

## Quantitative Analysis of Volatile Flavor Compounds in Two Transgenic Tomato Fruits using APCI-MS Technique

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### ABSTRACT

*The volatile flavor compounds of two transgenic tomato fruit (*Lycopersicon esculentum* Mill. cv. Ailsa Craig), transformed with either an ACC-oxidase (ACO1) antisense gene construct or a polygalacturonase (PG) sense suppression gene construct, were analyzed at various stages of tomato fruit ripening compared to non-transformed fruit. Nine key volatile flavor compounds released following maceration of the tomato tissue were measured, using Atmospheric Pressure Chemical Ionization-Mass Spectrometry (APCI-MS) in real-time analysis. ACO1 antisense fruits, which showed less activity of ethylene production, had lower levels of most volatiles measured throughout ripening compared to wild type and PG sense suppression fruits. PG sense suppression fruits, with low polygalacturonase activity, would be expected to have the same quality as wild-type fruits in terms of volatile components.*

**Keywords:** Flavor, Fruit ripening, Tomato, APCI-MS

### INTRODUCTION

The perceived flavor of fresh tomato fruits is a result of a complex interaction between sugars, organic acids, minerals, and aroma volatile compounds. The volatile composition during tomato fruit ripening has been studied extensively (Kazeniak and Hall, 1970; Buttery et al., 1971; Buttery et al., 1987; Buttery et al., 1988). Although over 400 compounds have been identified as volatile components of fresh tomatoes and tomato products (Petro-Turza, 1987), a small number of these compounds is involved in fresh tomato aroma. The following compounds are reported to contribute to fresh tomato aroma: hexanal, (*Z*)-3-hexenal, (*E*)-2-hexenal, 1-penten-3-one, 6-methyl-5-hepten-2-one,  $\beta$ -ionone, ethanol, methanol, (*Z*)-3-hexenol, 2- and 3-methylbutanal, and 2-isobutylthiazole (Buttery et al., 1987; Baldwin et al., 1991). Some of these compounds are formed during fruit ripening by deamination and decarboxylation of amino acids, for example,

3-methylbutanal and its corresponding alcohol, 3-methylbutanol (Yu et al., 1968). Other compounds are produced by lipid oxidation of unsaturated fatty acids only when the tissue is disrupted (e.g. hexanal and hexenal; (Galliard et al., 1977). Isomerase activity can convert (*Z*)-3-enals to (*E*)-2-enals with concomitant changes in aroma quality. All aldehydes can potentially be converted to the corresponding alcohols by alcohol dehydrogenase (ADH). All the C-6 aldehydes contribute to the “green” or “fresh” note to tomato aroma while 2-isobutylthiazole is a major character impact factor, formed from an amino acid precursor (Kazeniak and Hall, 1970).

Most analyses of tomato volatiles have used solvent extraction, followed by GC-MS, for identification and quantification. These techniques measure the total volatile composition, but the actual values obtained depend to some extent on the extraction method. Some have tried to deactivate the enzyme systems by adding calcium chloride prior to maceration. This may lead to underestimating the lipid oxidation products while prolonged incubation after maceration will lead to overestimating. Conventional extraction and GC-MS is also time-consuming and limits the number of samples that can be analyzed in one day. Given the variations in tomato fruits due to their position on the plant and the rapidity of post-harvest changes, it is desirable to analyze sufficient number of samples, with the same degree of ripeness, within a short space of time.

A method for the controlled maceration of tomato fruit with simultaneous analysis of the volatile compounds released from the fruit has been described previously (Linforth and Taylor, 1999; Boukobza et al., 2001). Using online Atmospheric Pressure Chemical Ionization-Mass Spectrometry (APCI-MS), up to 12 volatiles in the headspace above the macerate can be monitored continuously and quantitatively. Typically, after a brief maceration period (about 20 seconds), the volatile released can be measured over a 3-minute period and related to the actual composition in the liquid phase using macerate-air partition values.

In this study, high throughput analysis was used to monitor the volatile flavor compounds from different tomato fruits at various stages of fruit ripening. Two transgenic tomato fruits (one with polygalacturonase down regulated, and another with ACC-oxidase down regulated) were compared with wild-type tomato fruit.

## MATERIALS AND METHODS

### Plant materials

Tomato (*Lycopersicon esculentum* Mill. cv. Ailsa Craig) plants were grown and maintained under standard glasshouse conditions at the University of Nottingham, Sutton Bonington campus, during the winter season (October 2001-May 2002), following the university handbook procedure (BBSRC glasshouse user guide; the University of Nottingham, UK). Tomato fruits from wild type, sense suppression polygalacturonase (PG) transgenic lines (Smith et al., 1990) and antisense ACC-oxidase (ACO1) transgenic lines (Hamilton et al., 1990) were harvested at the same stage of ripening for measurement of volatile composi-

tion. Seven stages of ripening from mature green (MG), breaker (B), 3 days post-breaker (B+3), 7 days post-breaker (B+7), 10 days post-breaker (B+10), 14 days post-breaker (B+14), and 21 days post-breaker (B+21) of each genotype of tomato fruits were collected for analysis.

### **Volatile analysis**

Tomato samples were placed in a maceration device modified from a commercial blender and then properly sealed (Boukobza et al., 2001). Tomatoes were macerated for 10-20 sec and the headspace gas was continually flushed with air (170 ml/min) to rapidly remove the volatiles formed. The airflow, which carried all volatiles formed, was continuously sampled into the APCI-MS at a flow rate of 11.5 ml/min through a heated transfer line (0.53 mm i.d. fused silica tubule) held at 160°C. The headspace gas above the macerated fruit was carried out for a further 3 min. The release of nine selected volatile compounds (2-isobutylthiazole, 6-methyl-5-hepten-2-one, 1-penten-3-one, hexanal, hexenal, hexenol, methylbutanal, methylbutanol, and acetaldehyde) were monitored. Five replications were analyzed for each ripening stage.

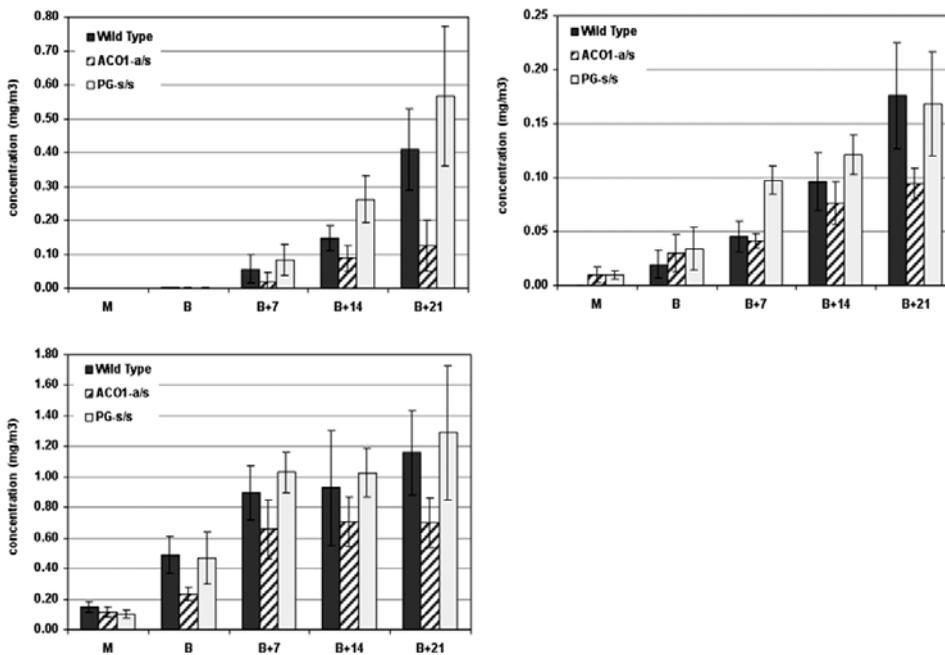
### **APCI-MS**

A Micromass Platform II quadrupole mass spectrometer (Micromass, Manchester, UK) operating in the gas phase using a positive ion, selective ion mode was fitted with a specifically designed air-sampling interface (Linforth and Taylor, 1998). Nine key volatile compounds in tomato fruits were monitored using the parameters described previously (Boukobza et al., 2001). For all volatile compounds, the corona pin was set at 4 kV and the dwell time was 0.5 sec. The cone voltages (CV) for each ion mass ( $m/z$ ) were adjusted to give a maximum sensitivity of  $[M+H]^+$  ion. All data were collected and analyzed by MassLynx software. The levels of volatiles monitored in each genotype were compared and expressed as maximum concentration observed during maceration (mg of volatile compound/m<sup>3</sup> of air) following calibration of the source with authentic standards.

## **RESULTS AND DISCUSSION**

The volatile components in wild type and transgenic tomato fruits at various stages of ripening were studied, beginning with some of the compounds reported to be formed during metabolism (Figure 1). Here, a clear pattern can be observed with increasing amounts of 2-isobutylthiazole, 6-methyl-5-heptenone and 1-penten-3-one as ripening proceeded from the mature green stage to the mature red stage. In mature green fruit, 2-isobutylthiazole was undetectable, both in wild type and transgenic tomato fruits. This compound occurs only in ripe tomato fruits and has not been detected in leaves or other parts of the tomato plant (Buttery and Ling, 1993). Wild-type tomato and PG sense suppression tomato had higher levels of this compound compared to ACO1 antisense fruit at the 21 days post-breaker stage of ripening (Figure 1a). In contrast, 6-methyl-5-hepten-2-one and 1-penten-3-one were detected in fruit at the mature green stage and the amounts increased with

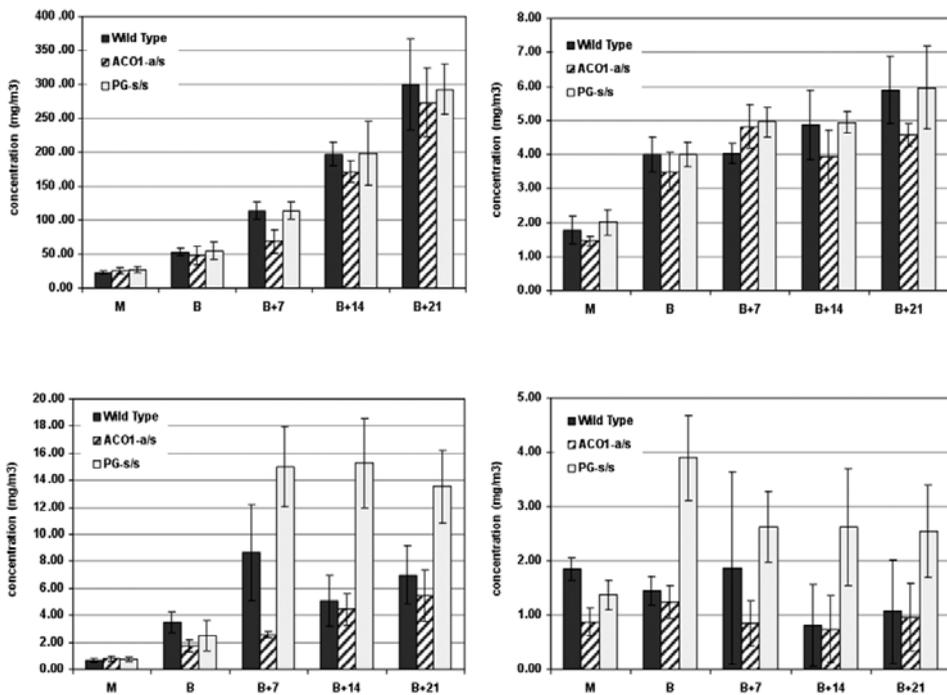
ripening. Again, the amounts were higher in wild type and PG sense suppression fruit compared to ACO1 antisense fruit at the ripening stage (Figure 1). The former volatile, a lycopene-derived volatile, was found at lower levels in mature green fruit with a dramatic increase during ripening, presumably due to the increased synthesis of lycopene as ripening progressed. Carotenoids and lycopene are both synthesized during fruit ripening and 6-methyl-5-hepten-2-one is derived from lycopene degradation. The origins of 1-penten-3-one are not so clear, but it is reported to be an oxidation product of metabolism. Although variation is quite high (as shown by the error bars), there was a clear trend in that the fruit with decreased ethylene production showed a reduced production of all three of these compounds over the whole ripening period.



**Figure 1.** The production of 2-isobutylthiazol (a), 6-methyl-5-hepten-2-one (b), and 1-penten-3-one (c) in wild-type, ACO1 antisense (ACO1 a/s), and PG sense suppression (PG s/s) fruit at the mature green (MG), breaker (B), 7 days post-breaker (B+7), 14 days post-breaker (B+14) and 21 days post-breaker (B+21) stages

Note: Data are the mean of five replicates. Error bars are  $\pm$  SD.

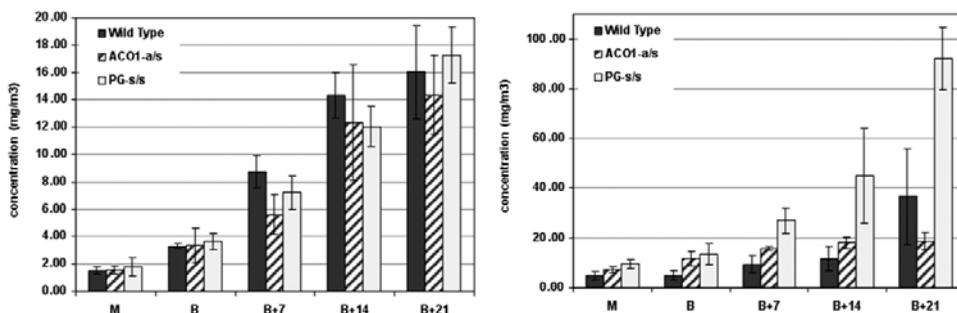
Figure 2 shows the amounts of aldehydes present in headspace above fruit during ripening. Hexanal and hexenal are produced by enzymatic oxidation of linoleic and linolenic acid, respectively, only when the fruit is macerated (Galliard et al., 1977). Hexanal and hexenal are reported to be important flavor volatiles of tomato with high odor unit values (Buttery et al., 1987; Petro-Turza, 1987; Buttery et al., 1988). The amount of hexanal showed a steady increase with ripening while hexenal levels remained steady after the mature green stage (Figures 2a and b). There were no differences between fruit types. This suggests that neither the amount of fatty acid substrate nor the relevant enzyme activities in the lipoxygenase pathway were affected by the genetic changes to the low ACO1 and PG fruits.



**Figure 2.** The production of aldehyde compounds; hexanal (a), hexenal (b) methylbutanal (c), and acetaldehyde (d) in wild-type, ACO1 antisense (ACO1 a/s), and PG sense suppression (PG s/s) fruit at the mature green (MG), breaker (B), 7 days post-breaker (B+7), 14 days post-breaker (B+14) and 21 days post-breaker (B+21) stages

Note: Data are the mean of five replicates. Error bars are  $\pm$  SD.

In contrast, the other two aldehydes, methylbutanal and acetaldehyde, which were formed during metabolism prior to maceration, showed significant differences between the down-regulated PG fruit and the wild type and ethylene suppressed fruit. Acetaldehyde concentrations from the down-regulated PG fruits showed the greatest difference from the wild type and ACO1 fruits. The presence of acetaldehyde in fruits and vegetables is often associated with anaerobic metabolism. One potential explanation is that transport of oxygen into the fruits is reduced because the cell walls in the down-regulated PG fruit maintain their integrity for a longer period or have altered hydration compared to the wild type or ACO1 antisense fruits (Smith et al., 1990). This might decrease the amount of oxygen available and the cell metabolism compensates by shifting to biochemical pathways that generate  $\text{NADH}^+$ . This is a well-known phenomenon in other temporary anaerobic situations, e.g., lactic acid formation in muscle and ethanol production in yeast. The production of 3-methylbutanal showed a similar increase during ripening to hexanal, but to a lesser degree. 3-Methylbutanal was significantly higher in PG sense suppression fruit than in wild type and ACO1 antisense fruits through the ripening period. The levels of these aldehydes were lower in ACO1 antisense fruit than in wild type and PG sense suppression fruit. It has been suggested that the volatile formation was effected from low ethylene production by ACO1 antisense fruit. If anaerobic condition was present in the down-regulated PG fruit, it might be expected that the levels of alcohols from the corresponding aldehydes might be increased by the aldehyde to alcohol conversion by the action of alcohol dehydrogenase (Eriksson, 1979). Measurements of hexenol (from hexenal) and methylbutanol (from methylbutanal) during the ripening period are shown in Figure 3.



**Figure 3.** The production of alcohols; hexenol (a) and methylbutanol (b) in wild-type, ACO1 antisense (ACO1 a/s) and PG sense suppression (PG s/s) fruit at the mature green (MG), breaker (B), 7 days post-breaker (B+7), 14 days post-breaker (B+14) and 21 days post-breaker (B+21) stages

Note: Data are the mean of five replicates. Error bars are  $\pm$  SD.

Although there were minor fluctuations in hexenol concentrations in Figure 3, they do not show a consistent trend or a significant difference; and this reflects the trends in hexenal content (Figure 2). For methylbutanol, there were again clear differences between the PG sense suppression fruits and the other two types of fruits. This supports the notion that conditions in the PG fruits during the breaker stage become anaerobic. Differences in acetaldehyde concentration could be seen from the breaker stage onwards. For methylbutanal/ol, the differences are only clear from the B+7 stage onwards.

### CONCLUSION

The general pattern of physical and biochemical alterations during ripening of tomato fruit was nearly identical in both the wild type and transgenic tomato fruits studied. However, ACO1 antisense tomato fruits showed lower performance with most of the volatile compositions compared to the other genotypes studied. The average amount of these volatile compounds in ACO1 antisense fruit was lower than those in the wild type and PG sense suppression fruits. Generally, ethylene production is often correlated with the synthesis of pigments and flavor volatiles. The levels of several of these volatiles were lower in ACO1 antisense fruits compared to wild type and PG sense suppression fruit. It is possible that tomato volatile formation may be directly or indirectly regulated by the ethylene. Conversely, PG sense suppression fruits would be expected to have the same quality as wild-type tomato fruit in terms of volatile compositions. Such a conclusion would have to be verified for the overall quality acceptance, not only by chemical analysis but also by sensory evaluation.

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