-type: none"> <i>Y. lipolytica</i> enhanced the defence responses by stimulating the activity of polyphenol oxidase, peroxidase, chitinase, and l-phenylalanine ammonia lyase Induced resistance mediated by a crosstalk between salicylic acid (SA) and ethylene/jasmonate (ET/JA) pathway 	Zhang et al., 2017
Orange (cv. Powell)	<i>P. digitatum</i> (10^6 conidia mL ⁻¹)	TOs & MOs	PCA, ANOVA	<ul style="list-style-type: none"> <i>P. digitatum</i> altered DEGs involved in carbohydrates metabolism and induced up regulation of stress/defence related genes, up regulation of hormone biosynthesis and signal transduction 	Tang et al. (2018)
Banana (cv. Brzail)	Fungicide (0.1% for 3min)	TOs, POs, & MOs	PCA, PLS-DA with VIP > 1.0	<ul style="list-style-type: none"> Ethylene and auxin signalling involved in banana ripening by regulating cell wall and starch metabolism 	Li et al., 2019B
Banana (cv. Brazilian)	Fungicide (melatonin, 10 nM)	TOs & MOs	PLS-DA and OPLS-DA, VIP > 1.0	<ul style="list-style-type: none"> Fruit senescence significantly delayed by melatonin treatment Melatonin induced metabolic process involved in synthesis of cell wall, starch degradation, and genes involved in cell wall metabolism 	Li et al., 2019b
Banana (cv. Carvendish)	Fungicide (0.05% for 3 min)	POs & TOs	PLS-DA, OPLS-DA, VIP > 1.0	<ul style="list-style-type: none"> Heat treatment accelerated loss of firmness and senescence, increase expression level of gene encoding cell wall degrading enzyme, and abundance of chlorophyll metabolism related protein 	Li et al., 2019c;
Sweet cherry (Lapins)	CaCl ₂ (0, 1, 2 & 4%) Heat treatment, HT (20, 40 & 50 °C)	TOs & MOs	KEGG, multivariate analysis of variance (MANOVA)	<ul style="list-style-type: none"> Combined calcium and HT enhance fruit quality, stimulated transcripts involved in sugar and amino acid metabolism Calcium treatment delay senescence, down regulated transcripts involved in sugar metabolism and secondary metabolism 	Michailidis et al. (2019)
Banana (cv. Brazilian)	0.1 mM 3-indoleacetic acid (IAA)	TOs, POs, & MOs	Two-way orthogonal partial least squares (O2PLS), Orthogonal-based clustering algorithm	<ul style="list-style-type: none"> Exogenous AII treatment accelerate the ripening of harvested banana explained by up regulation of genes related to auxin and ethylene signalling 5784 genes, 94 proteins and 133 metabolites DEs that are linked to ripening process DEs transcriptional facts were mainly ethylene response factor and basic helix-loop-helix family 	Yun et al., 2019
Pear (cv. Shuijing)	<i>W. anomalus</i> (1×10^8 cells mL ⁻¹)	POs & TOs	DEGs genes and proteins, GO and KEGG pathway	<ul style="list-style-type: none"> <i>W. anomalus</i> treatment up regulated genes and proteins involved in defence and stress response and plant hormone signal transduction, flavonoid biosynthesis, and carbohydrate metabolism Most resistance-related proteins including PR families' proteins, chitinase and β-1,3-glucanase increases in accumulation 	Zhang et al., 2020a; Zhang et al., 2020b; Zhang et al., 2020c
Strawberries (cv. Fengxiang)	<i>R. mucilaginosa</i> (1×10^8 cells mL ⁻¹) cultured with chitosan	POs & TOs	DEGs, GO, KEGG	<ul style="list-style-type: none"> <i>R. mucilaginosa</i> treatment induced disease resistance, the function of DEPs and DEGs identified related to JA/ET, ABA and GA signal transduction, synthesis of disease resistance, regulation of ROS 	Gu et al., 2021
Litchi (cv. Feizixiao)	Room temperature storage	TOs, POs, & MOs	TOs and POs – DEGs DEPs, GO and KEGG pathway MOs – PLS-DA model and HCA	<ul style="list-style-type: none"> Most of DEGs, DEPs and DEMs related to sugar and carbohydrate metabolic pathways were downregulated Most of the DEGs, DEPs, and DEMs related to 'pentose phosphate pathway, amino sugar and nucleotide sugar metabolism were down regulated 	Gu et al., 2021
Kiwifruit (cv. Hayward)	Storage condition (room temperature for 26 day)	POs & MOs	One way ANOVA and KEGG pathway	<ul style="list-style-type: none"> 71 DEPs identified as hormone -related protein that involve in ethylene synthesis, ethylene induced regulated responsive activated (IRRA) pathway, auxin IRRA pathway, abscisic acid IRRA pathway, jasmonate synthesis, degradation pathway and metabolism regulation 	Tian et al. (2021)
Purple passion fruit (cv. Tainong1)		TOs & MOs	PCA, POLS-DA	<ul style="list-style-type: none"> 295 unigenes identified involved in DE flavonoid Higher synthesis of flavonoids during ripening, regulation of flavonoid biosynthesis pathway 	Xin et al., 2021
Pear (Zaoshu Shanli)	Storage (25 °C, 18 days)	TOs & MOs	PCA, KEGG, DEGs	<ul style="list-style-type: none"> Glycerophospholipid linked to the postharvest pear softening Glycerophospholipid metabolism and gene associated glycerophospholipid metabolism was the most enriched pathway in metabolite and gene DEGs analysis, respectively 	Xu et al., 2021b

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Table 2 (continued)

Fruit type	Postharvest condition	Integrated omics	Method of data analysis*	Major outputs	Reference
Table grape (cv. Red Globe Grape)	<i>Y. lipolytica</i>	POs & TOs	DEGs, GO, KEGG	<ul style="list-style-type: none"> <i>Y. lipolytica</i> induce expression of gene involved in protection of host from pathogens, upregulated for gene encoding synthesis of phytoalexin, pathogenesis-related protein WRKY transcription factor, hypertensive protein compared to controls 	Zhao et al., 2021
Papaya (cv. Suiyou-2)	1-MCP	TOs & MOs	PCA, and hierarchical cluster (HCA), OPLS-DA, KEGG	<ul style="list-style-type: none"> 1-MCP delayed ripening and quality deterioration 1-MCP long term treatment (2hr) resulted ripening disorder, DEGs and DEMs indicated inhibition of basal metabolism and secondary metabolites that count for ripening disorder 	Zheng et al., 2021
Navel orange (cv. Lane Late)	Edible coating/waxing stored at room temperature (22 ± 2 °C) and 55-60% RH	POs & MOs	Co-expression network – WGCNA, Hierarchical clustering analysis (HCA)	<ul style="list-style-type: none"> 167 key hypoxia responsive proteins, involve in mitochondrial metabolism were identified using WGCNA HCA resulted differences in protein abundance and expression pattern between waxed and control fruit Low O₂ condition alter mitochondrial proteome resulted in mitochondrial metabolism (organic and amino acid) and accumulation of metabolites (succinic acid and GABA) 	Li et al., 2022
Green bell pepper (cv. Jingtian No.3)	Methyl jasmonate (MeJA) ($30 \mu\text{mol L}^{-1}$) for 10 min Storage (4 °C for 6 days)	TOs, POs & MOs	Transcriptomic/proteomics - KEGG/Metabolomics - PCA and OPLS-DA model, VIP > 1	<ul style="list-style-type: none"> MeJA alleviate chilling injury through down regulation of MYC2 expression, increased jasmonic acid; DEGs associated with abscisic acid changed differentially MeJA reduced cell wall modification, high expression of genes encoding pectate lyase, pectinesterase and polygalacturonase 	Fu et al., 2022
Plum (cv. Friar)	Melatonin (0.05% v/v)	TOs & MOs	GO, DEGs, Pearson's correlation	<ul style="list-style-type: none"> DEGs due to melatonin treatment involved in biosynthesis of flavonoids and anthocyanins Melatonin treatment inhibited the transcription of ethylene biosynthesis DEGs during storage High correlation was observed between MYB transcription factors and anthocyanin biosynthesis, correlated with flesh reddening caused by cold stress 	Xu et al., 2022

GO = Gene Ontology, DEGs = Differentially expressed genes, PCA = Principal component analysis, ANOVA = Analysis of variance, PLS-DA = Partial least square discriminant analysis, OPLS-DA = orthogonal partial least squares discrimination analysis, VIP = Variable importance on projection, KEGG = Kyoto Encyclopaedia of Genes and Genomes, WGCNA = Weighted gene co-expression network analysis, TOs = Transcriptomics, MOs = Metabolomics, POs = Proteomics.

auxin. Ethylene plays a crucial role in fruit ripening and a sharp increase preceded fruit softening, and colour change, however, fruit have a specific regulatory mechanism of ethylene signalling (Fernández-Cancelo et al., 2022). Detail analysis on the effects of ethylene on the postharvest quality and stress response of fruit was summarized by Ansari and Tuteja (2015). Several studies reported the integration of two or more omics techniques to identify the key player of fruit senescence during postharvest treatment and storage (Li et al., 2019c; Li et al., 2019b; Li et al., 2019a; Tian et al., 2021; Yun et al., 2019).

the metabolomic and transcriptomic integrated study done by Michailidis et al. (2019) utilised to characterise the effect of heat (40 °C), and calcium (2% CaCl₂ for 10 min) treatment during postharvest handling of sweet cherries (cv. Lapins). Targeted gene expression analysis and metabolic changes clearly identifies 30 genes, 52 primary and 28 secondary metabolites that involved regulation of various synthesis and metabolism pathways, but the study lack omics data integration. Similarly, Santin, Lucini, Castagna, Rocchetti, Hauser, & Ranieri et al. (2019) investigated the influence of UV-B radiation on the phenolic profile of peach (cv. Fairtime) skin treated for 36 hrs at different interval by combining transcriptomic and metabolomics data. This study demonstrated that genes involved in both UVR8 signalling and phenylpropanoid biosynthesis were upregulated by UV-B treatment, but the treatment resulted in a decrease of several phenolic compounds such as anthocyanin. Furthermore, Li et al. (2019b) reported the integration of metabolomic and transcriptomic to understand the senescence process of 'Brazil' bananas during 18 days of storage. According to the authors, *β-amylase 3* and *glycosyl transferase* were down regulated,

whereas, the late stage of ripening gene encoding *soluble starch synthase*, *polygalacturonase*, and *pectinesterase* were highly induced that might accelerate starch degradation.

Other omics integration approach such as, proteomic, and transcriptomic also used to demonstrate the key factors that are linked to the peel softening due to senescence of heat treated (30 °C) banana (cv. Carvendish) fruit based on 66 DEs proteins and 927 DEs genes for 21 days storage (Li et al., 2019c; Li et al., 2019b; Li et al., 2019a). With investigation of the DEs protein and gene involved stress response, energy metabolism and signalling reported as indicator of response to heat stress. Comparing with the control sample, heat treatment increased the expression level of genes encoding cell-wall degradation enzyme and altered cell wall components, thereby accelerating loss of banana peel firmness.

Recently, metabolomics and proteomics integration indicated accelerated senescence resulted a marked change in antioxidant enzymes, emission of linalool, terpenes geraniol, hotrienol as well as alcohols and aldehydes in ethylene treated papaya and suggested aminobutyric acid (GABA) could be used as a biological marker to reveal the effects of ethylene on the papaya colour, firmness, and aroma (Der Agopian, Fabi and Cordenunsi-Lysenko, 2020).

Postharvest senescence is a continuous and irreversible process; however, it is not simply an age dependent process rather it is an organised and controlled process where cell components degraded gradually. The degradative process linked to postharvest senescence that have a detrimental impact on fruit organoleptic and nutritional quality, which influences consumers' buying decision. Table 2 presents the

integrated omics studies that have been used to understand the mechanisms different postharvest treatments fruit postharvest storage conditions.

3.2. Postharvest pathogen defence

In addition to the physiological and physicochemical changes, invasion of fruit by pathogens causes a huge loss during postharvest handling. Over the past decades the use of physical treatments, natural compounds, plant hormones and biocontrol agents have been investigated as an alternative for the use of synthetic chemicals, such as fungicides as postharvest disease management strategy (Campos et al., 2022; Leng et al., 2022). According to Tian et al. (2021), plants have a wide variety of constitutive defence mechanisms to protect themselves against pathogens. In response to pathogenic infection, fruit can go through complex signalling and defence pathways (Hong et al., 2016).

Fruit defence response mostly includes the induction of cell wall alterations, deposition of callose, oxidative burst, and accumulation of pathogenesis-related proteins (chitinases, glucanases and proteases). The induction of host defence can be achieved by various postharvest treatments such as biocontrol, physical treatments (UV-C, light), natural and/or synthetic chemicals, sanitizing agents (ozone, electrolyzed water), and the use of volatile compounds. In recent years, considerable postharvest research has been conducted on the use of biocontrol strategies as safe and sustainable strategy (Matrose et al., 2021). Similarly, the induction of host resistance to pathogenic/spoilage microbes represents a novel strategy of disease management (Zhang et al., 2020c; Leng et al., 2022). Hence, the application of omics tools in the investigation of this phenomenon would better our understanding of the underpinning mechanisms and pathways.

4. Biocontrols as postharvest disease management strategy and the role of omics

Biocontrol agents involve the use of living organisms (micro/macro) to elicit defensive response when introduced into another host. The response could follow different antagonistic strategies depending on the antagonistic, pathogen of interest and the host. Postharvest biocontrol control against (BCAs) have been found to suppress the development of disease on the surface of the fruit. Extensive studies been reported using antagonistic to biologically control postharvest diseases by the complex interaction between microbial antagonistic, pathogen and the host (Dukare et al., 2018; Spadaro and Droby, 2016; Zhang et al., 2020a; Zhang et al., 2020b; Zhang et al., 2020c). Although these interactions have been identified by many studies, understanding the antagonistic mechanisms of the BCAs were limited. Recently, few studies utilized omics techniques for better understanding of the mechanisms and potential pathways postharvest biocontrol system for fruit (Zhang et al., 2017; Zhang et al., 2020d).

Proteins and genes related to the resistance and defence in *Wick-erhamomyces. anomalus* treated and control 'Shuijing' pear fruit was compared through proteomic and transcriptomic analysis during storage (Zhang et al., 2020d). Treatment of pear with *W. anomalus* induced up-regulation of disease-resistance-related genes and their corresponding proteins. *W. anomalus* treatment induced up regulation of *MYC4* and β -1,3-gluconase which is a transcriptional activator of JA and salicylic acid (SA). Zhang et al. (2017) used proteomics and transcriptomics techniques. The authors investigated the mechanism, possible pathways, and signal elicitors in the defence induction by *Yarrowia lipolytica* in 'Red Fuji' apple during postharvest. They reported the expression of pathogenesis-related proteins and several defence response genes downstream of SA, JA/ET signal pathways and up regulation of peroxidase, thaumatin-like protein, and chitinase. In another study, Zhang et al. (2020a), Zhang et al. (2020b), Zhang et al. (2020c) was able to identify the defence response of pear (cv. Shuijing) induced by *Wick-erhamomyces anomalus*. The authors found that the antagonistic

treatment induced genes associated with JA signalling pathways, flavonoid biosynthesis, and accumulation of resistance related protein.

Metabolomics and transcriptomic approaches were used to identify DEMs and DEGs in postharvest biocontrol study by Tang et al. (2018). The authors demonstrated that metabolomics and transcriptomic analysis aid their understanding of the defence mechanism of 'Powell' orange induced by *Penicillium digitatum*. After *P. digitatum* inoculation, the level of rhamnose and inositol significantly increases, putative involvement of G-protein signalling, JA, ethylene pathways, and transcriptional factors such as *WEKY*, *MYB* and *AP2/ERF* were activated as defence response. Based on the above cited articles, it is evident that integration of transcriptomic, proteomic and metabolomic analysis into biocontrol assessment of fruit would further expand our knowledge of their modes of action, efficacy, and functionality. This information would therefore enhance their viability for upscaling to pilot scale from basic laboratory research and increase their potential for commercialization.

5. Conclusions

This article summarised the potentials for integrated omics approach in the advancement of biocontrol research for postharvest fruit disease management application, with the emphasises on the need upscaling. The review provided an overview on the roles of integrated omics in the optimisation of postharvest handling practices for fruit, and trends on integrated omics data analysis, and interpretation tools were discussed. Many of the studies reported in this article focused only on the linear data analysis approach with few biological repeats because of high cost. Furthermore, the application of these approaches is at its early stages in postharvest research with very few information available on the transcriptional and protein profile associated with the maintenance of fruit quality and safety during postharvest storage. Majority of postharvest omics studies have focused on integration of gene-protein dataset and little to no information is available on gene-metabolites analysis studies. Advanced data integration tools such as, machine learning could play crucial in the future as research develops.

Most importantly, majority of integrated omics studies were focused on fruit response to storage temperature, and ethylene stress. Very few studies, have reported on the application of integrated omics technique for postharvest biocontrol approach till date. This implies that most research conducted on the use of biocontrols have been on presence or absence of symptoms on fruit, which offers a limited scope. Multiomics investigation can be an excellent opportunity to identify mechanisms underpinning the induction of fruit-host resistance to pathogenic infections and for further commercialization. In general, the information obtained can help in critical the successful implementation of appropriate postharvest management strategies for better fruit quality and extended shelf life. In addition, there are various types of postharvest treatments/technologies that has been used for fruit such as, modified atmosphere (O₂/CO₂) storage, thermal and non-thermal treatments, but their impacts on the fruit have not been explored to omics levels. Therefore, further research is needed in these areas to fully understand the impact of postharvest strategies on the whole fruit.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

- Ban, Z., Yan, J., Wang, Y., Zhang, J., Yuan, Q., & Li, L. (2018). Effects of postharvest application of chitosan-based layer-by-layer assemblies on regulation of ribosomal and defense proteins in strawberry fruit (*Fragaria × ananassa*). *Scientia Horticulturae*, *240*, 293–302.
- Belay, Z. A., Caleb, O. J., Vorster, A., van Heerden, C., & Opara, U. L. (2020). Transcriptomic changes associated with husk scald incidence on pomegranate fruit peel during cold storage. *Food Research International*, *109285*.
- Chen, G., Chen, C., & Lei, Z. (2017). Meta-omics insights in the microbial community profiling and functional characterization of fermented foods. *Trends in Food Science and Technology*, *65*, 23–31.
- Der Agopian, R. G., Fabi, J. P., & Cordenunsi-Lysenko, B. R. (2020). Metabolome and proteome of ethylene-treated papayas reveal different pathways to volatile compounds biosynthesis. *Food Research International*, *131*, Article 108975.
- Duan, W., Shao, W., Lin, W., Yuan, L., Lu, Q., Chen, L., & Li, J. (2021). Integrated metabolomics and transcriptomics reveal the differences in fruit quality of the red and white *Fragaria pentaphylla* morphs. *Food Bioscience*, *40*, Article 100896.
- Fu, A., Zheng, Y., Lv, Y., Watkins, C. B., Bai, C., Ma, L., ... Zuo, J. (2022). Multi-omics analysis reveals specific modifications associated with reduced chilling injury in bell pepper fruit by methyl jamonate. *Postharvest Biology and Technology*, *185*, Article 111799.
- Gu, N., Zhang, X., Gu, X., Zhao, L., Godana, E. A., Xu, M., & Zhang, H. (2021). Transcriptomic and proteomic analysis of the mechanisms involved in enhanced disease resistance of strawberries induced by *Rhodotorula mucilaginosa* cultured with chitosan. *Postharvest Biology and Technology*, *172*, Article 111355.
- Guo, X., Luo, T., Han, D., Zhu, D., Li, Z., Wu, Z., & Wu, Z. (2022). Multi-omics analysis revealed room temperature storage affected the quality of litchi by altering carbohydrate metabolism. *Scientia Horticulturae*, *293*, Article 110663.
- Lara, I., Bustamante, C. A., & Villarreal, N. M. (2022). Fruit responses to biotic and abiotic stressors during postharvest. *Frontiers in Plant Science*, *13*, Article 914841.
- Li, T., Wu, Q., Duan, X., Yun, Z., & Jiang, Y. (2019). Proteomic and transcriptomic analysis to unravel the influence of high temperature on banana fruit during postharvest storage. *Functional & integrative genomics*, *19*(3), 467–486.
- Li, T., Wu, Q., Zhu, H., Zhou, Y., Jiang, Y., Gao, H., & Yun, Z. (2019). Comparative transcriptomic and metabolic analysis reveals the effect of melatonin on delaying anthracnose incidence upon postharvest banana fruit peel. *BMC Plant Biology*, *19*(1), 1–15.
- Li, T., Yun, Z., Wu, Q., Qu, H., Duan, X., & Jiang, Y. (2019). Combination of transcriptomic, proteomic, and metabolomic analysis reveals the ripening mechanism of banana Pulp. *Biomolecules*, *9*(10), 523.
- Li, X., Bi, X., An, M., Xia, Z., & Wu, Y. (2020). iTRAQ-based proteomic analysis of watermelon fruits in response to Cucumber green mottle mosaic virus infection. *International Journal of Molecular Sciences*, *21*(7), 2541.
- Li, X., Tian, Z., Chai, Y., Yang, H., Zhang, M., Yang, C., ... Cheng, Y. (2022). Cytological and proteomic evidence reveals the involvement of mitochondria in hypoxia-induced quality degradation in postharvest citrus fruit. *Food Chemistry*, *375*, Article 131833.
- Mathabe, P. M., Belay, Z. A., Ndlovu, T., & Caleb, O. J. (2020). Progress in proteomic profiling of horticultural commodities during postharvest handling and storage: A review. *Scientia Horticulturae*, *261*, Article 108996.
- Matrose, N. A., Obikeze, K., Belay, Z. A., & Caleb, O. J. (2021). Plant extracts as alternative sources for post-harvest management of fungal pathogens: A Review. *Food Bioscience*, *41*, Article 100840.
- Michailidis, M., Karagiannis, E., Tanou, G., Sarrou, E., Stavridou, E., Ganopoulos, I., ... Molassiotis, A. (2019). An integrated metabolomic and gene expression analysis identifies heat and calcium metabolic networks underlying postharvest sweet cherry fruit senescence. *Planta*, *250*(6), 2009–2022.
- More, A. S., Ranadheera, C. S., Fang, Z., Warner, R., & Ajlouni, S. (2020). Biomarkers associated with quality and safety of fresh-cut produce. *Food Bioscience*, *34*, 100524.
- Oak, P., Jha, V., Deshpande, A., Tanpure, R., Dawkar, V., Mundhe, S., ... Gupta, V. (2022). Transcriptional and translational perturbation in abiotic stress induced physiological activities and metabolic pathway networks in spongy tissue disorder of mango fruit. *Postharvest Biology and Technology*, *188*, Article 111880.
- Pott, D. M., Lima, F. D. A., Soria, C., Willmitzer, L., Fernie, A. R., Nikoloski, Z., Osorio, S., & Vallarino, J. G. (2020). Metabolic reconfiguration of strawberry physiology in response to postharvest practices. *Food Chemistry*, *321*, Article 126747.
- Santin, M., Ranieri, A., Hauser, M. T., Miras-Moreno, B., Rocchetti, G., Lucini, L., ... Castagna, A. (2021). The outer influences the inner: Postharvest UV-B irradiation modulates peach flesh metabolome although shielded by the skin. *Food chemistry*, *338*, Article 127782.
- Si, J., Fan, Y. Y., Liu, Z. L., Wei, W., Xiao, X. M., Yang, Y. Y., ... Chen, J. Y. (2022). Comparative transcriptomic analysis reveals the potential mechanism of hot water treatment alleviated-chilling injury in banana fruit. *Food Research International*, *157*, Article 111296.
- Sirangelo, T. M., Rogers, H. J., & Spadafora, N. D. (2022). Multiomic approaches to investigate molecular mechanisms in peach post-harvest ripening. *Agriculture*, *12*(4), 553.
- Spadaro, D., & Droby, S. (2016). Development of biocontrol products for postharvest diseases of fruit: The importance of elucidating the mechanisms of action of yeast antagonists. *Trends in Food Science & Technology*, *47*, 39–49.
- Tang, N., Chen, N., Hu, N., Deng, W., Chen, Z., & Li, Z. (2018). Comparative metabolomics and transcriptomic profiling reveal the mechanism of fruit quality deterioration and the resistance of citrus fruit against *Penicillium digitatum*. *Postharvest and Technology Biology*, *145*, 61–73.
- Tian, X., Zhu, L., Yang, N., Song, J., Zhao, H., Zhang, J., ... Li, M. (2021). Proteomics and metabolomics reveal the regulatory pathways of ripening and quality in post-harvest kiwifruits. *Journal of Agricultural and Food Chemistry*, *69*(2), 824–835.
- Wang, Y., Sun, Y., Zhou, D., Zhang, Q., Pan, L., & Tu, K. (2022). Transcriptomics analysis provides insights into metabolisms of sugars and carotenoids of nectarine fruit subjected to different temperature storage. *Scientia Horticulturae*, *304*, Article 111262.
- Xin, M., Li, C., He, X., Li, L., Yi, P., Tang, Y., ... Sun, J. (2021). Integrated metabolomic and transcriptomic analyses of quality components and associated molecular regulation mechanisms during passion fruit ripening. *Postharvest Biology and Technology*, *180*, Article 111601.
- Xu, M., Zhang, X., Li, D., Gu, X., Godana, E. A., Dhanasekaran, S., ... Zhang, H. (2022). Transcriptome analysis of postharvest grapes in response to *Talaromyces rugulosus* O1 infection. *Postharvest Biology and Technology*, *178*, Article 111542.
- Xu, Y. J. (2018). Foodomics: A novel approach for food microbiology. *TrAC Trends in Analytical Chemistry*, *96*, 14–21.
- Yun, Z., Li, T., Gao, H., Zhu, H., Gupta, V. K., Jiang, Y., & Duan, X. (2019). Integrated transcriptomic, proteomic, and metabolomics analysis reveals peel ripening of harvested banana under natural condition. *Biomolecules*, *9*(5), 167.
- Zhang, F., Ji, S., Wei, B., Cheng, S., Wang, Y., Hao, J., ... Zhou, Q. (2020b). Transcriptome analysis of postharvest blueberries (*Vaccinium corymbosum* 'Duke') in response to cold stress. *BMC Plant Biology*, *20*(1), 1–20.
- Zhang, H., Chen, L., Sun, Y., Zhao, L., Zheng, X., Yang, Q., & Zhang, X. (2017). Investigating proteome and transcriptome defense response of apples induced by *Yarrowia lipolytica*. *Molecular Plant-Microbe Interactions*, *30*(4), 301–311.
- Zhang, Q. Y., Ge, J., Liu, X. C., Wang, W. Q., Liu, X. F., & Yin, X. R. (2021). Consensus Co-expression network analysis identifies AdZAT5 regulating pectin degradation in ripening kiwifruit. *Journal of Advanced Research*.
- Zhang, Q., Zhao, L., Li, B., Gu, X., Zhang, X., Boateng, N. A. S., & Zhang, H. (2020a). Molecular dissection of defense Response of pears Induced by the biocontrol yeast *Wickerhamomyces anomalus* Using transcriptomics and proteomics approaches. *Biological Control*, *104305*.
- Zhang, Z., Zhao, P., Zhang, P., Su, L., Jia, H., Wei, X., ... Jia, H. (2020c). Integrative transcriptomics and metabolomics data exploring the effect of chitosan on postharvest grape resistance to *Botrytis cinerea*. *Postharvest Biology and Technology*, *167*, Article 111248.
- Zhao, L., Wang, M., Li, B., Dhanasekaran, S., Wang, K., Gu, X., ... Zhang, H. (2021). Investigating proteome and transcriptome defense response of table grapes induced by *Yarrowia lipolytica*. *Scientia Horticulturae*, *276*, Article 109742.
- Zheng, S., Hao, Y., Fan, S., Cai, J., Chen, W., Li, X., & Zhu, X. (2021). Metabolomic and transcriptomic profiling provide novel insights into fruit ripening and ripening disorder caused by 1-MCP treatments in papaya. *International Journal of Molecular Sciences*, *22*(2), 916.