CHAPTER 5 : CONCLUSIONS

Tomato is an excellent scientific model to study the role of a wide range of ripening genes. There are a large numbers of studies carried out to identify the volatile components and their composition in tomato fruit. However, there is very little information focused on the volatile composition of transgenic tomato fruit. It is therefore essential to understand how volatiles of transgenic tomato fruits are affected, in order to improve or produce tomato fruits that give desired quality attributes. In the present work, a new and rapid technique using an Atmospheric Pressure Chemical Ionization-Mass spectrometry (APCI-MS) was used to monitor the real time flavour volatile compounds from two transgenic tomato fruits at various stages of fruit ripening. Two transgenic tomato fruits (one with ethylene down regulated, *ACO1* antisense and another with polygalacturonase down regulated, *PG* sense suppression) were compared with wild-type tomato fruit.

5.1 Overall conclusions

An APCI-MS has a considerable potential for monitoring the real time analysis of rapid volatile changes during plant tissue disruption. This technique was used for volatile analysis in cucumber and can be applied to investigate the alterations of volatile compounds composed in tomato as well as transgenic tomato fruits. This rapid technique was also successfully applied to study the biochemistry of flavour formation through a lipid oxidation pathway. C6 and C9 aldehydes in cucumber were generated only when tissue was macerated in the presence of oxygen. The sensitivity of the APCI-MS analysis in this experiment confirmed that there were no existences of C6 or C9 aldehydes in cucumber before maceration. An addition of linolenic acid [illegible] and linoleic acids increased the formation of nonadienal and nonenal, but the formation of these compounds was less when only one of the fatty acids was added. These results confirmed that the precursors of nonadienal and nonenal formations by cucumber were linolenic and linoleic acids, respectively. The amounts of volatiles present in the cucumber homogenates were estimated by microwaving a sample of cucumber to inactivate the enzyme systems that produce C6 and C9 volatile compounds. The amounts of volatile in fresh tissue were then obtained by comparing the sample results with a known authentic standard concentration in gas and liquid phases. However, the volatility of flavour molecules and mass transfer coefficient including air-water partition coefficient must be taken into consideration when the concentrations of fruit flavour were measured.

The APCI-MS technique was an efficient method to minimize fruit to fruit variation and was a rapid tool to compare flavour quality of large batches of fruits. However, this technique has some limitations such as some ion masses were corresponded to several compounds, whose individual time-profiles could not be measured. Thus, identification of compounds with the same mass is not possible to identify. In addition, positional isomers such as 2- and 3-methylbutanal can only be measured as methylbutanals. Moreover, the amount of volatile that could be measured was only in the headspace above the fruit maceration. The quantification of the amount of volatiles formed in the fresh tissue would be accomplished and translated to actual contents expressed as mg volatile compounds per kg fresh tissue rather than headspace concentration.

The release of nine key volatile compounds (2-isobutylthiazole, 6-methyl-5-hepten-2-one, 1-penten-3-one, hexanal, hexenal, hexenol, methylbutanal, methyl-butanol, and acetaldehyde) at various stages of ripening was monitored by an APCI-MS. A Mass Spectrometer equipped with a Liquid Chromatograph (LC-MS) interface was used for the quantitative analysis of many different non-volatile compounds composed in tomato fruit. There were no significant differences in sugars, calcium, [illegible] contents between fruit types [illegible] of citric acid was slightly higher in *ACO1* antisense tomato fruit than the other two varieties.

The general pattern of physical and biochemical alterations during ripening of tomato fruit was almost identical for both wild-type and transgenic tomato fruits that were studied. However, *ACO1* antisense tomato fruit showed lower performance with concern to 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 that in the wild-type and *PG* sense suppression fruits. Although the variation was quite high, there was a clear trend for a decrease in the ethylene production with reduction of the most volatile compounds, over the whole ripening periods. Generally, ethylene production is often correlated with the synthesis of pigments and flavour volatiles. The levels of several volatiles were lower in the *ACO1* antisense fruit compared to the *PG* sense suppression and wild-type tomato fruits. It is possible that tomato volatile formation may be directly or indirectly regulated by the ethylene. Conversely, the *PG* sense suppression fruit would be expected to have the same quality as the wild-type tomato fruit in term of volatile compositions. Although acetaldehyde concentrations for the down regulated *PG* fruit showed the greatest difference from the wild-type and *ACO1* antisense fruits. Such a conclusion would have to be verified for the overall quality acceptance not only by chemical analysis but also by sensory evaluation. A study about a correlation between volatile analysis and genetic molecular analysis, which was focused on the expression of ripening-related lipoxygenase (LOX) genes was carried out. The expression of the five tomato LOX clones during fruit ripening indicated that the flavour biogenesis in tomato fruit may be involved with a collaborated performnace of tomato LOX isoforms throughout the ripening stages. In the *ACO1* antisense fruit, the ethylene probably causes a reduction in expression of tomato LOX genes, especially *TomloxC* and *TomloxE*. These results led to the reduction of volatile formation in the intact of *ACO1* antisense tomato fruit.

Volatile compounds monitored in cucumber and tomato homogenate samples and by EI-MS mass spectral matching. The APCI-MS trace gave mainly molecular ions of the form $M+H^+$, whereas the EI-trace gave classical 70eV fragmentation patterns. The appearance of a particular ion in the APCI trace could be correlated with ions in the EI trace. Although a conventional GC-MS gave characteristics spectra from the identities of molecules and produced details structure information, these techniques were too slow when large numbers of samples had to be analyzed including for a real time analysis. To overcome the slow analysis times using the GC-MS methods, a rapid and real time technique was needed for such situations. The APCI-MS technique was suitable not only for a very rapid analysis but also for monitoring in a real time analysis. These techniques were possible to be used to study the biochemical pathway for volatile formation in cucumber and may apply for volatile analysis in other fruits.

5.2 Future works

Studying the components of volatile compounds in transgenic tomato fruits showed that there was an influence of transgene on flavour quality attributes. However, in the absence of sensory data, it was not possible to evaluate consumer perception and the differences in volatile compounds between fruit types, especially for a new cultivar developed with a desired quality via gene manipulation. Further work would involve sensory analysis of tomato volatile compounds. Moreover, the determination of lipid content would need to be correlated with the level of aroma compounds formation. An experimental model for volatile carbonyl compounds formation from fatty acids and lipoxygenase using an APCI-MS may be needed to be studied further to see the feasibility of providing a new natural source of fruit flavour production. Investigating the possibility of using these techniques for the volatile analysis in other fruits and vegetables could be another topic recommended for future studies.