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ABSTRACT BOOK

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KEYNOTE:
How packaging, technology and innovation can reduce food waste for fresh produce

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Tackling food waste requires collaboration across the entire food supply chain, from producers through to consumers. Maintaining food quality, nutrition, safety are also of paramount importance in the fresh produce sector. The role that packaging, technology and innovation plays will be discussed through case studies. Understanding consumer perceptions of packaging and technology will also be presented.

Apple fruit responses to phytosanitary X-ray treatment

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In the face of increasing global awareness around health and climate change, horticultural industries need alternative non-chemical disinfestation tools to maintain and gain new market access. X-ray ionizing radiation is an emerging postharvest treatment with internationally accepted standards under the International Plant Protection Convention (IPPC). With the disinfestation capability of the technology well established, it is important to determine the impact of X-ray treatment on product quality. In collaboration with the United States Department of Agriculture – Agricultural Research Services (USDA-ARS) in Hawaii, this study examined the potential use of X-ray as a phytosanitary treatment for New Zealand apples. Over the 4 years of research, we examined the effect of a range of phytosanitary X-ray doses across six New Zealand apple cultivars. Key postharvest variables were examined, including the storage time before and after X-ray. The results showed no significant impact on the external appearance, soluble solids content, weight or colour of the fruit. A minor decrease in titratable acidity and fruit softening was observed and X-ray treatment was found to induce varying degrees of internal browning between different cultivars. The impact of X-ray treatment on apple fruit quality is discussed, with a focus on the potential interaction between X-ray treatment, fruit ripening and internal browning observed in 'Royal Gala'. Overall, the results showed X-ray technology could be a viable phytosanitary tool for the New Zealand apple industry.

In-transit ripening and outturn quality for mango

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Mango fruit produced in Northern Territory (NT), Australia, are transported up to ~4000 km over ~4 days to ripening centres in southern states. Upon arrival, they are exposed to ethylene (C_2H_4) gas applied via ‘shot’ or ‘trickle’ systems to deliver uniformly ripe fruit for market. In response to the lost time under refrigerated transport, an alternative to current commercial ripening practices for cv. 'Honey Gold' mango was investigated to improve fruit supply and quality and reduce costly overheads associated with existing cooling and ripening infrastructure. In-transit ripening was explored for 10 commercial ‘Honey Gold’ mango consignments shipped from Katherine, NT to Wamuran in Queensland (QLD) and in simulated ripening laboratory experiments. Ripening was initiated inside six of the 10 refrigerated road containers using Ripestuff™, an inclusion complex (IC) powder that releases C_2H_4 gas. The study compared optimum and non-optimum ripening conditions of air temperature, fruit pulp temperature, air relative humidity, and air C_2H_4 and carbon dioxide (CO_2) gas concentrations inside the refrigerated containers. Fruit responses were measured as quality parameters of skin colour, fruit firmness, flesh titratable acidity (TA) and flesh total soluble solids (TSS) and time to eating ripe. The real-time and simulated in-transit modified atmosphere ripening rate and fruit quality data was used to develop a regression model to 'predict' mango fruit responses to in-transit ripening conditions and stage of fruit ripeness. The resulting 'prediction calculator' decision aid tool could be used by industry to effectively manage supply, marketing and improve quality of mango fruit ripened in-transit.

Effect of *Listeria monocytogenes* on fresh apples under long-term, low-temperature simulated sea-freight storage conditions

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Listeria monocytogenes (L. monocytogenes) is a pathogen of concern in many fresh produce supply chains. Fresh apples are not generally recognised as presenting a risk for *L. monocytogenes* transmission; however, the listeriosis outbreak in the USA in 2015 linked with caramelised apples resulted in 7 deaths. It not only highlighted the need for the development of preventive measures to control the presence of *L. monocytogenes* across the value chain, but it also re-emphasised that postharvest food safety is critical, and can often be overlooked. Little is known about the persistence of *L. monocytogenes* on apples during long-term, low-temperature storage, which is typical for apple exports from New Zealand. The aim was to determine the effect of long-term, low-temperature sea-freight from New Zealand to the USA and Europe, two key New Zealand markets, on the survival of *L. monocytogenes* on fresh apples. Temperature and humidity values were recorded during a shipment to each market (USA and Europe), then the observed variations around the 0.5°C target temperature were simulated in laboratory trials using open and closed calyx cultivars of apples inoculated with a cocktail of seven strains of *L. monocytogenes*. The cocktail was inoculated on the body and in the calyx of apple cultivars. Samples were analysed for *L. monocytogenes* quantification at various intervals during the simulation. On each occasion, an extra set was analysed after a subsequent 8 days at 20°C. The result showed that *L. monocytogenes* did not grow on apples, and the numbers declined in the calyx or on the body. Cultivars selected in this study had no significant effect on the survival of *L. monocytogenes* for both sea-freight simulations, either in the calyx or on the body ($P > 0.05$). There was also no significant difference in the survival behaviour of *L. monocytogenes* at 0.5 or 20°C. The results of this study provide useful insights into the survival of *L. monocytogenes* on different apple cultivars that can be used to develop effective risk mitigation strategies for fresh apples during long-term, low-temperature international sea-freight transportation.

Insights into fruit calcium deficiency using a novel immature tomato pericarp disc system

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Calcium has wide ranging effects on pre and postharvest fruit development, with calcium deficiency causing disorders such as blossom-end rot in tomatoes and bitter pit in apples. Blossom-end rot in tomatoes is often used as a model system for studying calcium deficiency disorders in fruit due to the prevalence of prior tomato research and the ease of growing the plants. In addition to calcium deficiency, blossom-end rot is associated with accumulation of reactive oxygen species and oxidative stress in fruit tissues. However, environmental effects at the plant level can also induce blossom-end rot, confounding research into the causes of this disorder at the cellular level. To better understand the effect of calcium deficiency on fruit cells, a novel pericarp disc system was developed using immature tomato fruit. In this system, discs from the blossom-end of tomatoes developed blossom-end rot symptoms over 4 days of sterile, high humidity storage. Discs from the stem-end did not develop symptoms. Enzymatic analysis confirmed that symptom development in discs follows similar biochemical trends compared to symptom development in whole fruit on the plant. Treating discs with 1% $CaCl_2$ and 500mM ascorbic acid eliminated symptom development. To assess the effect of calcium and ascorbic acid treatments on the spread of symptoms, discs were made from fruit showing blossom-end rot symptoms. Each disc contained approximately 1/3 blossom-end rot affected tissue and 2/3 visually healthy tissue. Time laps images showed symptoms spreading from the blossom-end rot affected tissue to the healthy tissue in discs treated only with water. In discs treated with calcium, symptoms did not spread. Moderate spread of symptoms was observed in ascorbic acid treated discs. Surprisingly, calcium treated discs had similar hydrogen peroxide content compared to control discs on days 1 and 2 of storage, and increased hydrogen peroxide content on day 3. This suggests increased calcium may help mitigate the damaging effects of oxidative stress rather than reducing the accumulation of reactive oxygen species. Disc respiration rates increased prior to symptom development, and a strong correlation was found between day 1 respiration (when no symptoms were visible) and day 2 color measurements. Phytohormone treatments that affect blossom-end rot at the whole plant level were not affective in the disc system, suggesting the effects of these compounds are at the whole plant level. These results indicate significant steps towards understanding the mechanism of cell death during fruit calcium deficiency disorders.

Based on JIM5 antibody, low methylesterified homogalacturonan was found accumulated in cell wall of loquat during postharvest storage, which may contribute to the firmness increase of loquat with lignification symptoms.

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Loquat fruit exhibits typical lignification symptoms during their natural senescence process, such as loss of fruit flavor, increased firmness and decreased juice yield. When stored at a cold environment after postharvest, these lignification symptoms can be severely intensified. The purpose of this study was to analyze the changes of pectin in cell wall and the relationship between pectin and loquat texture by using immunofluorescence labelling. JIM5 was used to recognize low methylesterified homogalacturonan (HG) in the cell wall of loquat fruit at different stages of ripening from green fruit through the mature stage to postharvest storage under 20 °C, 0 °C and low temperature conditioning (LTC), respectively. During the growth of loquat fruit, the fruit firmness gradually decreased and reached the lowest at maturity. Severe lignification symptoms occurred in loquat stored at 20 °C and 0 °C, accompanied with increase in firmness. The firmness of loquat increased fastest under stored at 0 °C. The lignification symptoms of loquat were suppressed under LTC treatment, for the smallest firmness increase among three groups. JIM5 labelling was found concentrated in the tricellular junction zones between cells and also presented in the middle lamella region. The fluorescence intensity of JIM5 increased during the fruit development, which indicated the accumulation of pectin is necessary for texture formation and fruit ripeness. With the increase of storage time, the fluorescence intensity of JIM5 in loquat stored at 20 °C and 0 °C increased quicker than the fluorescence intensity of loquat under LTC. Since the newly synthesized HG is usually highly methylesterified and is less susceptible to enzymatic attack, HG with a low degree of methylesterified can be cleaved by polygalacturonase and lyase. The JIM5 antibody results indicated that pectin underwent degradation during the lignification symptoms occurring in the loquat at three different kinds groups. On the other hand, low degree of methylesterified pectin can be crosslinked by calcium resulting in gel formation with strong hydraulic retention and can contribute to intercellular adhesion among parenchyma cells. The accumulation of low methylesterified homogalacturonan content in loquat cell wall without further degradation may contributed to the firmness increase and juice yield decrease of loquat during postharvest storage. It is the first-time immunofluorescence analysis was used to reveal the changes of pectin in fruit with lignification symptoms, which provide more comprehensive and holistic insight into the changes of loquat texture.

Postharvest accumulation of red apocarotenoid β-citraurin in a red-peeled huyou (*Citrus changshanensis*) affected by storage temperature and ethylene application

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Carotenoids mainly determine the mature citrus fruit color from yellow to orange and red by their different chemical structures. β-Citraurin is a kind of red apocarotenoid and it plays an important role in generating the red peel color of citrus fruit. Huyou (*Citrus changshanensis*), which is native to Changshan Country, Zhejiang Province in China, is a natural hybrid citrus with yellow peel, probably generated by a cross between pummelo and sour orange. Red-peeled huyou is a spontaneous mutant of ordinary huyou accidentally discovered in an ordinary huyou plantation, which was characterized by red peel of fruit at full maturity, especially after harvest. The distinct red peel color mainly due to the presence of red apocarotenoid β-citraurin as well as an increase in total carotenoids. The expression of *carotenoid cleavage dioxygenase 4b1* (*CCD4b1*) accounted for 99.0% of total transcript abundance of *CCD4s* in red-peeled huyou peel and was nearly 100 times higher than that in ordinary huyou. β-Citraurin accumulation and peel coloration was mostly favored at 15 °C but strongly inhibited at moderately high temperatures 20 °C and 25 °C. Exogenous ethylene application for 3 d had no obvious effect on β-citraurin accumulation in red-peeled huyou but holding fruit at moderately higher temperatures (20 °C and 25 °C) for 3 d had a significant adverse effect on β-citraurin accumulation. The expression of *phytoene synthase 1* (*PSY1*) and *CCD4b1* was higher at 10 °C and 15 °C and significantly lower at 20 °C and 25 °C. Besides, the main fruit quality attributes for the fruit stored at different temperatures were measured and it was observed that the quality of fruit stored at 15 °C is better or equal to that of fruit stored at 5 °C and 10 °C. Storage of fruit at ambient temperatures 20 °C and 25 °C is not ideal because of adverse effect on color development and much higher weight loss. In summary, red-peeled huyou should be stored at 15 °C for better color development as well as internal fruit quality; regular ethylene based degreening at moderately high temperatures did not favor the red color development.

KEYNOTE:
High performing VOC phenomics to improve the horticultural supply chain

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The current crucial mission of the agro-food industry is to guarantee food safety and, at the same time, improve perceived food quality and fulfil consumer expectations. To address this issue a broad and objective quality detection of food products is needed. Examples of key quality traits for the agro-food industry are the development of volatile organic compounds (VOCs) associated with the shelf life and taste quality of fruit and vegetables. However, the so called “phenotyping bottleneck”, caused by the absence of high-throughput and non-invasive methodologies, impedes an effective evaluation and prediction of food VOCs. The extreme complexity of food VOC aroma, both families of compounds and concentration ranges, is a challenging issue for any existing analytical technology. The rapid development of mass spectrometry (MS) application in metabolomic studies had a significant impact in the field of VOC analysis. The progress of MS techniques is mostly focused on instrumental improvements of mass resolution, mass accuracy, sensitivity, and enhanced reproducibility. Direct injection mass spectrometric (DI-MS) techniques, such as Proton Transfer Reaction – Time of Flight - Mass Spectrometry (PTR-ToF-MS) and Selected Ion Flow Tube – Mass Spectrometry (SIFT-MS), have opened new possibilities for food aroma analysis by decreasing the time needed for sample preparation and analysis, and by providing the possibility of non-destructive, real time and high-throughput volatilome analysis. These achievements enhanced the relevance of VOC assessment into horticultural modelling, for instance to better predict the product shelf-life or to estimate the final quality of a processed food based on the raw material. This presentation aims to review several prototypical analytical approaches, based on chemical ionization mass spectrometry, suitable to address the aroma complexity of agro-food products in different situations: i) non-destructive VOC assessment; ii) high-throughput automated headspace analysis; iii) dynamic destructive analysis; iv) real-time process monitoring. Tailored pre- and post-harvest studies confirmed the potentials of DI-MS application into the whole agro-food production chain, from breeding to consumers. These studies allowed us to estimate the interaction between genetic variability, ripening stages and storage condition on the perceived quality of several fruit species (i.e. apple, strawberry, and blueberry). Another important outcome was the development of putative VOC biomarkers linked with fruit spoilage caused, for instance, by the occurrence of postharvest disorders (i.e. superficial scald or anoxic stress) or by fresh-cut processed deterioration.

Non-destructive identification and quantification of internal disorder in of Conference pears using X-ray CT imaging and Deep Learning

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Long term storage in controlled atmosphere is used to deliver high quality pear fruit year-round. However, internal disorders, e.g., internal browning and cavities, can develop under suboptimal storage conditions and are invisible from the outside. We demonstrate a non-destructive inspection method to identify and quantify internal disorders in X-ray CT scans of pear fruit. Herein, a deep neural network is used for semantic segmentation. Conference pears were stored in control and suboptimal storage conditions for 8 months to obtain healthy and defect fruit with variation in internal disorder degrees. After storage, an X-ray CT dataset was collected and manually annotated. Thereafter, a U-net based model was trained to automatically segment healthy tissue, core and internal disorders, i.e., cavities and internal browning. The quantitative data obtained from the segmentations was used to quantify the severity of internal disorders. Classification accuracies of 99.4 and 92.2 % were obtained for the classification of “consumable” vs “non-consumable” fruit on the one hand and “healthy” vs “defect but consumable” vs “non-consumable” fruit on the other hand. The identification of “defect but consumable” fruit showed to be the most difficult. The presented method might be of great interest to researchers and industry working on postharvest quality assurance, product analysis and foreign object detection in foods and other industries.

Low oxygen stress effect on the metabolism of pear fruit (*Pyrus communis* `Conference´)

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Belgium’s pome fruit market suffers from huge economical losses due to low oxygen associated physiological disorders occurring during storage before commercialization. A thorough understanding of the metabolism of the fruit during the stress inducing low oxygen storage can provide knowledge on their adaptive survival ability. Recent findings indicate a major role of an O_2 sensor that detects even small changes in the ambient O_2, inducing a dynamic adaptation of the central metabolism circumventing the induction of critically low oxygen levels inside the fruit. Stable isotope labelling has been extensively used in plants for the elucidation of metabolic pathways and their regulation under biotic and abiotic stress. To elucidate the striking adaptation behavior of pear fruit a ^{13}C-labeling feeding experiment was performed. Pear tissue discs from early, commercial and late maturity stages were exposed to 21 % of O_2 for 24 hours followed by 0.2 % O_2 levels for another 24 hours under a constant temperature of 18 ^{o}C in a liquid medium containing U-^{13}C sodium pyruvate. Changes in concentrations and ^{13}C enrichment of citric acid cycle, fermentation, amino acid and γ-aminobutyric acid shunt associated metabolites were monitored to compare the metabolic effects under normoxic and hypoxic conditions among the three harvesting periods and the propagation of label through the metabolic pathways using gas chromatography coupled to mass spectrometry analysis. Primary results showed successful utilization of ^{13}C pyruvate from pear tissue accompanied by rise in organic acids such as fumarate, succinate and 2-ketoglutarate with a very small increase in lactate during hypoxia, while in contrast, sugar alcohols and sugars except glucose decreased. Hypoxia led to a reduction of abundances of most amino acids in early and commercial stages, however, alanine was slightly increased in late maturity stages. No γ-aminobutyric acid accumulation was seen under hypoxic treatment.

In-Situ Respiration Measurement of Fresh Produce Using a Modular Sensor and a Respirometer

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It is crucial to monitor respiration (*R*) and respiratory quotient (RQ) in situ, continuously and in real-time for identifying the optimal conditions for the long-term storage of fresh produce. In this study, the application of a gas sensor (RMS88) and a modular respirometer for in situ real-time monitoring of gas concentrations and respiration rates of strawberries during storage in a lab-scale controlled atmosphere chamber (190 L) and of Pinova apples in a commercial storage facility (170 t) is reported. The RMS88 consisted of wireless O_2 (0% to 25%) and CO_2 sensors (0% to 0.5% and 0% to 5%). The modular respirometer (3.3 L for strawberries and 7.4 L for apples) consisted of a leak-proof arrangement with a base plate containing a water channel and closed by a glass jar on top. Continuous recording of gas concentrations was performed by the RMS88 at regular intervals of 1 min for strawberries and 5 min for apples and, in real-time, transferred to a terminal program to calculate respiration rates (RO_2 and RCO_2) and RQ. Respiration measurement was performed in cycles of flushing and measurement periods. A respiration measurement cycle with a measurement period of 2 h up to 3 h was shown to be useful for strawberries under air at 10 °C. The start of anaerobic respiration of strawberries due to low O_2 concentration (1%) could be recorded in real-time. RO_2 and $RCO_2$of Pinova apples were recorded every 5 min during storage and mean values of 1.6 and 2.7 mL kg^{-1} h^{-1}, respectively, were obtained when controlled atmosphere (CA) conditions (2% O_2, 1.3% CO_2, and 2°C) were established. The modular respirometer was found to be useful for in situ real-time monitoring of the respiration rate during the storage of fresh produce and offers great potential to be incorporated into the RQ-based dynamic CA storage system.

Kiwifruit responses to ethylene in controlled atmosphere storage

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Controlled atmosphere (CA) with elevated level of carbon dioxide (5% CO_2) and reduced level of oxygen (2% O_2) has commonly been used by the industry to maintain the firmness of kiwifruit (*Actinidia deliciosa*). Kiwifruit is known to be very sensitive to ethylene. Increases in the ethylene concentration within the storage environment can induce rapid softening. However, the response of kiwifruit stored under CA conditions to exogenous ethylene remains unknown. The aim of this study was to determine whether the presence of ethylene impacts the beneficial effect of CA. 'Hayward' kiwifruit from 3 growers were stored in air and CA (2% O_2 + 5% CO_2) conditions at 0 °C for up to 13 weeks. Fruit were exposed to exogenous ethylene (&lt1, 10, 100 and 1000 $nL \cdot L^{-1}$) from the third week of storage onwards. Fruit quality (firmness, soluble solids content and disorder incidence) was assessed fortnightly from the start of ethylene exposure. Kiwifruit stored under CA conditions had a higher firmness in comparison to air at the same assessment time from early stage of storage, irrespective of the ethylene concentration. Ethylene effects were first observed on air-stored kiwifruit soon after initiation of ethylene exposure, while the same did not occur on CA-stored fruit until after longer period of ethylene exposure. The low concentration of ethylene in CA did not differentiate fruit quality from straight CA treatment throughout the experiment. Meanwhile, ethylene at higher concentrations induced advanced softening compared to lower concentrations. This study demonstrates that CA reduces the sensitivity of kiwifruit to ethylene induced softening. However, ethylene exposures at high concentration for long durations should be avoided even in CA in order to reduce undesired softening.

Exogenous Melatonin Treatment Alleviates Chilling Injury by Maintaining Membrane Integrity and Reducing Oxidative Stress in Cucumber (*Cucumis sativus* L.).

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Melatonin (N-acetyl-5-methoxytryptamine, MT) is an amine hormone involve in multiple physiological functions in plants. In the current study, exogenous treatment of MT (100 μM) on chilling injury (CI), quality parameters, antioxidant activities, and scavenging capacity of cucumber fruit was studied at 4oC+1 for 15 days. In comparison with control, this result demonstrated that MT treatment effectively delayed CI development. Furthermore, quality characteristics of cucumber fruits, which are ascorbic acid content, total soluble solids (TSS) and soluble proteins were moderately increased in MT treated fruits. Meanwhile, MT treatment inhibited ion leakage and malondialdehyde (MDA) content, which further resulted in membrane integrity during cold storage. Moreover, compared with the control group, MT application also stimulated antioxidant activities, specifically catalase (CAT), ascorbate peroxidase (APX), superoxide dismutase (SOD) and peroxidase (POD) activities. Moreover, increased antioxidant capacity facilitated to reduced hydrogen peroxide (H2O2) and superoxide anion (O2•–) production level by MT treatment. Whereas, exogenous MT induced 2, 2-Diphenyl-1picryl hydrazyl (DPPH) activity and hydroxyl (OH•) radical scavenging which inhibit reactive oxygen species (ROS) accumulation often lead to minimize oxidative stress. In conclusion, the result suggests that chilling injury reduced by MT action in the coordinated approach of maintaining membrane integrity, enhancing antioxidant activity and avoiding oxidative damage in cucumber fruit. Consequently, MT is an effective postharvest treatment in protecting against chilling injury and extending shelf life at cold storage condition.

BMSB injuries detection using a laser backscattering image system

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Brown marmorated stink bug (BMSB, *Halyomorpha halys* Stål) causes economic loss to the kiwifruit industry in many countries, such as Italy, Greece, USA and China. BMSB injured kiwifruit, generally with symptoms of white corky spots, are not accepted by consumers. Currently, BMSB injury assessment is destructive and there is no standard scale for evaluating the severity. As one approach to non-destructively detect BMSB injuries, laser backscattering image (LBI) technique was tested on 200 kiwifruit for this purpose. LBI records the emitted light after a single laser beam interacts with the fruit tissue in the visible and near-infrared (NIR) region. In LBI, the quality attributes related to cell structure and chemical composition are represented using two optical properties: absorption coefficient (μ_a) and reduced scattering coefficient (μ_s'). This study investigated whether μ_a and μ_s' at 3 wavelengths (532 nm, 660 nm, 785 nm) can detect BMSB injury for ''Zesy002' and 'Hayward' kiwifruit. The LBI images and quality attributes of kiwifruit were collected for both healthy (HF) and injured fruit (IF). A BMSB injury prediction model was created using the estimated μ_a and μ_s' after pre-classification based on reference objects (phantoms) with known μ_a and μ_s'. For 'Zesy002', the prediction model had a moderate true positive rate of 94%. However, 'Zesy002' kiwifruit were assessed at 14 days after harvest, thus LBI images may be affected by both BMSB injury and postharvest storage. For 'Hayward' the prediction model was poorer, assumably due to the long trichomes on the fruit surface and the low injury severity compared to 'Zesy002'. Additionally, as a "point" measurement technique, LBI has the limitation of undersampling the whole fruit tissue. In order to improve the results obtained in the current study, image analysis that allows image acquisition from the whole kiwifruit is required to be developed.

KEYNOTE:
The fruit ENCODE project reveals the origins of fruit ripening mechanisms

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Fleshy fruit ripening has evolved many times throughout angiosperm history, and many species require the plant hormone ethylene. Much of what we know about this process comes from the study of tomato, where ethylene, transcription factors and whole-genome demethylation are critically involved. But the precise molecular mechanism and whether it is conserved in other species remains largely unknown. Inspired by the human ENCODE project, we began to systematically profile gene expression, chromatin accessibility, H3K27me3 and DNA methylation dynamics during fruit development in 11 fleshy fruit species. When we analyzed the fruit ENCODE data, we realized that climacteric fruits without a recent whole-genome duplication (WGD) such as peach, melon and papaya utilize a common angiosperm senescence-related NAC transcription factor to create a positive feedback loop to synthesize the autocatalytic ethylene. For plants that have undergone a recent WGD like tomato, apple and pear, they evolved a loop by neofunctionalization of the duplicated MADS-box transcription factors. Banana, a monocot climacteric species that diverged from eudicot over 100 myr and has undergone three recent WGDs, uses a leaf senescence-related NAC transcription factor to generate a positive feedback loop and an additional loop with three MADS-box transcription factors that makes its ripening ethylene independent once initiated. It turns out that DNA methylation changes associated with ripening genes is unique for tomato. All climacteric fruits we examined, including tomato, appears to have utilized H3K27me3 to regulate key ripening and ethylene synthesis genes. One of the lessons we learnt from this project is that developmental processes such as ripening are controlled by a complex and redundant regulatory network. If we want to fully understand it, we might need to adopt a more holistic approach considering regulatory inputs from multiple dimensions such as 3D chromatin organization, DNA methylation, chromatin accessibility, histone modification and TF regulatory networks.

Transcriptome analysis of red mango fruit reveals induced defense against Colletotrichum gloeosporioides

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Mango fruit (Mangifera indica L.) exposed to sunlight develops red skin and are more resistant to biotic and abiotic stresses. Here we show that red mango fruit is more resistant than green fruit to Colletotrichum gloeosporioides. LCMS analysis showed high amounts of antifungal compounds, as glycosylated flavonols, glycosylated anthocyanins, and mangiferin in red vs. green mango skin, correlated with higher antioxidant and lower ROS. However, also the green side of the red mango fruit that has low levels of flavonoids was resistant, indicated induced resistance. Transcriptomes of red and green fruit inoculated on their red and green sides with C. gloeosporioides were analyzed. Overall, in red fruit skin, 2,187 genes were upregulated in response to C. gloeosporioides. The green side of red mango upregulated 22 transcription factors and 33 signaling-transcripts related to induced resistance. The RNA-Seq analysis suggests that resistance of the whole red fruit involved upregulation of ethylene, brassinosteroid, and phenylpropanoid pathways. To conclude, red fruit resistance to fungal pathogen was related to both flavonoid toxicity and primed resistance of the fruit that was previously exposed to sunlight at the orchard.

Linalool synthesis related *PpTPS3* expression was regulated by transcription factor *bHLH* and changes in epigenetic modifications during peach fruit ripening

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The monoterpene linalool contributes to aroma of peach (*Prunus persica*) fruit, however, molecular mechanism associated with linalool synthesis remains to be investigated. Here, we characterized a novel terpene synthase 3 (*PpTPS3*) responsible for linalool formation based on QTL mapping. Recombinant PpTPS3 catalyze synthesis of linalool in *vitro*, both transient overexpression of *PpTPS3* in peach fruit and stable transgenic overexpression in tomato led to linalool accumulation in *vivo*. To investigate the transcriptional regulation of *PpTPS3*, we screened a series of transcription factors (TFs) and found that a TF of bHLH family, *PpbHLH1*, had the highest activation effect on the promoter of *PpTPS3*. Yeast one-hybrid assay and EMSA showed that PpbHLH1 directly binds to E-box (CACATG) in the *PpTPS3* promoter. Transient overexpression of *PpbHLH1* resulted in linalool accumulation in peach fruit. Significant correlation was observed between *PpbHLH1*, *PpTPS3* and linalool among different peach cultivars. Taken together, these results indicate that *PpbHLH1* activates the *PpTPS3* promoter thereby regulating linalool synthesis in peach fruit. In addition, epigenetic modifications such as DNA methylation was also associated with *PpTPS3* transcript and linalool content during peach fruit ripening.

Are CBFs associated with chilling tolerance in kiwifruit?

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Kiwifruit maybe susceptible to chilling injury (CI) yet they rarely show symptoms store at 0˚C commercially. The biological basis for the chilling susceptibility of kiwifruit is not clear. The *C-REPEAT BINDING FACTOR (CBF)* genes have been extensively studied in Arabidopsis, with a suggested role in protecting the plants from freezing tolerance. The function of *CBFs* in the postharvest chilling tolerance of kiwifruit has yet to be determined. We previously identified *AcCBF2/3* in kiwifruit, for which induction by cold temperatures was dependent on fruit maturity. In this study, the expression of *AcCBF2/3* in response to chilling temperature were characterized in relation to fruit maturation, storage time, pre-storage temperature conditioning, and associated with the incidence of chilling injury after storage. Using transgenic rapid flowering *Actinidia chinensis* 'Hort16A' plants, we generated transgenic *AcCBF3* overexpressing lines using CAM35S promoter and edited *AcCBF2/3* using CRISPR-Cas9 mediated transformation to assess the function of these genes. These double-transformed plants will be used to understand the relationship between temperatures and chilling response of kiwifruit.

Postharvest dipping with 3,5,6-trichloro-2-pyridiloxyacetic acid solutions delays senescence of mandarins and oranges

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The effects of postharvest treatment of three citrus fruit types with 3,5,6-trichloro-2-pyridyloxyacetic acid (TPA) on the deterioration of calyx quality, decay incidence and internal quality parameters in long-term storage were investigated. Navel oranges and 'Afourer' mandarins were treated with TPA concentrations of 2, 4, 8, 16 and 32 µM, while Valencia oranges were treated at concentrations of 0, 15, 30, 60 and 120 µM. Fruits were stored in air at 20˚C for 32 and 28 days, respectively. TPA treatment exhibited a concentration-dependent effect on fruit quality, with higher concentrations resulting in a greater reduction in the incidence of calyx deterioration and decay, a lowering of respiration rate, ethylene production and ethanol accumulation, and inhibition of change in TSS and TA levels and hence maintaining the TSS/TA ratio. The results show that postharvest TPA treatment can be used to alleviate calyx senescence and maintain postharvest quality in citrus fruits.

Phenylalanine: Improve fruit quality and resistance to chilling injury and postharvest fungal pathogens

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The fruit quality is important for the marketability. More than one-third of harvested fruits and vegetables are lost; major part of it is due to decay caused by fungal pathogen. The restrictions on fungicides that control those fungal pathogens, call for economical, safe and eco-friendly alternatives to control the postharvest decay. Fruit's natural resistance depends majorly on flavonoids and anthocyanins synthesized from the phenylpropanoid pathway, which have antioxidant and antifungal activity, and play a part in fruits aroma. In this work, we hypothesized that exogenous application of phenylalanine, a precursor of phenylpropanoid pathway, will increase natural defense response to pathogen and chilling injury, and improve fruit quality. Indeed, postharvest application of phenylalanine to mango, avocado and citrus fruits reduced fruits decay caused by *Colletotrichum gloeosporioides*, *Alternaria alternata*, *Lasiodiplodia theobromae*, or *Penicillium digitatum*. Similarly, pre-harvest application of phenylalanine reduced postharvest decay in mango. Additionally, phenylalanine treatment on mango fruit reduce chilling injury and maintain fruit quality in mango fruit during storage at sub-optimal temperature. Interestingly, phenylalanine also improve the fruits natural aroma, and increase the soluble sugars. Thus, phenylalanine activated the phenylpropanoid and anthocyanin pathways allowing the production of specific antioxidant and antifungal flavonoids. This novel application of the amino acid, phenylalanine enhance fruit quality, natural resistance and health properties.

Postharvest treatment with double-strand RNA loaded within nano-clay sheets reduces *Botrytis cinerea* colonization and gray-mold in harvested fruits

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Postharvest fruit and vegetable loss is estimated at more than 40%. Pathogenic fungi cause a major part of this loss. During storage, along ripening, the fruit becomes more susceptible to fungal pathogen and the development of postharvest disease. Currently, the common treatment against postharvest diseases is fungicides. However, there is a growing concern for their harmful influences on the environment and human health. Therefore, there is a need to develop new strategies to control postharvest pathogens. One of the most common pathogens, *Botrytis cinerea*, infects over 200 plant species, causing grey mold disease. Recently, it was demonstrated that *B. cinerea* naturally uptakes small double-strand RNA (dsRNA) molecules from the host plant. This small dsRNA is downregulating genes through the RNA interference system. Thus, in this study, we aimed to develop dsRNA (targeted to essential genes), which will be applied postharvest to reduce fungal colonization and fruit rot. We synthesized dsRNA consists of three genes involved in the ergosterol biosynthesis pathway. Due to the low stability of dsRNA, it was loaded on layered double hydroxide (LDH) clay nano-sheets, which should protect the dsRNA from degradation and serve as a slow-release device. Our results show that the growing part of *B. cinerea* germination tube and hyphae can uptake dsRNA when applied alone or loaded in LDH. The dsRNA decreased fungal growth and germination both in-vitro and in-vivo in various fruits (e.g. grape, strawberry, bell-pepper cherry and more). Notably, treatments with dsRNA reduced by 100 fold the concentration needed to control *B. cinerea* of several fungicides targeting ergosterol. In contrast, addition of external ergosterol restores the phenotype, which indicates that the reduction in germination and fungal growth is due to activation of RNA interference system. These results also supported the down-regulation of all three related genes after dsRNA treatment, as shown by qPCR. When loading the dsRNA within LDH, we were able to reduce the necessary dsRNA concentration to obtain the same decay control and inhibition of fungal growth. Overall, our results indicate that the combination of dsRNA and LDH may serve as a safe alternative to the postharvest application of fungicides.

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