Elucidating physiological and molecular mechanisms of ethylene induced ripening and low temperature modulated ripening in kiwifruit

In order to extend “eating window”, the optimum ripening phase suitable for eating, combination of treatment with propylene (ethylene analog) and 1-methylcyclopropene (1-MCP; ethylene inhibitor) was assessed in three kiwifruit cultivars ‘Rainbow Red’ Actinidia chinensis, ‘Sanuki Gold’ A. chinensis, and ‘Hayward’ A. deliciosa. Propylene treatment initiated ripening process by inducing fruit softening, increase in soluble solids content (SSC), and decrease in titratable acids (TA) accompanying with or without endogenous ethylene production, depending on duration of exposure. Once endogenous ethylene was induced, it accelerated fruit ripening resulting in over ripening phase and shortening of “eating window”. ‘Rainbow Red’ and ‘Sanuki Gold’ fruit treated with propylene continuously or for 48 h initiated endogenous ethylene production that led to “eating window” for only 2 days (ranging 3-5 days after start of treatment) whereas it was for 7 days (ranging 3-10 days) in ‘Hayward’ fruit. Limited propylene treatment for 24 h to three cultivars induced ripening without detection of ethylene production suggesting that the optimum ripening phase suitable for eating can be attained without endogenous ethylene production, resulting in longer “eating window”. ‘Rainbow Red’ and ‘Sanuki Gold’ fruit treated with propylene for 48 h followed by 1-MCP treatment had extended “eating window” and shelf-life with suppression of endogenous ethylene. These results illustrates the practicability of different durations of propylene treatment in facilitating kiwifruit ripening and additional benefit of 1-MCP treatment to extend shelf-life of new high quality kiwifruit cultivars, ‘Rainbow Red’ and ‘Sanuki Gold’.

To assess kiwifruit ripening using non-destructive acoustic method, mature ‘Rainbow Red’, ‘Sanuki Gold’ and ‘Hayward’ kiwifruit were treated with propylene at 5000ppm continuously then stored at 20°C for 7 day. Non-destructive firmness was measured using resonance measuring device (RMD) and resonance frequency of individual fruit was translated to elasticity index (EI). Destructive fruit firmness of core and outer-pericarp was measured using a penetrometer then ethylene production, SSC and TA were also determined. The progression of on-vine kiwifruit
ripening was monitored at interval of two weeks from pre-optimum harvesting stage. Non-destructive firmness, destructive firmness, ethylene production, SSC and TA were measured. Propylene treatment induced ethylene biosynthesis, softening of core and outer pericarp, increase in SSC and decrease of TA in the three kiwifruit. For non-destructive firmness, resonance frequency and elasticity index (EI) decreased in kiwifruit cultivars after propylene treatment. Coefficient of correlation between core and outer pericarp destructive firmness and EI values achieved ranging from 0.78-0.97. Non-treated control kiwifruit did not depict ripening characteristics. Similarly, on-vine fruits portrayed ripening characteristic by fruit softening, decrease in elasticity index, increase of SSC and decrease in TA but this ripening occurred gradually for 2 months after commercial harvesting time. Interestingly, on-vine kiwifruit did not produce ethylene yet ripening progressed gradually while fruit was still attached to the vine. High coefficient of correlation between destructive firmness and EI shows that kiwifruit ripening can be determined reliably using non-destructive acoustic method both in accelerate ripening after propylene/ethylene treatment and in gradual ripening while fruit is still attached to the vine.

Kiwifruit is a climacteric fruit and ripening is typically mediated by ethylene. Ethylene induced ripening occurs via ethylene signaling pathway that subsequently modify transcription factors and genes responsible for acquisition of ripening qualities. To compare ethylene-dependent and low temperature ethylene-independent ripening in kiwifruit, ‘Sanuki Gold’, ‘Rainbow Red’ and ‘Hayward’ kiwifruit were: (1) treated with propylene (ethylene-dependent ripening); (2) subjected to 20°C and 5°C (ethylene-independent ripening and (3) on-vine ripening. Continuous propylene (ethylene analog; 5000 ppm) treatment induced fruit ripening by softening, increase in SSC and decrease in TA compared to control fruit. Storage at low temperature (5°C) also induced ripening compared to 20°C stored fruit irrespective of 1-MCP treatment. Ethylene production was detected in propylene treated fruit but not in fruit stored at 5°C and 20°C. 1-MCP treatment did not suppress fruit ripening in 5°C stored fruit indicating fruit ripening was solely due to low temperature exposure. On-vine ripening was observed in kiwifruit although the at different rates with ripening occurring earlier in early-harvesting cultivars (‘Rainbow Red’ and ‘Sanuki Gold’) compared to late-harvesting cultivar (‘Hayward’). To further investigate molecular characterization of ethylene-dependent and ethylene-independent fruit ripening induced by low temperature, ‘Sanuki Gold’ kiwifruit was analyzed using RNA-sequencing technique by Next Generation Sequencing. RNA sequencing analysis using Kiwifruit Genome database as reference genome identified >30,000 differentially expressed genes (DEGs) were either up or down regulated by propylene or by low temperature. Assessment of DEGs with >3 or <0.33 fold yielded 3837 significant DEGs. Venn analysis displayed eight distinct patterns of gene expression; 2111 DEGs were up-regulated solely by ethylene, 467 DEGs up-regulated by low temperature only, while 636 genes were up-regulated by both ethylene and low temperature. Other genes 439 DEGs were down-regulated by propylene and low temperature treatment. Interestingly, 184 DEGs showed antagonistic expression between propylene and low temperature regulation. Functional annotation classified DEGs linked to ethylene biosynthesis (15 genes); cell wall modifying (44 genes); carbohydrates metabolism (12 genes); and other genes associated with cellular functions. DEGs associated to Transcription Factors (TFs) identified 495 genes which included 20 ERF, 21 NAC, 17 MYB-HB, 10 bZIP and other TFs. Real-time PCR analysis was done which validated RNA-seq results obtained confirming reliability of these results. On-vine ripened kiwifruit had similar gene expression as low temperature stored fruit in most of the genes analyzed. These results show that kiwifruit can ripen mediated by ethylene through ethylene signaling
pathway and also in ethylene-independent manner facilitated by low temperature stimulus. Assessment of gene expression confirms presence of distinct (only by ethylene or low temperature) and overlapping ripening mechanisms implying existence of ethylene and low temperature ripening stimulating systems in kiwifruit.

The response of ‘Sanuki Gold’, ‘Rainbow Red’ and ‘Hayward’ kiwifruit cultivars to different storage temperatures was assessed at physiological and molecular level. Kiwifruit was assessed at three categories: ethylene-dependent (continuous propylene treatment at 5000ppm), ethylene-independent low temperature modulated ripening (storage at 5°C, 10°C, 15°C or 22°C with or without 1-MCP treatment) and progressive assessment of on-vine ripening (1, 2 and 3 months after optimum harvesting time). Kiwifruit treated with propylene ripened within 5 days depicting increase in ethylene production, decrease in firmness and TA, and an increase in SSC. Fruit stored at 5°C, 10°C and 15°C also exhibited a decrease in firmness and TA, and an increase in SSC independent of ethylene during storage. However, fruit stored at 5°C and 10°C ripened faster than those stored at 15°C (modest) and 22°C (slowest). Ripening also occurred during on-vine with a seasonal decrease in temperature without detection of ethylene. Real-time PCR analysis showed that propylene treatment induced the expression of ethylene biosynthesis and signaling genes (ACS, ACO1, ETR), cell wall modifying-related genes (PGC, EXP1, XET1, PL, PMEi), carbohydrate metabolism-related genes (βAMY1, SUSA), GAO, and transcription factors ERF6 and bZIP. Fruit stored at 5°C showed increased expression of PGC, XET2, EXP1, EXP6, EXP17, PMEi, βAMY2, SUSA, ACO2, GAO, CBP) and transcription factors (ERF15, ERF17, NAC1, NAC2, MADS, bZIP). Genes that were expressed in fruit stored at 5°C were also slightly but significantly induced in fruit stored at 10°C and at 15°C to a lesser extent while the expression was low in fruit stored at 22°C. Some genes such were expressed in both ethylene and ethylene-independent systems. High expression of ripening associated genes was also observed during on-vine ripening after 3 months similar to low temperature modulated ripening except ethylene biosynthesis genes ACS and ACO1 accounting for the lack of ethylene production. Storage of fruit at 5°C highly induced ripening associated genes such as PGC, EXP1, EXP6, EXP17, PMEi, βAMY1, βAMY2, SUSA, GAO, MADS, ERF17, NAC1, NAC2 and bZIP gene expression hence the stimulated fruit ripening. Storage at 10°C induced slight gene expression but it was significant enough to cause fruit ripening. Ripening associated genes were stimulated by low temperature and during on-vine ripening, noteworthy, the rate of gene modulation took longer time compared to propylene treated fruit. These results show that low temperature modulates fruit ripening independent of ethylene by inducing the expression of ripening-related genes and transcription factors in kiwifruit. ‘Rainbow Red’ and ‘Sanuki Gold’ kiwifruit were more sensitive to chilling temperatures of 5, 10 and 15°C compared ‘Hayward’ fruit which responded to 10°C to induce ripening independent of ethylene.