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Aroma of peaches and nectarines: interaction between maturity at harvest, postharvest conditions and fresh-cut processing

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Look deep into nature,
and then you will understand everything better

Albert Einstein

Abstract

Peach is one of the most appreciated temperate fruit worldwide with high potential value on the market. However, consumers are not satisfied with the quality attributes of peach and nectarine at consumption and are not stimulated in repurchasing the fruit. This induced a strong decrease in sales in both local and export markets with the consequent drop of revenues for producers.

In addition to fresh consumption, the offer of fresh-cut peaches and nectarines is increasing and represent a valid alternative for stone fruit commercialization to match the increasing demand of ready-to-eat product on the market.

The aim of this work was to explore the interaction between the maturity at harvest and the development of the fruit flavour during postharvest, including the volatile organic compounds (VOCs) responsible for the aroma. Different peach and nectarine cultivars were used as well as different technologies such as SPME-GC-MS and PTR-ToF-MS to characterize the aroma profile of the fruit. Diverse cold storage lengths were applied to simulate short and long-distance export in which fruit are generally submitted to fulfil market demands. Furthermore, VOCs were also investigated on different cultivars of nectarines submitted to fresh-cut processing.

When fruit were submitted to cold storage the volatile profile of the fruit was generally enhanced, weakening the differences present at harvest between maturity classes.

Fresh-cut processing, induced a major variation in the volatile profile of the fruit such as the immediate release of VOCs associated with positive sensations, and the production of off-flavour volatiles over time in cold storage. A quality-oriented storage, processing and packaging should include the aroma volatiles to ensure the success of the peach and nectarine industry.

Keywords: *Prunus persica*, SPME-GC-MS, PTR-ToF-MS, Flavour, VOCs

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Table of Content

1. Introduction

1.1 Peach and nectarine market

Peach (*Prunus persica* L. Batsch) is an economically important crop, with a worldwide production over 22 million tonnes (FAOSTAT, 2014) featuring high nutritional attributes and pleasant flavour (Wang et al., 2006).

Europe is the major producing area with over 4.5 million tonnes produced in 2014. Among the European countries, Spain and Italy are the major producers with over 1.57 and 1.37 million tonnes of production, respectively. About a third of the total European production is exported with the corresponding export value of 1.9 billion dollars (FAOSTAT 2014).

However, the consumption of peaches and nectarines is facing a reduction primarily due to the poor quality and flavour characteristics of the fruit at consumption (Bruhn et al., 1991; Crisosto et al., 2006).

This reduction in the demand has not paired a reduction in the fruit supply (Bianchi et al., 2017) which has caused a substantial product surplus with the consequent drop in the price and revenue for producers.

The low economic return impacted negatively the final product quality since growers and pack houses are not willing to invest to grow and store the crop under the optimal conditions.

In addition, the perishable nature of peaches and nectarines impose major restrictions on the storability of the product. In fact, fruit must be handled and shipped within a short period of time, limiting the possibility of selling it in the most profitable periods of the year or to the most profitable markets worldwide.

In this complex scenario, achieving and maintaining high quality fruit on the market, ensuring consumer satisfaction upon consumption becomes vital for the peach and nectarine industry.

1.2 Peach and nectarine quality: flavour and aroma

Fruit quality involves organoleptic, nutritional, nutraceutical traits (Abbott, 1999) and some aspects not directly related to consumption, such as the sustainability of the production methods. Indeed, fruit should be free of biotic and xenobiotic contaminants and rich in nutritional and nutraceutical compounds important for the human health.

At the moment of the purchase, the quality of fresh peaches and nectarines is judged by consumers upon their appearance (Kader, 1999) which is mainly determined by the surface colour and the

absence of defects. Fruit appearance has been an important target for peach and nectarine breeding in the last decades (Iglesias and Echeverría, 2009), resulting in the release of several highly attractive fruit for consumers.

After purchase, consumer satisfaction is determined by the fruit flavour (Brückner, 2008) as it stimulates consumer to repurchase the fruit (Kader, 1999). Flavour is defined as the combination of taste and aroma sensations (Pérez and Sanz, 2008). Taste is mainly determined by the combination of organic acids and sugars providing sweetness and sourness sensations and for less extent bitterness, astringency or saltiness (Pérez and Sanz, 2008). Aroma results by a complex mixture of several volatile organic compounds emitted by the fruit and is influenced by genetics and environmental factors, cultural practices and postharvest handling (Pérez and Sanz, 2008).

Research has widely investigated the aroma composition of peaches and nectarines and more than 100 volatile compounds were identified (Aubert et al., 2003; Cano-Salazar et al., 2013, 2012; Robertson et al., 1990; Wang et al., 2009), each of them with a specific contribution to the aroma perception. However, the different volatiles possess different odour notes and not likely to be attributable to peach-like odours if smelled singularly.

Contributing to this aroma complexity are mainly esters, aldehydes, terpenes, alcohols, and lactones (Eduardo et al., 2010; Wang et al., 2009).

C6-Aldehydes (e.g. Hexanal isomers) are the most representative of the aldehydes and are characterized by 'green' odour notes (Hatanaka, 1993) that are predominant in immature fruit (Visai and Vanoli, 1997) and tend to decrease during ripening (Engel et al., 1988; Robertson et al., 1990). Green odour notes are also given by the presence of C-6 alcohols, (e.g. (Z)-3-hexenol and (Z)-2-hexenol) synthesized from the corresponding aldehydes (Z)-3- Hexenal and 2-Hexanal.

The progression of ripening leads also to the production of off-flavour alcohols (for the most Ethanol) produced via fermentative metabolism.

Esterification of alcohols and carboxylic acids determines the production of volatile esters (Pérez and Sanz, 2008) mainly related to fruity notes (Sumitani et al., 1994) that are generally increasing with the progression of ripening. Examples of common esters present in peaches and nectarines are Ethyl Acetate and Methyl Acetate (Wang et al., 2009).

Terpenes are an important volatiles group of peaches and nectarines and are mainly represented by Linalool (Li et al., 2015) α-Terpineol (Spencer et al., 1978), Limonene and β-Myrcene (Sánchez et al., 2012). Terpenes are generally associated with pleasant odour notes and are present in higher concentration in ripe fruit.

Lactones (e.g. δ-Decalactone, γ-Decalactone, γ-Octalactone, γ-Hexalactone, γ-Undecalactone) are reported to be major contributors of the peachy aroma (Horvat and Chapman, 1990; Jia et al., 2005;

Lavilla et al., 2002; Robertson et al., 1990; Visai and Vanoli, 1997) and are associated with pleasant and fruity notes (Rizzolo et al., 2006; Zhang et al., 2011). Lactones are increasing with fruit ripening (Robertson et al., 1990).

1.3 Cold storage and maturity as factors determining peach and nectarine quality

A fruit would express its maximum quality properties if grown under the optimal conditions, harvested at the physiological maturity and consumed at the optimal ripening stage such as immediately after short storage, to ensure superior flavour and nutritional values (Whitaker, 2008).
This scenario is uncommon as the peach and nectarine industry relies on global markets with the need of shipping fruit for long distances.
Peach and nectarine are highly perishable fruit and are characterized by a drastic loss of firmness at ambient temperature (Crisosto et al., 1999). For decades the supply chain has primarily focused on prolonging fruit storability to allow long distance export (Martínez-Romero et al., 2000). This included the development of new cultivars, such as the stony-hard, selected mainly for their better storage characteristics rather than for their quality attributes.
Low temperature during storage and shipping is widely used to preserve peaches for both local and export markets (Cano-Salazar et al., 2012). However, long term cold storage may induce a substantial reduction in sensorial quality (Infante et al., 2008), related with the decrease of the flavour characteristics (Robertson et al., 1990). Peaches and nectarine during storage may also incur in the development of physiological storage disorder such as chilling injury (Lurie and Crisosto, 2005) identified in the deterioration of textural properties (mealiness, woolliness, leatheriness), loss of juiciness, browning of the flesh, and loss of the ability to ripen (Crisosto et al., 1999; Luza et al., 1992). Chilling injury induces also a deterioration of aroma properties with the reduction of pleasant volatiles such as lactones (Xi et al., 2012).
As function of the fruit quality, peach and nectarine volatiles in relation with cold storage were widely investigated (Ortiz et al., 2010; Xi et al., 2012; Zhang et al., 2011, Cano-Salazar et al., 2013, 2012; Crisosto et al., 2006). However, the industry is still in needs of guidelines or technologies to increase, or at least, maintain the fruit quality throughout the whole supply chain.
The maturity at harvest is a factor that greatly influences the market life potential and quality of the fruit (Costa et al., 2009). A fruit that is harvested immature, featuring high level of firmness, will not

express the best eating quality at consumption, but is less subjected to external damages when handled in postharvest.

To the best of our knowledge, only few studies present on literature focused on the relation between the maturity of the fruit at harvest and the evolution of the organoleptic properties (Infante et al., 2012) and the volatile aromas (Robertson et al., 1990) during postharvest.

1.4 The determination of peach and nectarine maturity

Peach and nectarines are climacteric fruit and therefore characterised by a sharp rise in respiration rate and ethylene emission at the onset of ripening (Ziosi et al., 2008) determining a fast evolution of the texture and flavour properties.

Ethylene is also involved in the development of aroma-related volatiles either by modulating the activity of VOCs producing enzymes, such as alcohol acyltransferase (Defilippi et al., 2005), or lipoxygenase (Zhang et al., 2011), or determining the availability of the precursors involved in VOCs biosynthesis (Defilippi et al., 2005).

Current literature investigating the trends of VOCs emission during either ripening, or storage identifies the stage of maturity through skin ground colour (Infante et al., 2012; Robertson et al., 1990), distance from full bloom of the harvesting date (Cano-Salazar et al., 2013; Ortiz et al., 2009; Visai and Vanoli, 1997), or fruit firmness (Prinsi et al., 2011). All these parameters are either indirectly, or only partially correlated with fruit maturity and, therefore, are intrinsically biased indicators of fruit maturity stage when considered independently. Furthermore, flesh firmness and other more reliable maturity parameters are assessed by destructive assays. Thus, they do not allow a precise monitoring of quality on the same fruit along the duration of the experiment.

A recent proposed methodology suggests the identification of the maturity stage in peaches and nectarines by the combination of fruit ethylene emission and the index of absorbance difference (I_{AD}) measurable non-destructively with DA-Meter (Bonora et al., 2014, 2013; Ziosi et al., 2008).

The I_{AD} expresses the chlorophyll content in the fruit skin and flesh. Lower values of I_{AD} are indicates a lower content of chlorophyll and therefore a more advanced fruit maturity (Ziosi et al., 2008).

The I_{AD} showed to be a robust parameter to monitor the evolution of fruit maturity in peaches and nectarines (Bonora et al., 2014, 2013; Reig et al., 2012; Ziosi et al., 2008) and it has been successfully used for research purposes in plums (Infante et al., 2011), apricots (Costa et al., 2008) and apples (Busatto et al., 2014; Farneti et al., 2017).

However, being an indicator of chlorophyll contents, the I_{AD} requires to be strengthened by a parameter more closely associated to the physiology of ripening. Therefore, the combined measurement of I_{AD} and the ethylene emission provides a more accurate and reliable assessment of the maturity of the fruit (Ziosi et al., 2008).

1.5 Fresh-cut processing as alternatives to peach and nectarines fresh consumption

The modern life-style aligns more with fast and ready to eat products. The fruit industry, over the last decades, has increased the offer of fresh-cut fruit (Denoya et al., 2015) with the aim of providing a more practical product as alternative to fresh consumption. Due to the complexity in optimizing the factors affecting fruit quality in the supply chain, the convenience of the fresh-cut product may represent a valuable alternative to the traditional consumption of peaches and nectarines.

Indeed, minimally processed fruit are meant to have maintained or improved the physiochemical, nutraceutical and flavour characteristics as the fruit commercialized intact, hence representing a technological challenge for the industry.

Processing results from several mechanical operations including cutting, slicing and coring that are critical to determine the potential shelf life of the fruit (Soliva-Fortuny and Martín-Belloso, 2003). These operations induce the disruption of the cell compartmentalization bringing enzymes and substrates in contact, speeding up fruit perishability, softening and inducing surface browning (Artés and Gómez, 2006). The latter represents a major challenge for the commercialization of the fresh-cut fruit as consumers are reluctant to purchase a poor looking fruit, regardless of the quality traits such as texture, flavour and taste.

As per the fresh consumption, fresh-cut products must be handled for several days along the supply chain, therefore lengthening the shelf life and maintaining at the optimum the quality properties until consumption is mandatory to deliver premium fresh-cut products on the market.

For this reason, several research efforts aimed at extending the shelf life of processed peaches and nectarines. These studies were mostly focused on the suitability for processing of different cultivars, heat treatments (Koukounaras et al., 2008), the application of edible coatings (Pizato et al., 2013), the enzyme inactivation by high pressure processing (Denoya et al., 2016, 2015), low temperature storage, and modified atmosphere packaging (MAP) (Koukounaras et al., 2008).

As per the fresh consumption, consumer's acceptability is driven by fruit taste and aroma, therefore, a thorough understanding of the VOCs behaviour in fresh-cut peaches and nectarines is pivotal to ensure repetitive repurchases.

Several studies focused on deciphering the VOC profile of peaches and nectarines in relation to the genotype and to cold storage (Aubert et al., 2003; Cano-Salazar et al., 2012; Cano-Salazar, López and Crisosto, 2013; Ortiz et al., 2010) or chilling disorders (Xi et al., 2012), or the maturity at harvest (Robertson et al., 1990). However, for fresh-cut products, the implication of slicing, packing and storing on the development of the volatile aromas has not been investigated yet.

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2. Aim of the thesis

The fruit aroma is among the most important features affecting the quality of peaches and nectarines on the market and due to its complexity, is difficult to be controlled both in pre- and post-harvest conditions. Several factors along the production chain affects the fruit aroma and among them, the temperature and length of the cold storage and shelf life, the maturity of the fruit at harvest and processing techniques such as fresh cut, are believed to play a crucial role in regulating the fruit flavour perceived at consumption.

Therefore, the aim of this work was to explore the interaction between the fruit maturity at harvest, the postharvest conditions and fresh-cut processing on the development of the fruit flavour during postharvest, including the volatile organic compounds (VOCs) responsible for the aroma. To characterize the aroma profile, different peach and nectarine cultivars were used as well as different analytic technologies such as solid-phase micro extraction-gas chromatography-mass spectrometry (SPME-GC-MS) and proton transfer reaction-time of flight-mass spectrometry (PTR-ToF-MS).

2.1 Structure of the thesis

This thesis includes three core chapters (3 to 5) structured as scientific papers, with a title, introduction, materials and methods, results, discussion, conclusions, references, tables and figures. Chapter 3 focuses on investigating the 'Stark Red Gold' nectarine aroma volatiles in relation to the maturity of the fruit at harvest by PTR-ToF-MS whilst chapter 4, on investigating 'August Flame' peach aroma volatiles in relation to the maturity of the fruit at harvest by SPME-GC-MS. Both chapter involved different experimental designs, simulating different postharvest conditions commonly applied in the peach and nectarine industry.

Chapter 5 involved the investigation of aroma volatiles emitted by three cultivars of nectarine, harvested at different maturity stages, in response to fresh-cut processing, packing and storage.

Chapter 6 highlights the general conclusion of the thesis.

3. Is the maturity at harvest influencing nectarine flavour after cold storage?

Abstract

Peach is a highly perishable fruit which drastically restricts the marketing possibilities due to the short storage potential. Although characterized by pleasant flavour when fully ripe, peaches and nectarines are not well accepted by consumers due to the poor texture and flavour characteristics developed by fruit harvested before physiological maturity. In fact, harvest maturity, which is the most suitable for postharvest handling and storage usually does not coincide with physiological maturity. Moreover, during post-harvest and shelf life, despite fruit volatile organic compounds (VOCs) are associated with the consumer acceptance, they are not considered among the sensorial characteristics monitored in the optimization of the pre- and post-harvest fruit management.

The aim of this research was to investigate the effect of the fruit maturity at harvest on the development of VOCs during a simulated post-harvest chain.

To do so, fruit of 'Stark Red Gold' nectarine, harvested at different maturity stages, determined by the "index of absorbance difference" (I_{AD}), were stored at 1 °C (90-95% RH) for 21 days, then maintained 6 days in shelf-life at 20 °C. The evolution in aroma production was monitored each day of shelf life by proton transfer reaction-time of flight- mass spectrometry (PTR-ToF-MS). Besides the VOC measurement, fruit where assessed for soluble solids content and flesh firmness.

The analysis of VOCs by PTR-ToF-MS allowed the detection and identification of 37 compounds tentatively identified in relation to internal databases and literature references.

Maturity classes presented differences of I_{AD}, firmness, and ethylene at harvest, although differences in the development of VOCs during shelf life were not clear between the maturity classes. The prolonged cold storage period to which the three maturity classes were subjected before shelf life might have weakened possible differences in aroma volatiles at consumption.

Keywords: *Prunus persica*, Aroma, VOCs, PTR-Tof-MS, postharvest

3.1 Introduction

Peach and nectarine production is not paired by a corresponding demand (Bianchi et al., 2017), as consumers are often unsatisfied with the texture and flavour properties of the fruit. The mismatch between expected and real quality at consumption drives consumers to avoid repurchases impacting negatively the sales and the net return for producers. Various factors are believed to be responsible for peach and nectarine poor quality, and may derive from a non-optimal management of the fruit at harvest and during postharvest. Furthermore, consumers are unable to differentiate among the different cultivars due to their similarity in external traits (colour and size) together with the absence of a market classification based on cultivars quality (Bianchi et al., 2017) such as texture and flavour. Texture and flavour are crucial quality parameters determing conusmers acceptibility and are strongly influenced by the maturity of the fruit at harvest and the duration of cold storage.

Flavour includes the perception of fruit taste and aroma. The latter results from the complex interaction of several classes of VOCs and is affected by the maturity of the fruit at harvest (Robertson et al., 1990) and the duration and type of cold storage (Aubert et al., 2003; Cano-Salazar et al., 2012; Ortiz et al., 2010, 2009).

Thus, optimizing the peach and nectarine management throughout the pre- and postharvest supply chain is crucial for the product quality and, ultimately, for the success of the industry.

This study aimed at understanding the effects of different harvest maturity of the fruit the development of VOCs in simulated postharvest conditions.

To determine non-destructively the maturity stage of the fruit at harvest, a novel approach was adopted in this study. Maturity was determined as a relation between the index of absorption difference (I_{AD}) measured non-destructively with the DA-Meter (TR-Turoni, Forlì, Italy) and by the emission of ethylene (Bonora et al., 2014, 2013; Ziosi et al., 2008), which is the key hormone in modulating the ripening syndrome in climacteric fruit, such as peach (Defilippi et al., 2005; Zhang et al., 2011). The I_{AD} showed to be a robust parameter to monitor the evolution of fruit maturity in peach (Bonora et al., 2014, 2013; Ziosi et al., 2008) and has been successfully used for research purposes in plums (Infante et al., 2011), apricots (Costa et al., 2008) and apples (Busatto et al., 2014; Farneti et al., 2017). However, being an indicator of chlorophyll contents, the I_{AD} is just as indirect as other traditional maturity indexes, and may require to be validated by a parameter more closely associated to the physiology of ripening. Therefore, the combined use of the I_{AD} and the fruit ethylene emission was adopted to provide a more accurate description of the fruit maturity (Ziosi et al., 2008).

3.2 Materials and methods

3.2.1 Plant material

This study was conducted on fruit of 'Stark Red Gold' nectarine (*Prunus persica* L. Batsch) grafted on GF677 rootstock, grown at the experimental orchard of the University of Bologna located in the province of Bologna, Italy. Trees were grown following standard agronomical practices for thinning, pruning and pathogen control.

3.2.2 Maturity classes and storage conditions

Nectarine maturity classes were identified analytically with the DA-Meter (TR, Forli, Italy), a VIS-spectrometer non-destructively measuring chlorophyll-a content in the fruit flesh (Ziosi et al., 2008). The output results in an Index of Absorbance Difference (I_{AD}) ranging from 0.0 to 2.0, with the lower values indicating a more advanced fruit maturity (Bonora et al., 2014, 2013; Ziosi et al., 2008). Since ethylene has been reported as a pivotal hormone in regulating most of the ripening processes in climacteric fruit (Defilippi et al., 2005), in this trial the maturity classes were identified by relating the I_{AD} values to fruit ethylene emission. Ethylene was measured from 5 replicates of 1 fruit per each decimal value of I_{AD} from 1.5 to 0, by placing the intact fruit in a 1 L air-tight jar at room temperature for 2 h. A 10 mL headspace sample was then withdrawn and ethylene quantified with a Dani HT 86.01 (Dani, Milan, Italy) packed-gas chromatograph.

The combination of I_{AD} and ethylene measurement allowed to segregate fruit in three maturity classes at harvest: pre- climacteric (immature, C-I: I_{AD} 0.9 – 0.8); onset of climacteric (mature, C-M: I_{AD} 0.7– 0.5) and climacteric (ripe, C-R: I_{AD} 0.4– 0.3).

3.2.3 Experimental design and storage conditions

Fruit I_{AD} was monitored every 2-3 days *in planta* to determine the progression of fruit maturity. When the population of fruit was comprehensive of the three maturity classes, harvest was performed.

720 nectarines sorted in three maturity classes (240 fruit each) were stored in temperature controlled rooms at 1 °C (90-95% RH) for 21 days. Fruit were then removed from cold storage and kept at 18 °C for 6 d to simulate shelf-life.

At harvest (T0) and on each day of shelf life (T1-6), a batch of fruit was removed and assessed for quality traits (soluble solids content, flesh firmness, titratable acidity) and VOCs.

3.2.4 Quality traits measurement

Quality measurement (flesh firmness, soluble solids content, titratable acidity) were carried out on 20 fruit per each maturity class. Flesh firmness was determined with a penetrometer (FTA Guss, South Africa) and soluble solids content with a digital refractometer (ATAGO, Tokyo, Japan).

3.2.5 Sample preparation for VOC analysis

Three biological replicates of 2 fruit each were used for VOC measurement. Nectarine were peeled, sliced and immediately frozen in liquid nitrogen. Glass vials of 20 mL equipped with PTFE/silicone septa were filled with 1 g of powdered frozen flesh sample mixed with 2.5 mL of deionized water, containing 1 g of sodium chloride, 12.5 mg of ascorbic acid, and 12.5 mg of citric acid to prevent tissue oxidation. Samples were then preserved at 4 °C until the analysis.

3.2.6 VOCs analysis by PTR-ToF-MS

VOC measurements of nectarine pulp tissue were performed in three replicates with a commercial PTR-ToF-MS 8000 apparatus (Ionicon Analytik GmbH, Innsbruck, Austria). The conditions in the drift tube were the following: 110 °C drift tube temperature, 2.25 mbar drift pressure, 550 V drift voltage. This leads to an E/N ratio of about 140 Townsend (Td) (E corresponding to the electric field strength and N to the gas number density; 1 Td = 10^{-17} Vcm^2). The sampling time per channel of ToF acquisition was 0.1 ns, amounting to 350,000 channels for a mass spectrum ranging up to m/z = 400. Every single spectrum is the sum of about 28.600 acquisitions lasting 35 µs each, resulting in a time resolution of 1 s. Sample measurements were performed in 60 cycles resulting in an analysis time of 60 s/sample.

Each measurement was conducted automatically after 20 min of sample incubation at 40 °C by using an adapted GC autosampler (MPS Multipurpose Sampler, GERSTEL), and lasted for around 2 min. During the measurements, 100 sccm of gaschromatography grade air was continuously injected into the vial, through a needle heated to 40 °C, and the outflow going through a second heated needle was delivered via Teflon fittings to the PTR-ToF-MS.

The analysis of PTR-ToF-MS spectral data and compound annotation, counting of correction of the spectra through Poisson statistics, internal calibration, noise reduction, baseline removal, and compound concentration quantification proceeded according to (Cappellin et al., 2012, 2011a, 2011b)

3.2.7 Data analysis

The detection of the array of masses with PTR-ToF-MS was reduced by applying noise and correlation coefficient thresholds. The first removed peaks not significantly different from blank samples (Farneti et al., 2015), while the latter excluded peaks having over 99 % correlation, which mostly correspond to isotopes of monoisotopic masses (Farneti et al., 2017).

Data analysis was performed with R.3.3.3 software using internal functions and external packages such as "mixOmics" and "ChemometricswithR", for multivariate statistical analysis and "ggplot2" for graphic representations. Anova analysis were performed by the external package "Agricolae".

3.3 Results and discussions

3.3.1 Maturity, cold storage and shelf life influence on fruit quality traits

The main quality traits of nectarines, determined at harvest (T0) in relation to the maturity class, are reported in table 3.6.1. A significantly higher production of ethylene was reported for the C-R class (I_{AD} 0.3-0.4) which is correspondent to the climacteric phase for this variety as previously reported by Ziosi et al. (2008). The three maturity classes differed significantly in flesh firmness at harvest, with C-I characterized by firmer fruit than C-M and C-R (table 3.6.2). No significant differences in the content of soluble solids were detected at harvest between maturity classes.

The effect of maturity and cold storage on quality traits was determined by the comparison of the data recorded at the first assessment after removal from cold storage (T1) with the corresponding values at T0 (table 3.6.2).

I_{AD} drastically decreased during cold storage, with a two-fold reduction irrespective of the maturity class. However, the maturity classes remained different in I_{AD}, maintaining the pattern of segregation detected at harvest. Firmness and soluble solid content were not affected by cold storage, maintaining similar values as detected at harvest.

The combined effect of cold storage and shelf life temperatures had the strongest effect in regulating the quality traits. I_{AD} decreased during shelf-life (from T0 to T5) showing separate trends between the three maturity classes until day 3. From that point until the end of shelf life, the three maturity classes showed similarly low I_{AD} values.

Fruit firmness also decreased consistently during shelf life maintaining initial differences among maturity classes until the day 4.

The optimal firmness for nectarine consumption (8.8 to 13.2 N; Crisosto, 2002) was attained by C-M and C-R fruit classes after 4 days of shelf life, whilst C-I took one more day. This result highlights

the fast perishability of nectarines, as few days of shelf life consequent to a 3 weeks cold storage determined a fast fruit softening. To ensure consumers satisfaction in relation to firmness, a maximum shelf life of 5 days is envisaged for 'Stark Red Gold' nectarines, particularly when fruit are exported overseas and therefore cold stored for long periods.

As common for peaches, the content in soluble solids was not affected by either cold storage and shelf life, since in peaches and nectarines, soluble solids are generally increasing in shelf life just consequently to fruit shrivelling (Cano-Salazar et al., 2012; Infante et al., 2008).

3.3.2 VOCs profiling by PTR-ToF-MS

The characterisation by direct injection allowed the identification of most volatile compounds responsible for nectarine aroma (table 3.6.3), recently described in literature by PTR-ToF-MS analysis (Bianchi et al., 2017). The PTR-ToF-MS setting adopted in this study allowed the detection of the full VOCs spectra in 1 s. Only the first 30 s of the full measurement (120 s) were analysed and averaged, to avoid possible measurement inaccuracies caused by an excessive dilution of the sample headspace. The whole VOCs spectra, assessed in three replicates for each sample, were reduced from 244 to 70 masses, applying noise and correlation coefficient thresholds. For 43 of these masses, the chemical molecular formula was identified, while 37 were tentatively identified using internal databases and correspondence with literature data.

Amongst the compounds composing the VOC profile, aldehydes formed the most representative group with 12 fragments detected, followed by esters (6), alcohols (3), ketones (4), sulphur compounds (2), lactones (2) and monoterpenes (1).

3.3.3 Maturity, cold storage and shelf life influence on VOCs emission

To describe the effect of the maturity class on VOCs emission at harvest and during shelf life, a principal component analysis (PCA) was carried out with VOCs data resulting from PTR-ToF-MS analysis (fig 3.7.1 A). The PCA accounted for 91% of the total variance with the first two principal components and PC1 (85.6%) mostly described changes of VOCs over time in shelf life, whilst differences due to cold storage and maturity class contributed to PC2 (5.43%).

As per the loading plot (fig 3.7.1 B), positive values of PC2 were mainly associated with higher concentration at harvest of the masses *m/z* 81.07001 (Hexenal isomers and Linalool) and *m/z* 69.0700

(aldehyde fragment) and to the mass related to monoterpenes (*m/z* 137.1350) tentatively associated with (-)-Limonene, (+-)-Limonene, β-Myrcene, Linalool, 4-Terpineol and Geraniol.

Positive values of PC1 were mainly explainable by the increase in magnitude of the masses *m/z* 45.0329 (Acetaldehyde), *m/z* 33.0326 (Methanol), *m/z* 75.0435 (Methyl acetate) and *m/z* 87.0440 (Butyrolactone) during shelf life ripening.

By analysing the trends of the significant masses impacting the total variability described in the abovementioned PCA, the molecular fragment associated with monoterpenes (*m/z* 137.1350) was found to be higher in concentration at harvest (T0) in C-R fruit in comparison with C-I and C-M (fig 3.7.2). After 21 days of cold storage, the concentration dropped and never recovered during shelf life ripening irrespective of the segregation into maturity classes.

This may indicate a negative effect of cold storage on the volatile aroma of 'Stark Red Gold' nectarine, as monoterpenes are generally associated with pleasant odour notes. Among these, Linalool is a frequently detected compound in peaches and nectarines, generally reported to be higher in mature fruit (Horvat and Chapman, 1990; Robertson et al., 1990) and negatively affected by cold storage (Cano-Salazar et al., 2013; Robertson et al., 1990).

Overall, cold storage seemed to have weakened the differences in monoterpene emissions present between maturity classes at harvest, when ripe fruit had the most abundant concentration, as expected.

The concentration of the fragment related to C6-aldehydes (*m/z* 81.07001) was highest at harvest and decreased significantly after cold storage in a similar trend among the three maturity classes (fig 3.7.3). C6-aldehydes are commonly associated with "green" odour notes. Their concentration is highest in immature fruit and tends to decrease with the progression of ripening (Visai and Vanoli, 1997). In this work, no differences between maturity classes either at harvest or during shelf life ripening were found.

The concentration of Acetaldehyde (*m/z* 45.0329) was similar among the maturity classes at harvest, decreased in response to cold storage, and recovered during shelf life ripening with a higher intensity for the C-R fruit up to T4 (fig 3.7.4). Acetaldehyde is generally described as an off-flavour metabolite, and occurs in fruit with the progression of ripening (Porat and Fallik 2008; Whitaker, 2008) as a result of fermentative metabolism (Porat and Fallik, 2008).

The emission of Methanol (*m/z* 33.0326) increased in the last 2 days of shelf-life (fig 3.7.5) possibly deriving from the degradation of pectin in the cell walls (Fall and Benson, 1996) occurring during fruit softening (Ortiz et al., 2010). The production of Methanol was linked with a similar trend with the production of Methyl acetate (*m/z* 75.0435) (fig 3.7.6) indicating a possible conversion of the alcohol in the corresponding methyl ester. This compound is associated to fragrant and fruity notes, therefore positively contributing to the aroma.

Lactones were mainly represented by the Butyrolactone (*m/z* 87.0440), and increased with the progression of ripening during shelf life. Also in this case, the trends (fig 3.7.7) did not suggest differences in concentration induced by the segregation into maturity classes at harvest.

3.4 Conclusions

The present work described the behaviour of nectarine flavour with the combination of maturity stage at harvest and the exposition to cold storage. Unlike I_{AD}, firmness, and ethylene emission, which presented differences among the maturity classes since harvest, we did not report substantial differences in the development of aroma VOCs in shelf life. However, the prolonged cold storage period to which the fruit were subjected might have reduced the differences existing in aroma volatiles emitted during shelf life ripening. Further studies to unravel the link between fruit maturity at harvest and aroma development are required. A better definition of the optimal time of harvest for peaches and nectarines may be useful to maximize their quality at consumption and, consequently, the consumer likelihood of repurchase.

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3.6 Tables

Table 3.6.1: fruit segregation in the maturity classes C-I (immature), C-M (mature) and C-R (ripen) at harvest according to the IAD values and ethylene production. Values are reported as means. Lower case letter indicates significant differences among maturity classes performed with ANOVA and LSD test $p<0.05$.

I_{AD} range	Class	Ethylene production ($nl\ L^{-1}\ h^{-1}\ g^{-1}$ FW)	Fruit firmness ($kg \cdot cm^{-2}$)	Total soluble solids (°Brix)
1 – 0.8	C-I	0.15 b	7.5 a	10.4 a
0.7 – 0.5	C-M	0.41 b	6.6 b	11.0 a
0.4 – 0.3	C-R	2.62 a	6.0 c	10.9 a

Table 3.6.2: Maturity and quality characteristics of 'Stark Red Gold' nectarines at harvest (0) and during shelf life for C-I (immature), C-M (mature) and C-R (ripen) fruit. Lower case letter indicates differences (LSD) for both maturity classes and days of assessment.

Shelf life (days)	**I_{AD}**			**Firmness (N)**			**Brix (%)**		
	C-I	C-M	C-R	C-I	C-M	C-R	C-I	C-M	C-R
0 (Harvest)	0.77 a	0.41 c	0.27 d	73.7 a	65.4 b	59.4 c	10.9 a	11.3 a	11.9 a
1	0.38 b	0.23 d	0.13 e	71.9 a	64.2 b	58.4 c	10.6 a	11.3 a	11.8 a
2	0.19 d	0.11 ef	0.08 g	69.5 ab	65.8 b	49.9 d	11.0 a	11.3 a	11.3 a
3	0.05 f	0.06 gh	0.04 h	47.1 d	30.8 e	23.3 f	11.0 a	11.6 a	11.9 a
4	0.06 i	0.04 i	0.04 i	20.9 f	13.8 g	13.0 g	11.1 a	11.2 a	11.5 a
5	0.04 i	0.03 i	0.06 i	9.4 h	8.5 h	7.2 hi	11.4 a	11.1 a	11.6 a
6	0.04 i	0.03 i	0.06 i	6.7 i	5.4 i	5.6 i	11.4 a	11.1 a	11.6 a

Table 3.6.3: Volatile compounds detected by PTR-ToF-MS. Values are reported as concentration (ppbv). Compounds identification occurred with internal database references and by the revision of Bianchi et al., 2017.

m/z	Formula	Tentative identification	Min	Max	Mean
ms28.0194		n.i.	0.038	0.388	0.118
ms33.0326	CH_4OH^+	Methanol	14.446	3715.594	705.516
ms40.0275		n.i.	0.041	0.318	0.137
ms41.0386	$C_3H_5^+$	Common fragment	2.073	24.208	10.002
ms42.0118	$C_2H_2O^+$	n.i.	0.005	0.091	0.033
ms42.0350	$C_2H_3NH^+$	Acetonitrile	0.245	3.243	1.627
ms43.0178	$C_2H_3O^+$	Common fragment	5.112	59.919	18.392
ms43.0544	$C_2CH_7^+$	Common fragment	0.983	5.701	3.251
ms45.0329	$C_2H_4OH^+$	Acetaldehyde	15.677	507.079	180.922
ms47.0142	C_2H6OH^+	Ethanol	4.072	8.708	6.326
ms49.0113	CH_4SH^+	Methanethiol	0.026	0.350	0.163
ms51.0427		n.i.	0.376	76.481	14.934
ms57.0699		n.i.	1.408	7.468	3.902
ms59.0489	$C_3H_6OH^+$	Acetone	4.024	21.495	14.372
ms61.0280	$C_2H_4O_2H^+$	Fragment of Acetic acid, Acetoin, common ester fragment	4.139	49.239	10.349
ms62.0290		n.i.	0.193	1.207	0.375
ms63.0271		n.i.	0.075	0.302	0.192
ms63.0424	$C_2H_6SH^+$	Dimethyl sulfide, Ethanethiol	0.069	2.082	0.813
ms67.0542	$C_5H_7^+$	n.i.	0.083	1.121	0.468
ms68.0552		n.i.	0.004	0.080	0.040
ms69.0328	$C_4H_4OH^+$	Furan	0.081	0.276	0.175
ms69.0700	$C_5H_9^+$	Aldehyde fragment	0.380	16.938	4.634
ms71.0491	$C_4H_6OH^+$	Butenal	0.134	1.022	0.510
ms71.0855	$C_5H_{11}^+$	3-methyl-1-butanol + 2-methyl-1-butanol, 3-Pentanol, 1-Pentanol	0.136	1.762	0.796
ms73.0646	$C_4H_8OH^+$	Butanal, isobutyraldehyde	0.447	3.940	2.249
ms75.0435	$C_3H_6O_2H^+$	Methyl acetate	0.447	173.938	26.538
ms79.0532	$C_6H_7^+$	Benzene	0.442	0.757	0.543
ms80.9934		n.i.	0.014	0.171	0.053
ms81.0304		n.i.	0.018	0.056	0.033
ms81.0701	C_6H9^+	Fragment of aldehydes (Hexenals); fragment of terpenes (Linalool)	0.328	25.630	6.657
ms82.0737		n.i.	0.021	1.571	0.440
ms82.9443		n.i.	0.005	0.040	0.016
ms82.9880		n.i.	0.003	0.061	0.017
ms83.0477	$C_5H_6OH^+$	Methylfuran	0.071	0.362	0.175
ms85.0266		n.i.	0.112	0.221	0.161
ms85.0650	$C_5H_8OH^+$	2-Pentenal	0.179	2.266	0.860
ms85.1007		n.i.	0.042	0.809	0.336
ms87.0440	$C_4H_6O_2H^+$	Butyrolactone	0.591	8.805	2.065
ms87.0802	$C_5H_{10}OH^+$	2-methyl butanal+3-methyl butanal, Pentanal	0.102	3.042	0.983
ms93.0366	$C_3H_8OSH^+$	n.i.	0.424	1.532	0.818
ms95.0474		n.i.	0.278	0.454	0.353
ms95.0862		n.i.	0.063	1.941	0.652
ms97.0644	$C_6H_8OH^+$	2,4-Hexadienal	0.029	0.254	0.104
ms97.1020	$C_7H_{13}^+$	Heptanal, fragment	0.044	0.391	0.184
ms101.0601	$C_5H_8O_2H^+$	2,3-Pentanedione	0.090	0.257	0.171

ms107.0850	$C_8H_{10}H^+$	Ethyl Benzene, Xylene	0.078	0.331	0.186
ms108.9565		n.i.	0.152	0.223	0.194
ms109.1026	$C_8H_{13}^+$	n.i.	0.143	0.890	0.448
ms111.0808		n.i.	0.018	0.248	0.128
ms111.1178		n.i.	0.045	0.179	0.119
ms113.0968	$C_7H_{12}OH^+$	Heptenal	0.027	0.542	0.239
ms115.0759	$C_6H_{10}O_2H^+$	Ethyl Crotonate (Ethyl (2E)-2-butenoate) , 5-Ethyldihydro-2(3H)-Furanone	0.053	0.493	0.219
ms115.1117	$C_7H_{14}OH^+$	Heptanone, Heptanal	0.026	0.105	0.059
ms117.0918	$C_6H_{12}O_2H^+$	Isobutyl Acetate, Butyl Acetate, Hexanoic Acid	0.035	0.151	0.098
ms121.1022	$C_9H_{13}^+$	n.i.	0.018	0.084	0.049
ms123.1186	$C_9H_{15}^+$	Nonenal	0.022	0.148	0.089
ms123.9447		n.i.	0.024	0.049	0.040
ms125.0974		n.i.	0.014	0.143	0.080
ms127.0710		n.i.	0.013	0.040	0.027
ms127.1136	$C_8H_{14}OH^+$	1-octen-3-one, 6-Methyl-5-Hepten-2-one, (E)-2-Octenal	0.038	0.561	0.229
ms129.1283		n.i.	0.032	0.385	0.152
ms131.1087	$C_7H_{14}O_2H^+$	Isoamyl Acetate+Ethyl Benzene, Amyl Acetate, Heptanoic Acid	0.006	0.055	0.036
ms135.1180		n.i.	0.031	0.122	0.068
ms136.0244		n.i.	0.011	0.088	0.059
ms137.1350	$C_{10}H_{17}^+$	(-) -Limonene, (+-) -Limonene, Beta Myrcene, Linalool, 4-Terpineol, Geraniol	0.049	4.947	0.400
ms139.1150		n.i.	0.019	0.148	0.074
ms141.1293	$C_9H_{16}OH^+$	2-Nonenal [a]	0.009	0.090	0.059
ms143.1062	$C_8H_{14}O_2H^+$	cis-3-Hexenyl Acetate, 2,5-Octanedione, trans-2-Hexenyl Acetate, 5-Butyldihydro-2(3H)-Furanone	0.015	0.170	0.081
ms145.1247	$C_8H_{16}O_2H^+$	Ethyl Hexanoate, Hexyl Acetate, 2-Ethyl Hexanoic Acid	0.015	0.059	0.042
ms153.0806		n.i.	0.005	0.022	0.014

3.7 Figures

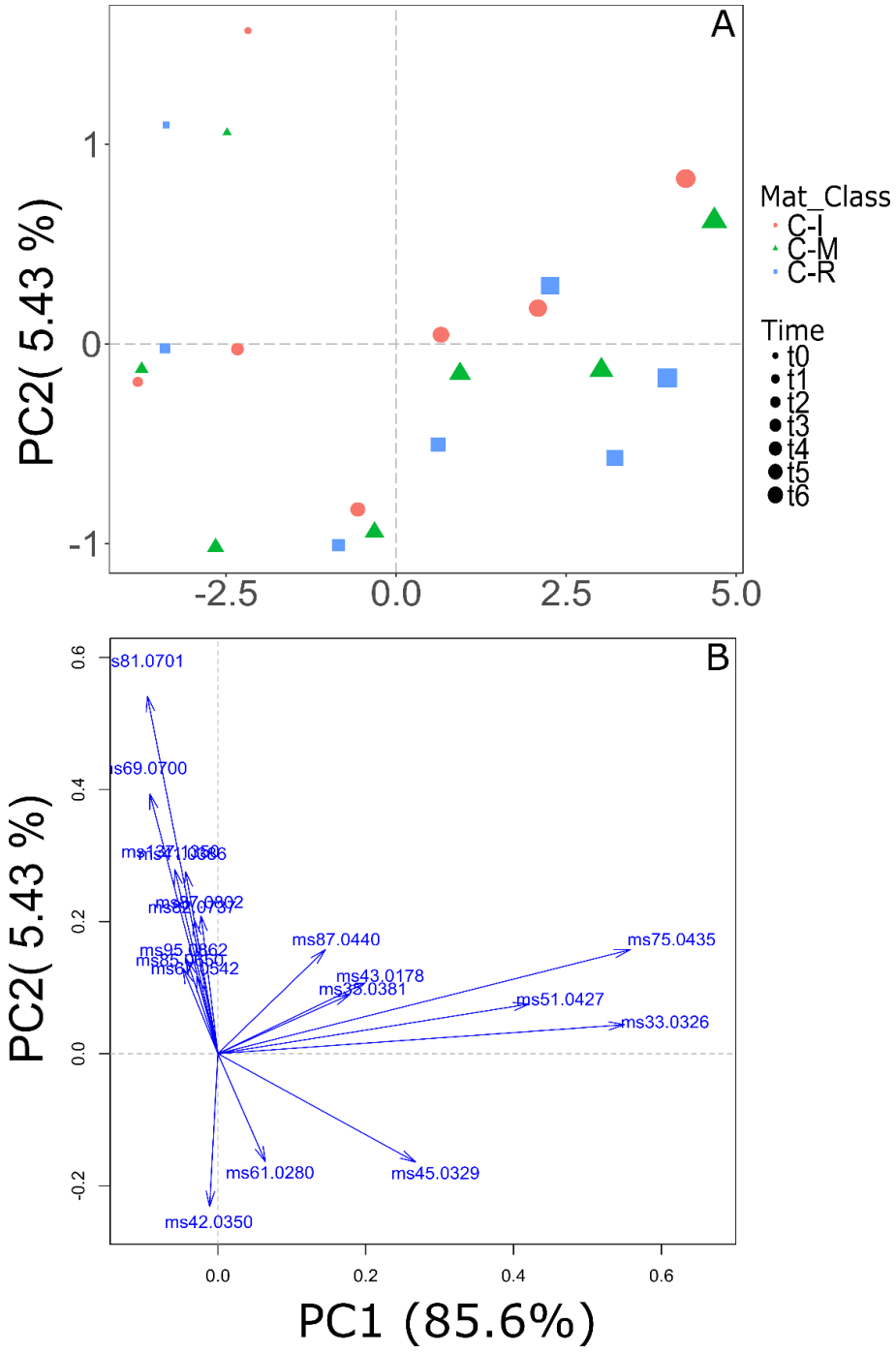

Figure 3.7.1: (A) two-dimensional principal component analysis (PCA), elaborated using the VOCs quantified by the PTR-ToF-MS during simulated shelf life for the three maturity classes (C-I: immature; C-M: mature; C-R: ripe). **(B)** Loading plot projecting the variables (VOCs) identified by PTR-ToF-MS during simulated shelf life. The tentative identification of the VOCs is reported in Table 2.

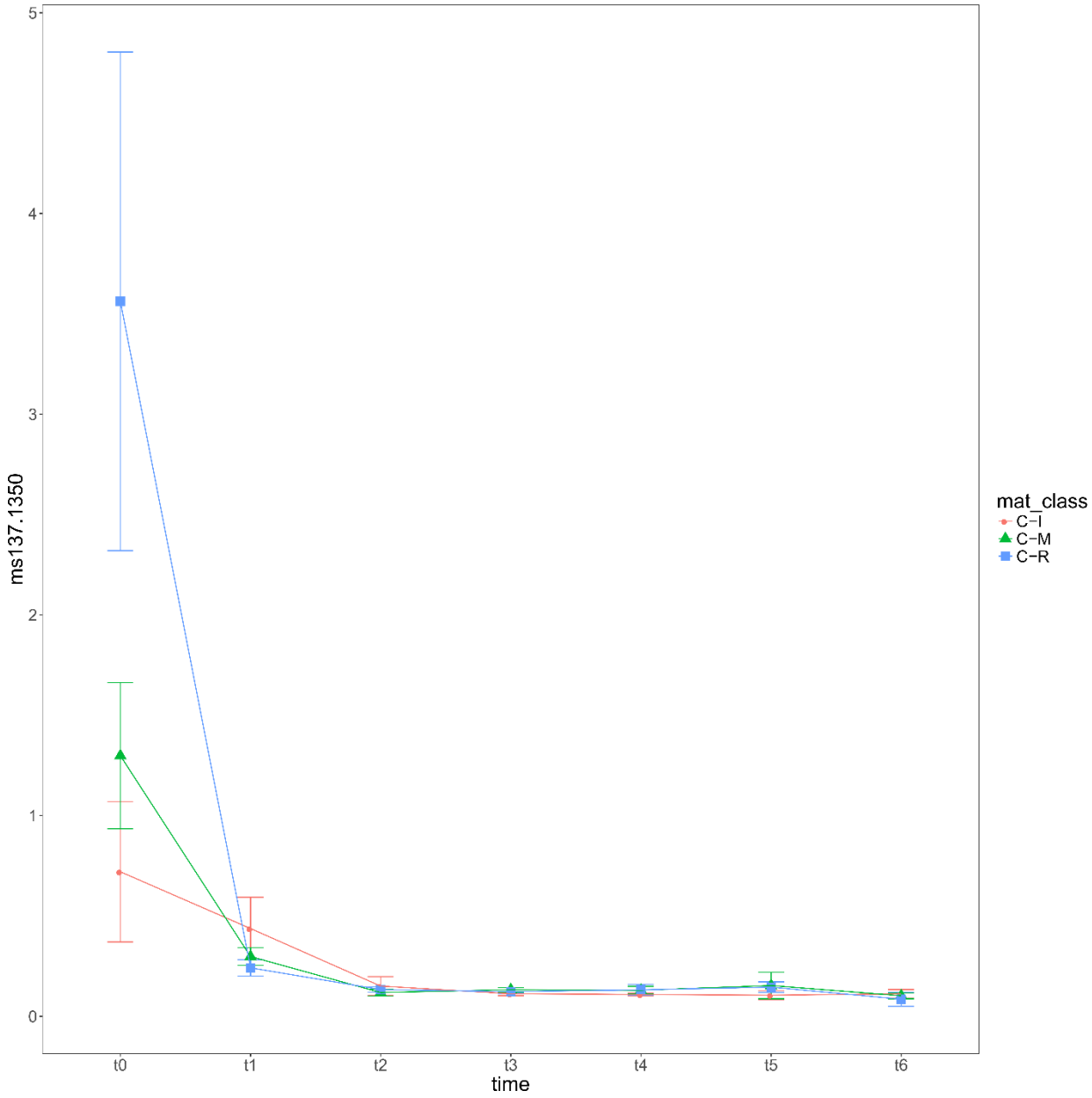

Figure 3.7.2: concentration (ppbv) trends of the mass associated with monoterpenes *m/z* 137.1350 ((-)-limonene, (+-)-Limonene, Beta Myrcene, Linalool, 4-Terpineol, Geraniol), during shelf life days from fruit of three maturity classes (C-I: immature; C-M: mature; C-R: ripe). Bars represent standard error.

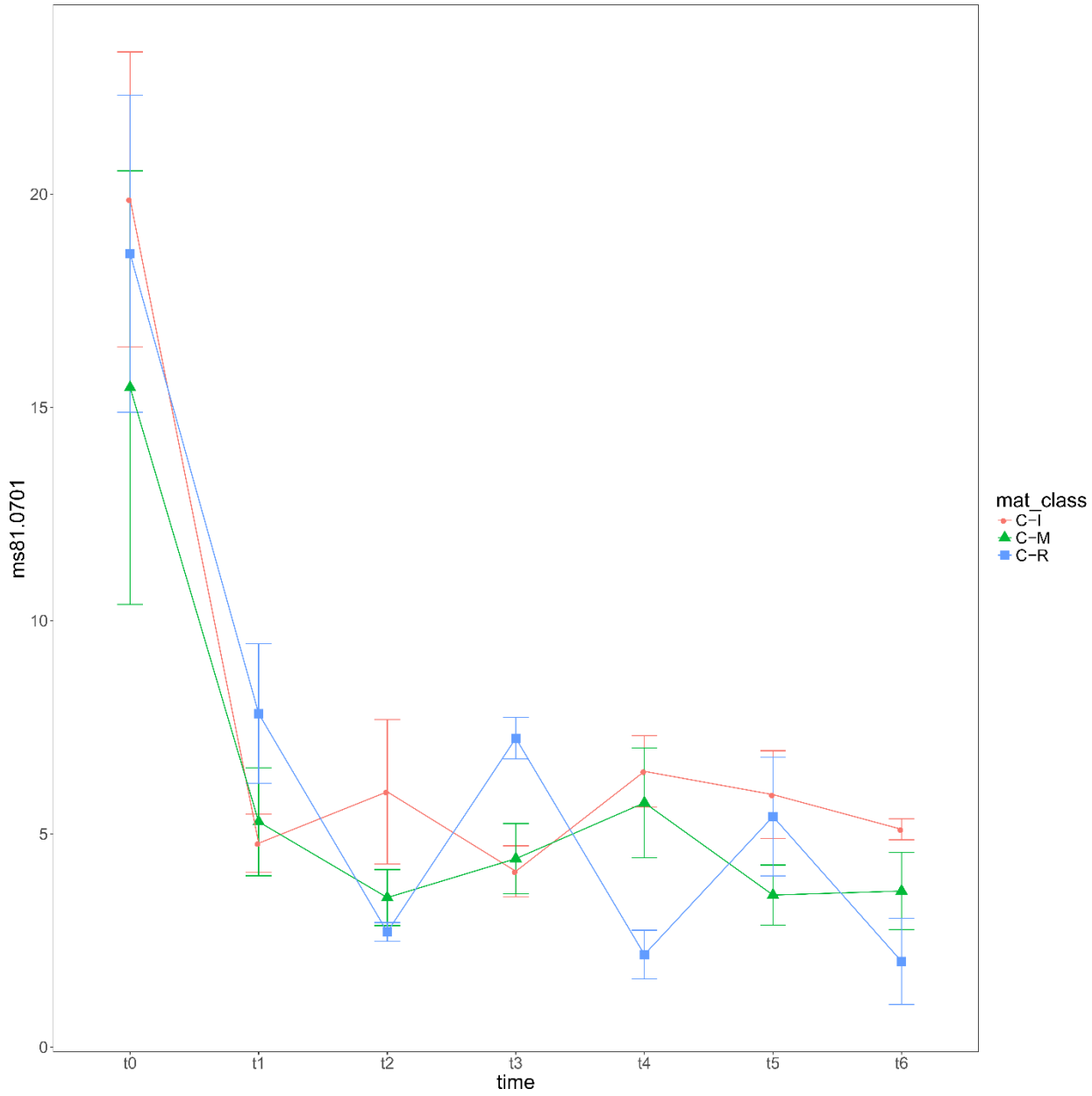

Figure 3.7.3: concentration (ppbv) trends of the mass *m/z* 81.0701, associated with C-6 aldehydes (Hexenal isomers), during shelf life days from fruit of three maturity classes (C-I: immature; C-M: mature; C-R: ripe). Bars represent standard error.

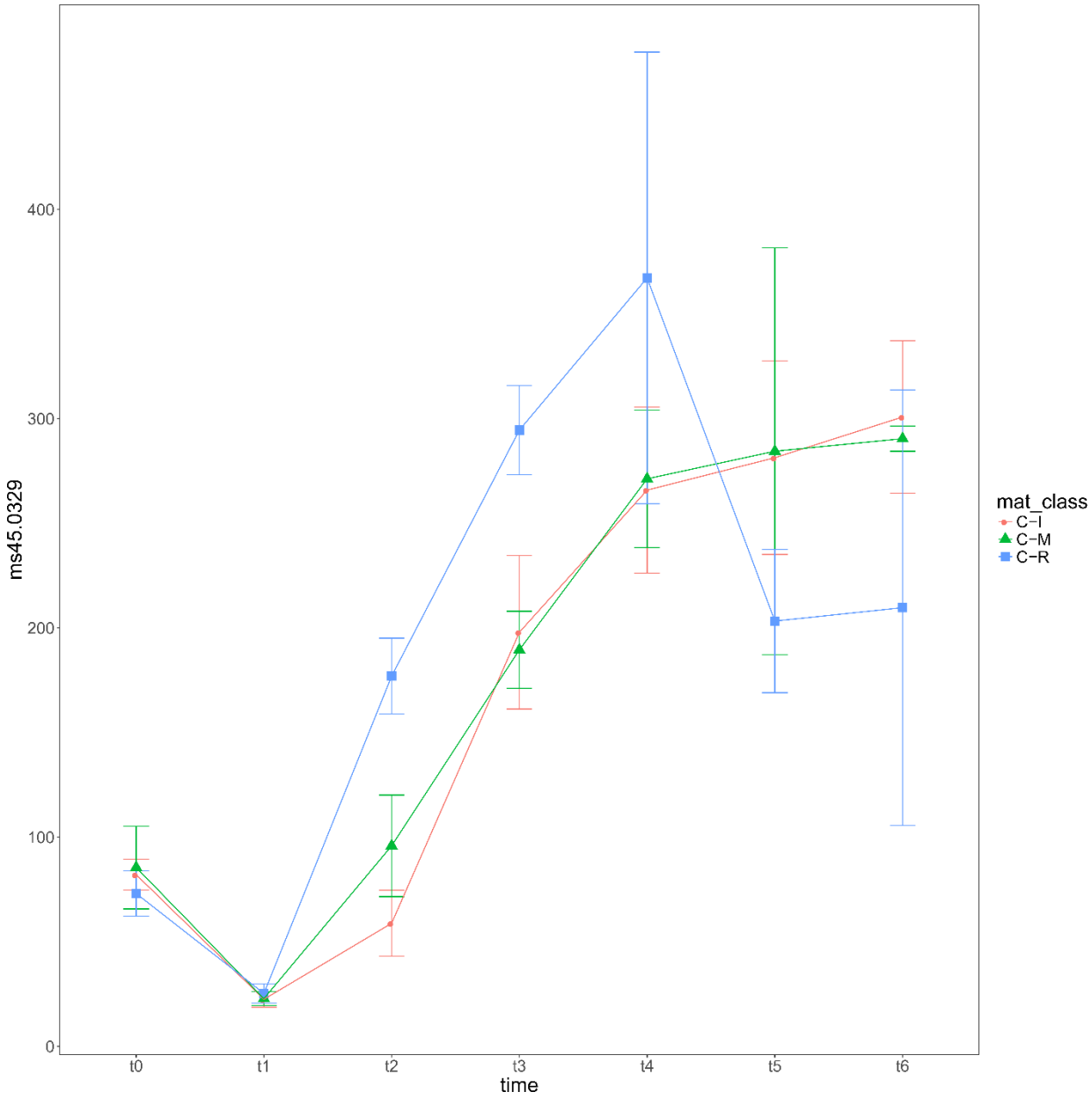

Figure 3.7.4: concentration (ppbv) trends of the mass *m/z* 45.0329 associated with Acetaldehyde, during shelf life days from fruit of three maturity classes (C-I: immature; C-M: mature; C-R: ripe). Bars represent standard error.

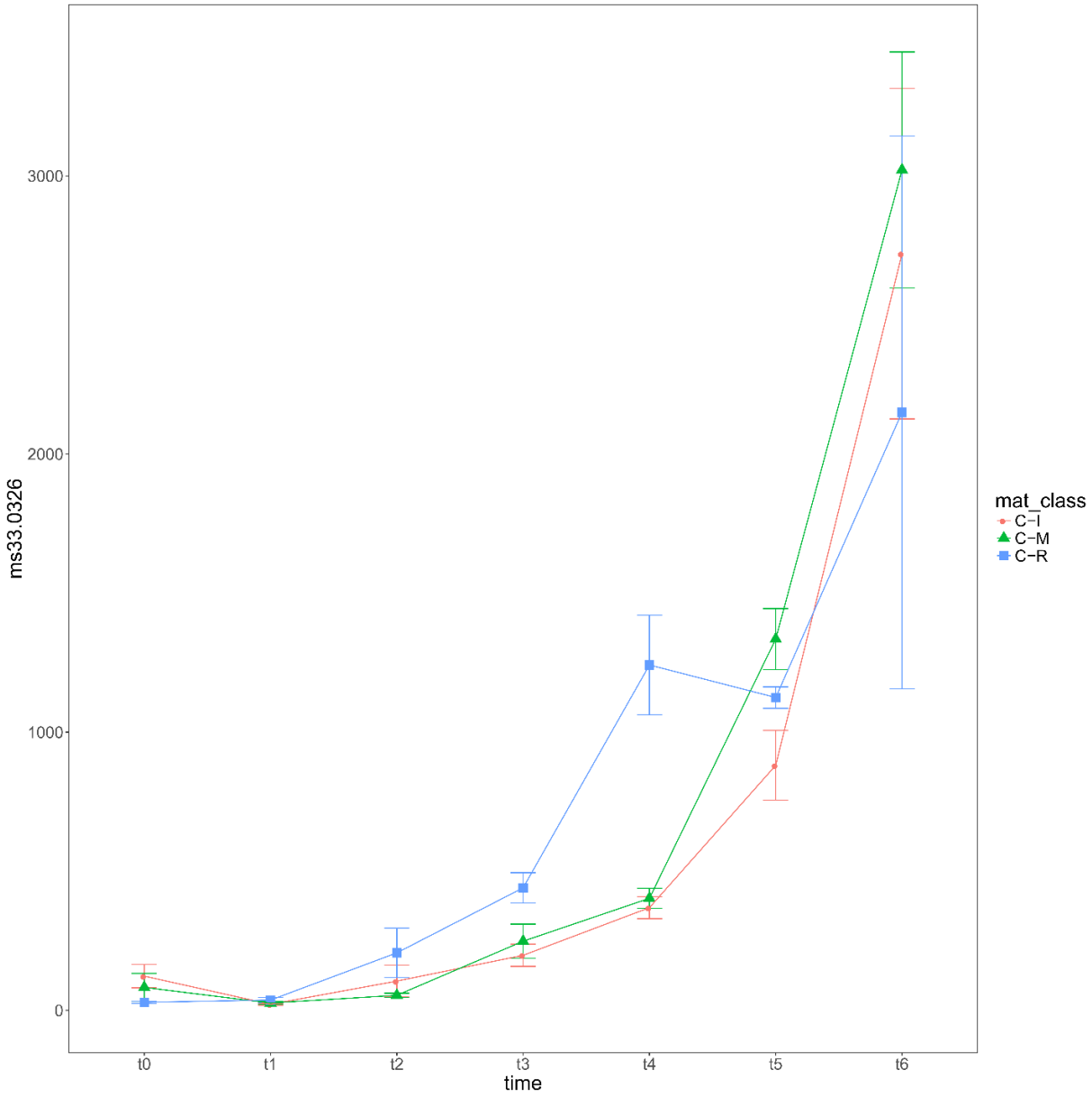

Figure 3.7.5: concentration (ppbv) trends of the mass *m/z* 33.0326 associated with Methanol, during shelf life days from fruit of three maturity classes (C-I: immature; C-M: mature; C-R: ripe). Bars represent standard error.

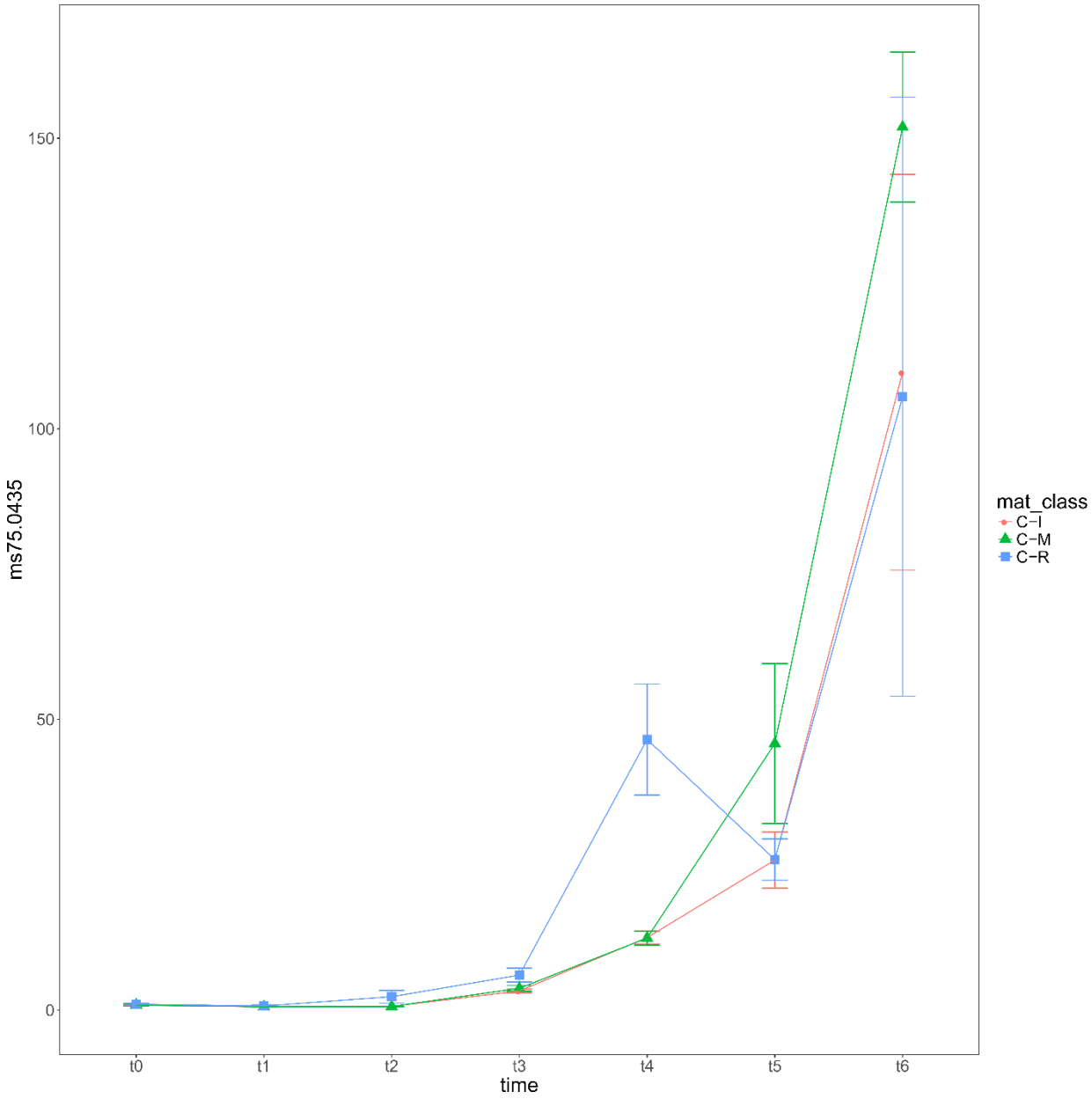

Figure 3.7.6: concentration (ppbv) trends of the mass *m/z* 75.0435 associated with Methyl acetate, during shelf life days from fruit of three maturity classes (C-I: immature; C-M: mature; C-R: ripe). Bars represent standard error.

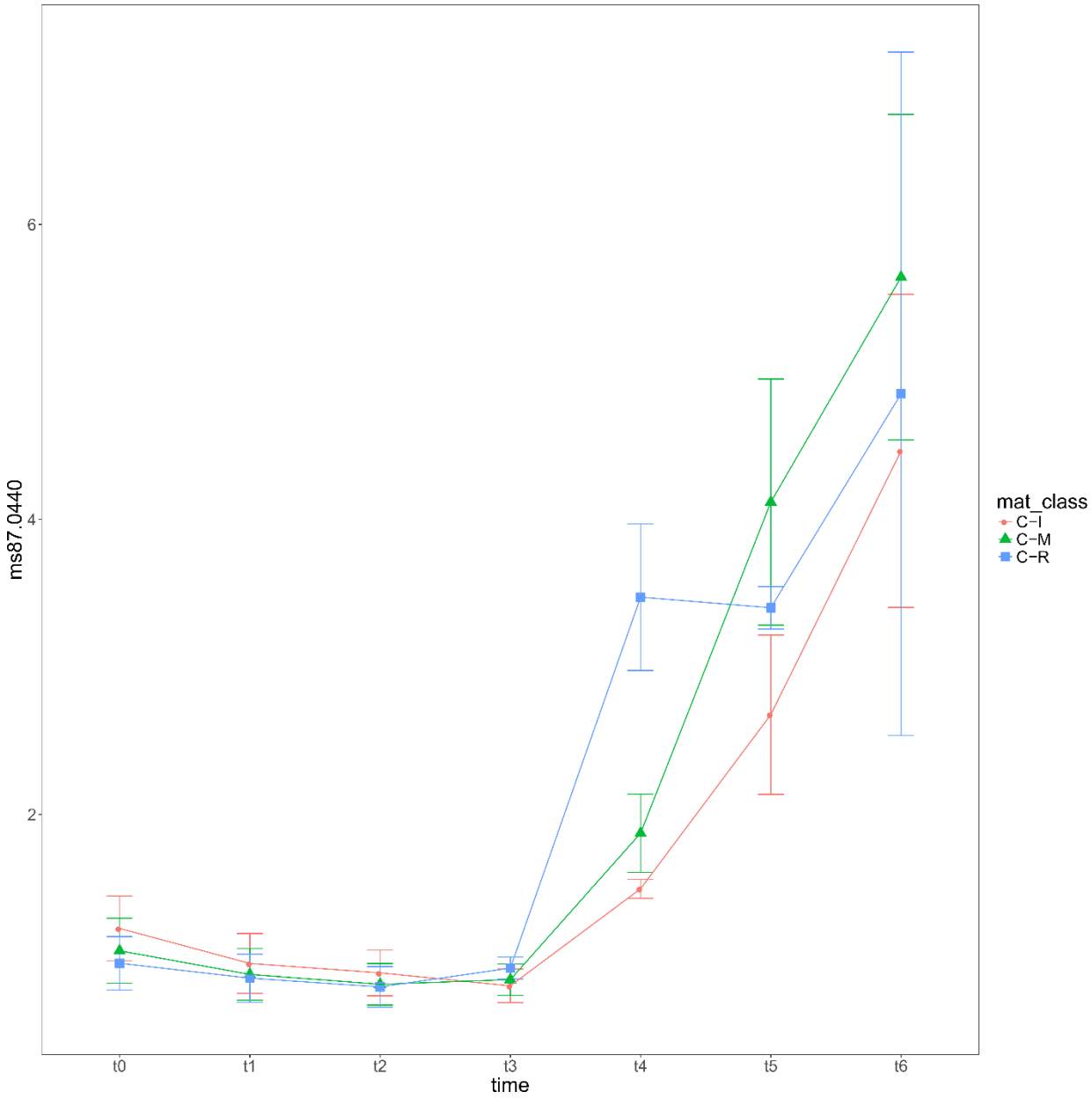

Figure 3.7.7: concentration (ppbv) trends of the mass *m/z* 87.0440 associated with Butyrolactone, during shelf life days from fruit of three maturity classes (C-I: immature; C-M: mature; C-R: ripe). Bars represent standard error.

4. Maturity stage at harvest and length of cold storage influence on flavour development in peach fruit

Abstract

Peach is one of the most appreciate temperate fruit worldwide. However, peach market is facing a constant decrease due to the poor fruit quality at consumption. Aroma is among the most important drivers of consumer's preference. The fruit ripening stage at harvest and the post-harvest management greatly affect aroma development. Nonetheless, these aspects have been often neglected in pre- and post-harvest management of peaches. The present work aimed at clarifying the influence of maturity at harvest on the evolution of peach aroma and quality during shelf-life after prolonged cold storage. August Flame peaches were harvested at three maturity stages, determined by the I_{AD} (index of absorption difference) and fruit ethylene emission. Fruit were stored for four weeks at 0 °C. Every week of cold storage was followed by 6 days simulated shelf-life (18 °C). During shelf-life fruit quality traits (firmness, soluble solids, acidity), ethylene and VOCs emission were monitored.

The different maturity classes presented at harvest clear differences of VOCs, ethylene, and quality traits, suggesting the segregation based on coupling the I_{AD} and ethylene emission was successful. Cold storage enhanced the aroma development of 'August Flame', primarily increasing the emission of esters and lactones associated with pleasant aroma. Cold storage also reduced the differences in aroma between the maturity classes. The role of ethylene, which is also influenced by cold storage, in regulating the VOCs emission is discussed.

Keywords: *Prunus persica*, cold storage, volatile compounds, aroma, $I_{AD,}$ fruit maturity

4.1 Introduction

Peach (*Prunus persica* L. Batsch) is an economically important crop, with a worldwide production over 22 million tonnes (FAOSTAT, 2014) featuring high nutritional attributes and pleasant flavour (Wang et al., 2006). However, peach consumption has been facing a constant drop due to the poor textural and flavour characteristics of some recently selected cultivars (Bruhn et al., 1991; Crisosto et al., 2006). The reduction of demand has not paired a reduction in fruit supply (Bianchi et al., 2017) which has caused a substantial surplus in production and a consequent drop in the revenue for producers. The development of new post-harvest strategies enabling to preserve, or even increase, fruit quality is among the main tools to overcome this problem. Peaches are a highly perishable fruit and the supply chain has primarily focused so far on prolonging their storability to allow long distance export (Martínez-Romero et al., 2000). Consequently, new cultivars, such as the stony-hard varieties, have been selected mainly for their better storage characteristics, more than for their quality attributes. Furthermore, the current cultivars are harvested before the optimal maturity, featuring high level of flesh firmness, low soluble solids concentration, strong acidity, and scarce aroma, regardless of the fact that fruit maturity at harvest greatly affect aroma at consumption (Infante et al., 2012). After harvest, fruit undergo storage at low temperature to be preserved for both local and export markets (Cano-Salazar et al., 2012). Long term cold storage may induce a substantial quality decay (Infante et al., 2008), often identified in drastic deterioration of the textural properties (Giné-Bordonaba et al., 2016; Zhang et al., 2010). However, no coherent information is available regarding the effects of the cold storage on volatile organic compounds (VOCs) emission of peach.

Aroma is considered by consumers a key component in determining peach quality (Bruhn, 1994; Lavilla et al., 2002; Wang et al., 2009) and depends on the interaction of several classes of VOCs, including esters, C6 aldehydes, terpenes, alcohols, and lactones (Eduardo et al., 2010; Wang et al., 2009). The latter are reported to be main contributors of the peach aroma (Horvat and Chapman, 1990; Jia et al., 2005; Lavilla et al., 2002; J. A. Robertson et al., 1990; Visai and Vanoli, 1997). During the progression of maturity, C6 aldehydes (mostly associated with “green” odour) generally decrease, whilst lactones, esters and terpenes increase (Aubert et al., 2003; Chapman et al., 1991; Engel et al., 1988b; Horvat and Chapman, 1990; Izumi et al., 2015; Lavilla et al., 2002). The progression towards senescence leads to the production of off-flavour alcohols (mainly ethanol and methanol) due to the insurgence of fermentative metabolism.

Ethylene represents a key hormone in regulating the synthesis of aroma-related volatiles, either modulating the activity of VOCs producing enzymes, such as alcohol acyltransferase (Defilippi et al., 2005) or lipoxygenase (Zhang et al., 2011), or determining the availability of the precursors involved

in VOCs biosynthesis (Defilippi et al., 2005). This research aimed at clarifying the relation between the harvest maturity and the evolution of fruit quality in postharvest, considering all the aspects influencing the organoleptic traits including the emission of VOCs responsible for the aroma.

Previous studies aimed at identifying the effects of cold storage on peach aroma volatiles (Cano-Salazar et al., 2013; Cano-Salazar et al., 2013; Ortiz et al., 2009; Zhang et al., 2011) but, to the best of our knowledge, only few focused on the relation between the maturity stage at harvest and the duration of cold storage (Aubert et al., 2003; Robertson et al., 1990).

In this study, maturity was determined as a relation between the index of absorption difference (I_{AD}) measured non-destructively with the DA-Meter (TR-Turoni, Forlì, Italy) and by the emission of ethylene (Bonora et al., 2013, 2014; Ziosi et al., 2008), which is a key hormone in modulating the ripening syndrome in climacteric fruit (Defilippi et al., 2005; Zhang et al., 2011) such as peach.

4.2 Materials and methods

4.2.1 Plant material

Fruit from 3 years old 'August Flame' peach trees (Prunus persica L. Batsch) grafted on 'Elberta' rootstock were used. Plants were grown at the Stone Fruit Field Laboratory at the Agriculture Victoria experimental research station located in Tatura, Victoria, Australia. Trees were managed following local standard agronomical practices for thinning, fertigation, pruning and pathogen control.

4.2.2 Maturity class definition

Peach maturity classes were identified by the combination of the I_{AD}, measured non-destructively with the DA-Meter (TR, Forli, Italy) and fruit ethylene emission. The I_{AD} represents an indirect measure of skin and flesh chlorophyll content and ranges from 2.0 to 0.0 where the lower values corresponds to a lower content of chlorophyll and therefore a more advanced fruit maturity (Ziosi et al., 2008).

Ethylene was measured in five replicates of a single fruit per each decimal value of I_{AD} from 1.7 to 0.0. Intact fruit were placed in a 1L air-tight glass jar and maintained at room temperature for one hour. Thereafter, 1 mL of the headspace was sampled and injected in the gas chromatograph (Shimadzu GC-14B, column Packed Alumina SS 80/100 180 cm; Shimadzu, Kyoto, Japan).

The combination of I_{AD} and ethylene measurement allowed to segregate fruit at harvest in three maturity classes: pre-climacteric (immature, C-I: I_{AD} 1.6 – 1.3); onset of climacteric (mature, C-M: I_{AD} 1.2 – 0.8) and climacteric (ripen, C-R: I_{AD} 0.7 – 0.0).

4.2.3 Experimental design and storage conditions

At harvest, 1820 peaches were collected and sorted by I_{AD} value into the previously described maturity classes (C-I, C-M and C-R). For each fruit, fresh weight was recorded before being placed in carton trays containing 20 fruit belonging to the same maturity class. Trays were stored at 0 °C for up to four weeks under normal atmosphere (95 % RH). Before storage (week 0) and at weekly intervals of cold storage, 120 fruit per each maturity class were taken from cold storage and kept at 18 °C for 6 days to simulate shelf-life. The evolution of quality traits and VOCs emission was assessed immediately after cold storage (day 0) and after 3 and 6 days of shelf life. For each assessment and maturity class, 5 fruit were individually sampled for VOCs emission and 35 were assessed for quality traits.

4.2.4 Fruit quality assessment

The standard fruit quality traits, such as flesh firmness (FF), soluble solids concentration (SSC) and titratable acidity (TA), were assessed by using standard methods (Bonora et al., 2013). FF was determined with a Food Texture Analyser (FTA Guss, South Africa), SSC with a digital refractometer (ATAGO, Tokyo, Japan) and TA was determined on 1 mL flesh juice (titration with 0.1 N NaOH to end point of pH 8.2) with a potentiometric titrator, Titrex Act2 with AS23 micro auto-sampler (Steroglass, Perugia, Italy).

4.2.5 Sample preparation for VOCs analysis

Five biological replicates per each maturity class, consisting of one individual fruit, were used for VOCs measurement. Peaches were peeled, sliced and immediately frozen in liquid nitrogen and ground with a commercial stainless-steel blender (Waring, USA). One gram of powdered frozen fruit was transferred into a 20-mL glass vial sealed with 18 mm PTFE/silicon septa (Agilent technologies). To each vial, 1 ml of antioxidant solution (400 g/l of sodium chloride, 5 g/L of ascorbic acid, and 5

g/L of citric acid) was added to prevent tissue oxidation (Farneti et al., 2014). Samples were spiked with 20 µL of 2-octanol (0.23 mg/L) used as internal standard, before placement in the autosampler.

4.2.6 Headspace SPME and GC-MS setup

For VOCs analysis, a Varian 3800 gas chromatograph equipped with a CTC Combi-PAL autosampler interfaced to a Varian 1200L mass spectrometer was used. VOCs extraction and analysis was performed according to Aprea et al. (2011) with the following modifications.

Samples were incubated at 40 °C and agitated at 250 rpm for 10 min prior to the introduction into the headspace of the SPME fiber (Supelco 57298-U). The SPME was exposed for 30 min to absorb the volatiles and then desorbed in the injector held at 250 °C for 10 min. Analytes were separated on a 30 m x 0.25 mm ID 0.25 µm DB-Wax capillary column (Agilent Technologies) operated with helium carrier gas at a constant flow of 1.2 mL min^{-1}. The GC oven temperature was held at 40 °C for 3 min, then programmed to 220 °C at 4 °C min^{-1}, then to 250 °C at 10 °C min^{-1} and held for 1 min. Electron impact ionisation mass spectra were collected from 40 to 500 amu with a 0.6 s scan time.

VOCs identification was performed by comparing each mass spectra and linear retention with the ones classified in NIST/EPA/NIH Mass Spectral Database (NIST 08, National Institute of Standards and Technology, Gaithersburg, MD, USA).

VOCs quantification was performed by rationing the peak area of each analyte and the internal standard (2-Octanol).

4.2.7 Statistical analysis

Data analysis was performed using Genstat 18.1 as ANOVA. VOCs were further evaluated with R statistical software version 3.2.3 using the external packages “MixOmics” for principal component analysis (PCA) and variable plots representation, “ggplot2” for line graphics and “FactoMineR” for multiple factor analysis (MFA).

4.3 Results

4.3.1 Maturity, cold storage and shelf life influence on fruit quality traits

At harvest assessment, significant differences of I_{AD} were present among the 3 maturity classes with lower values characterizing the C-R class, therefore indicating a more advanced maturity. Differences in firmness were recorded in C-R, with significant lower values at harvest in comparison with fruit of C-M and C-I (table 4.7.1). The content of soluble solids was also significantly higher in C-R in comparison with C-I. No differences in TA were found, at harvest, among the maturity classes (table 4.7.2).

Soluble solids concentration and titratable acidity were ratioed as SSC/TA (Aubert et al., 2003; Robertson et al., 1990) to predict the fruit sweetness perception. At harvest, no differences in SSC/TA ratio between the maturity classes were yet observed (table 4.7.2).

The effect of cold storage on quality traits was determined by the comparison of the data recorded at the first assessment after removal from each length of cold storage (day 0). Maturity classes remained significantly different in I_{AD} at every day 0 assessment, maintaining the pattern of segregation described at harvest (table 4.7.1). Cold storage induced a significant decrease over time of I_{AD} for C-I and C-M, whilst C-R maintained stable values after the second week. C-R had significant lower values of FF at all time tested after storage, whilst C-M and C-I started to present differences after 3 weeks of cold storage. The exposure to cold temperature caused a decrease in FF over time especially for C-R with a significant reduction within the first 2 weeks, then maintaining similar values for the third and fourth week of storage. Although C-R was consistently softer than other maturity classes, C-M and C-I became different after 3 weeks of storage, with C-I significantly firmer than C-M.

The content in soluble solids did not vary significantly due to cold storage exposure without displaying significant differences over time and between maturity classes (table 4.7.2). At week 4, significantly lower values of TA were detected for C-M and C-R in comparison with values at harvest. C-R had also significantly lower values of TA than that of C-I at week 4.

SSC/TA increased significantly at 3 and 4 weeks for C-M and C-R, however no significant difference was observed among maturity classes.

The combined effect of cold storage and shelf life temperatures had the strongest effect in regulating the quality traits. While significant differences were found in firmness between maturity classes at every evaluation immediately after cold storage, these differences were reduced once the fruit entered simulated shelf life. Higher levels of firmness were detected after 4 weeks of storage for C-I and C-M at the third day of shelf life (table 4.7.1). The trend of firmness reduction matched those of the I_{AD}

value with significant differences between maturity classes present just upon removal from cold storage.

SSC did not change significantly during shelf life, and did not show any significant differences among maturity classes (table 4.7.2). Higher SSC values were detected in C-M and C-R at the last day of shelf life after 4 weeks of cold storage.

SSC/TA was found to be significantly higher in C-R samples during the last day of every shelf life period in comparison with C-I.

Chilling injury symptoms, with altered flesh texture properties, including the complete loss of juice in the flesh, affected 20 % of the fruit during shelf-life starting after 3 weeks of storage. The number of affected fruit increased up to 70 % during shelf life after 4 weeks of cold storage. Indeed, it was not possible to collect data on SSC and TA for those fruit since they did not produce sufficient juice to perform the analysis (table 4.7.2).

4.3.2 Maturity, cold storage and shelf life influence on fruit ethylene production

Fruit belonging to C-R maturity class presented a higher ethylene emission than the ones in the other maturity classes at harvest, and after every exit from cold storage (table 4.7.1). During shelf life, fruit from C-R maturity class consistently had the highest ethylene emissions except for those stored for 3 weeks when fruit belonging to C-M maturity class showed the highest ethylene production rate in the last day of shelf life. In all maturity classes, the fruit removed after 2 weeks of storage showed during shelf life a notable increase in ethylene production.

4.3.3 Maturity, cold storage and shelf life influence on VOCs emission

Nineteen VOCs were identified and quantified through SPME-GC-MS analysis (table 4.7.3). A principal component analysis (PCA) was performed to analyses the influence of VOCs emission on the differentiation of the maturity classes at harvest (fig 4.8.1 A). The first two principal components accounted for 62 % of the total variance of the peach volatile profile.

According to the variable plot (fig 4.8.1 B) C-R was characterized by a higher level of lactones (i.e. δ-Decalactone, γ-Decalactone, γ-Octalactone, γ-Hexalactone and 6-Amyl-α-Pyrone), whilst C-M by a higher level of aldehydes (i.e. (E)-2-Hexenal, Pentanal and Furfural), alcohols (i.e. 2-Ethylhexan-1-ol, 1-Pentanol) and the ester (Z)-3-Hexenyl acetate. C-I showed higher levels of the aldehyde (Z)-3-Hexenal and the derived alcohol (Z)-3-Hexen-1-ol.

A second PCA analysis was performed to analyze the combined influence of the maturity classes at harvest and exposure to cold storage (fig 4.8.2 A). The first two principal components account for 69 % of the total variance and the PCA plot displays the VOCs emitted by the 3 maturity classes at each day 0 (beginning of shelf life), following the different periods of cold storage. VOCs emitted after 1 week of storage were comparable with those of week 0 (without cold storage) with a higher influence of lactones (i.e. δ-Decalactone, γ-Decalactone, 6-Amyl-α-Pyrone and 5-Ethyl-(5H)-Furan-2-one), the ester (Z)-3-Hexenyl acetate and the alcohol (Z)-3-Hexen-1-ol (fig 4.8.2 B).

Exposure to cold storage for longer periods led to a variation in VOCs emission. In fact. after 2 and 3 weeks of storage, the volatile profile showed a higher influence on aroma by aldehydes such as Pentanal and Furfural, and the alcohols 1-Pentanol and 2-Ethylhexan-1-ol. After 4 weeks, the predominant VOCs were the esters Ethyl Acetate, Methyl Acetate and Methyl Butanoate, the aldehydes (Z)-3-Hexenal, (E)-2-Hexenal and the lactone γ-Octalactone.

A third PCA was carried out to evaluate he effect of maturity, cold storage and shelf life on VOCs emission by the three maturity classes (fig 4.8.3 A). The PCA displays the emission of VOCs at the 6^{th} day of shelf-life following the different periods of cold storage.

The first two principal components accounted for 60 % of the total variance. Exposure to cold storage and shelf life determined a variation in the VOC profile of the three maturity classes (fig 4.8.3 B). VOCs emitted after 4 weeks of cold storage differed substantially from the ones produced in other time points, showing a lower influence of lactones and a higher one of esters in determining the overall fruit volatile profile.

Among maturity classes, C-R showed to maintain consistently higher levels of volatile lactones (i.e. δ-Decalactone, γ-Decalactone, γ-Octalactone, γ-Hexalactone and 6-Amyl-α-Pyrone) and esters (i.e. Ethyl and Methyl acetate), whilst C-I higher aldehydes (i.e. (Z)-3-Hexenal and Hexanal). C-M fruit always clustered in an intermediate position amongst C-R and C-I.

The evolution of fruit aroma in shelf life as a function of cold storage was analysed by grouping the main VOCs in four chemical classes (i.e. lactones, esters, alcohols, aldehydes), sharing similar odour descriptors. At the beginning of shelf life, these VOCs classes presented similar baseline levels for the 3 ripening classes, independently from the duration of cold storage. However, during the following 6 days in shelf life, VOCs emission rates differed depending on the maturity class.

Lactones were intensely emitted by C-R fruit even without cold storage, while C-M and C-I fruit required at least one week of cold storage to reach comparable emission rates (fig 4.8.4).

One week in cold storage maximised the ester production rate in shelf life for C-M and C-R samples, while 3 weeks were required by C-I fruit to achieve a level comparable to C-M (fig 4.8.5). However, C-R samples presented emission rates twice as high compared to the other classes.

Alcohols mostly showed decreasing trends for the shorter cold storage treatments, but this trend evolved with time, resulting in positive emission rates after the fourth week of cold storage. After one week of refrigeration, C-R samples presented consistently the highest emissions of alcohols (fig 4.8.6).

Aldehydes emission showed a decreasing trend during shelf life, with rates depending on the duration of cold storage and maturity class. Aldehydes reduced in two main phases, corresponding to 0 to 1 and 2 to 4 weeks of cold storage, in which C-M and C-R samples, showed the steepest decreases in aldehyde emission. C-I resulted in a positive production of aldehydes after 4 weeks of refrigeration (fig 4.8.7).

VOCs and ethylene emission, fruit chemical (SSC, TA) and physical parameters (flesh firmness) and overall maturity (I_{AD}) are the main descriptors of fruit organoleptic quality. The interaction of all these variables was evaluated by a multiple factor analysis (MFA) (fig 4.8.8). The variable plot depicted an inverse correlation with ethylene emission and the I_{AD}, fruit firmness and the emission of volatile aldehydes (i.e (E)-2-Hexenal, (Z)-3-Hexenal and Pentanal). On the other hand, the increase in ethylene emission directly correlated with that of volatile lactones (i.e. δ-Decalactone, γ-Decalactone, γ-Octalactone, γ-Hexalactone and 6-Amyl-α-Pyrone) and esters (i.e. Ethyl and Methyl acetates).

4.4 Discussion

4.4.1 Quality parameters are affected by maturity class and storage

In this trial, the loss of firmness was the most important change affected by maturity class, cold storage and shelf life temperature. Every period of cold storage, followed by 3 d of shelf life was enough to even out any differences of FF among maturity classes.

The “ready to eat” phase, identified with values of flesh firmness ranging from 8.8 to 13.2 N (Crisosto, 2002) was reached firstly by fruit in C-R class during the shelf life without cold storage. Fruit belonging to C-M and C-I classes reached the same value during shelf life, but after 1 week of cold storage. Longer exposure to cold storage determined an excessive drop in firmness during in shelf life. Higher values of firmness were detected for C-I and C-M fruit at the third day of shelf life after 4 weeks of cold storage. This could be explained by the insurgence of chilling injury which may have caused leatheriness of the fruit flesh (Cano-Salazar et al., 2012; Lurie and Crisosto, 2005; Luza et al., 1992).

This chilling injury symptoms in the last period of storage were unexpected, as the cultivar employed in this work is considered not susceptible to chilling disorder and the storage was performed at 0 °C in storage, which is the temperature generally recommended to maximize the peach storage potential (Crisosto et al., 1999; Lurie and Crisosto, 2005). In some cases, the chilling injury also affected the SSC and TA results, since juice was difficult to obtain from some samples.

As common for peaches, the content in soluble solids was not affected either by cold storage or shelf life, since soluble solids are generally increasing in shelf life as a consequence of fruit shrivelling (Cano-Salazar et al., 2012; Infante et al., 2008).

To better describe the perception of fruit sweetness, data were presented also as SSC/TA. During ripening this ratio generally increases as the titratable acidity in the fruit decreases (Aubert et al., 2003), therefore, with the progression of ripening, the perception of sweetness increases. The higher values of SSC/TA in the ripen class during shelf life may reflect a higher perception of sweetness of this fruit at consumption (Crisosto and Crisosto, 2005).

4.4.2 Evolution of VOCs profiles in shelf life

The analysis of VOCs confirmed the emission of some of the main compounds contributing to the peach aroma (Cano-Salazar et al., 2012; Cano-Salazar et al., 2013; Wang et al., 2009).

At harvest, the higher quantity of lactones emitted by the ripen fruit (C-R), reflected a more pleasant aroma in comparison with the other maturity classes (C-M and C-I). In fact, lactones are commonly described as main VOCs determining the peach aroma and are associated with pleasant and fruity notes (Rizzolo et al., 2006; Zhang et al., 2011).

Fruit belonging to the immature class (C-I) were characterized, at harvest, by a higher abundance of (Z)-3-hexen-1-ol and (Z)-3-Hexenal, having respectively “grassy-green” (PubChem Compound Database; CID=5281167) and “green” (The Good Scents Company Information System) odor properties,

The influence of cold storage length on VOCs emission by fruit belonging to the different maturity classes was evaluated by measuring VOCs emission at day 0 of shelf life.

Cold storage induced a variation in the volatile profile of the three maturity classes especially by reducing the volatile lactones (i.e. δ-Decalactone, γ-Decalactone, 6-Amyl-α-Pyrone and 5-ethyl-(5H)-furan-2-one). This observation may reflect a negative effect of prolonged cold storage on this group of volatiles (Robertson et al., 1990). However, with the progression of shelf life, the emission volatile lactones was restored to level higher than that emitted by C-R during shelf life without cold

storage. The maturity classes reached comparable emission rates of lactones during shelf life after two weeks of cold storage, suggesting similar fruity odor characteristics.

The higher rate of emission of esters by the ripen fruit (C-R) may be associated with a more pleasant aroma than that of immature (C-I) and commercially mature (C-M) fruit. In fact, esters contribute to the the fragrant, fruity and apple-like odors of the fruit (PubChem Compound Database; CID=6584, 8857 and 12180).

Among the alcohols detected, (Z)-3-Hexen-1-ol was the most abundant. This compound is characterized by a "strong green" (PubChem Compound Database; CID=5281167) odor, whilst 2-Ethylhexan-1-ol and 1-Pentanol with, "oily", "sweet" (PubChem Compound Database; CID=7720.) and "mild" (PubChem Compound Database; CID=6276) odors, respectively. (Z)-3-Hexen-1-ol emission decreased with the progression of shelf life determining the overall decreasing trend of alcohols, since 2-Ethylhexan-1-ol and 1-Pentanol showed an increasing trend.

In our experimental conditions, the emission of (Z)-3-Hexen-1-ol and (Z)-3-Hexenal, which show similar effects on the perceived aroma, were associated (fig 4.8.3).

Aldehydes are known for their contribution to "green" notes of perceived fruit aromas (Engel et al., 1988; Sánchez et al., 2012). These compounds were the predominant ones in the VOCs profile of 'August Flame' peaches. The progression of maturity led to a general decrease of the aldehydes.

The unexpected increasing trends of alcohols and aldehydes after week 4 of cold storage was possibly caused by the chilling injury.

4.4.3 Control of fruit ripening and quality through cold storage

The results presented in this work may allow to tailor the length of cold storage on the fruit maturity at harvest to maximise the quality traits development during shelf life. The duration of cold storage affected ethylene release, and for each maturity class the highest ethylene emission rates were associated with the maximum SSC/TA ratios. In addition, our results suggest that the rise in ethylene emission, induced by cold storage, may enhance the emission of volatile lactones by the fruit.

In fact, ethylene has shown to be directly associated with the emission of volatile lactones as displayed in the multiple factor analysis (fig 4.8.8), suggesting its possible role in modulating the synthesis of lactones in a similar manner among the three maturity classes. These results are supported by the findings of Zhang et al., (2011) who also reported a direct association between ethylene emission and fruity volatiles (esters and lactones) by peaches in shelf life after cold storage.

Volatile esters (i.e. Methyl acetate, Ethyl acetate and Methyl Butanoate) were not detected at harvest, and became detectable in increasing concentrations with the progression of shelf life after cold

storage. This could also be linked with higher emission of ethylene after cold storage. Defilippi et al., (2005) reports low volatile esters emission in the 'Greensleeves' apples under ethylene suppression conditions and the consequent recovery after exposure to exogenous ethylene. Furthermore, Cano-Salazar et al. (2012) reported that 20 days of cold storage improved esters emission of 'Early Rich' and 'Elegant Lady' during shelf life.

In contrast, C6 aldehydes and alcohols are generally inversely proportional to ethylene emission. These compounds have often been linked to mechanical damage to plant tissues, as a part of the signalling network resulting in the activation of plant defences (Matsui, 2006). Thus, an incipient cold stress, possibly impairing overall fruit quality, may be postulated upon their appearance.

4.5 Conclusions

This work demonstrated the possibility to manipulate peach flavour by tailoring the exposure to cold storage on the maturity stage at harvest assessed non-destructively. In general, cold storage enhanced the overall fruity notes of 'August Flame' peach aroma and contributed to the reduction of the differences between the maturity classes. Thus, cold storage may be improving peach flavour of those fruit harvested too immature (i.e. before physiological maturity). Nonetheless, the maturity at harvest remain the key component in determining the evolution of peach quality, as C-R fruit were characterized by better flavour properties even prior cold storage.

Cold storage also enhanced the reduction in average flesh firmness value, which represent a limit to commercialization as fruit became overly soft after 3 days at room temperature. Soft fruit on the market shelf may also incur in damages on the skin and flesh caused by fruit handling. Finally, prolonged cold storage (4 weeks) induced severe chilling injury, which represent a conspicuous storage impediment, especially if the cultivar is exported to far-off destinations.

A quality-oriented storage strategy should account for peach ripening stage at harvest, to influence the aroma bouquet by a timely cold storage-induced ethylene release.

4.6 References

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4.7 Tables

Table 4.7.1 Maturity and quality characteristics of 'August Flame' peaches at harvest (week 0) and after storage (0 °C) for up to 4 weeks and shelf life (ambient, 18 °C) for up to 6 d for C-I (immature), C-M (mature) and C-R (ripen) fruit. LSD indicates significant differences for both maturity classes and days of assessment.

Storage (weeks)	Shelf life (days)	I_{AD}			Firmness (N)			Ethylene (nL L^{-1} h^{-1} g^{-1} FW)		
		C-I	C-M	C-R	C-I	C-M	C-R	C-I	C-M	C-R
0	0	1.28	0.95	0.39	68.4	64.6	54.3	0.10A	0.39A	2.76B
	3	0.8	0.53	0.17	56.1	41.1	30.9	1.03	1.70	2.80
	6	0.34	0.23	0.06	33.2	29.8	8.7	0.85A	2.34A	15.76B
1	0	1.23	0.74	0.33	57.2	54.6	39.4	0.72A	1.60A	3.88B
	3	0.47	0.11	0.09	23.1	13.4	10.2	4.43A	17.68A	38.62B
	6	0.11	0.08	0.12	8.6	7	5.3	33.15	32.08	65.93
2	0	1.04	0.67	0.25	54.5	53.9	36.9	8.84	8.74	13.40
	3	0.12	0.08	0.08	8.7	8.4	7.8	13.53A	13.76A	57.80B
	6	0.06	0.06	0.09	5.8	6.1	6	56.48	79.49	95.20
3	0	0.9	0.56	0.26	43.3	36.1	25.9	4.95	8.41	10.27
	3	0.12	0.07	0.07	8.8	8.4	6.5	25.73A	54.99B	33.37AB
	6	0.06	0.04	0.1	4.6	4.9	3.9	54.40	113.63	84.19
4	0	0.83	0.52	0.25	44.8	35.6	21.8	0.73A	2.12A	6.10B
	3	0.27	0.12	0.1	28.8	12.5	7.8	5.55	11.62	35.19
	6	0.18	0.13	0.11	6.3	7.4	4.4	18.13A	31.31AB	56.26B
	LSD	0.07 (P<0.001)			6.4 (P<0.001)			(P<0.05)		

Table 4.7.2 Quality characteristics of 'August Flame' peaches at harvest (week 0) and after storage (0 °C) for up to 4 weeks and shelf life (ambient, 18 °C) for up to 6 d for C-I (immature), C-M (mature) and C-R (ripen) fruit. LSD indicates significant differences for both maturity classes and days of assessment.

Storage (weeks)	Shelf life (days)	SSC (%)			TA (g/L malic acid)			SSC/TA ratio		
		C-I	C-M	C-R	C-I	C-M	C-R	C-I	C-M	C-R
0	0	15.2	16	17.3	10.8	11	9.9	1.38	1.4	1.53
	3	15.8	16.3	16.2	11	11.2	8.8	1.39	1.39	1.81
	6	15.8	15.9	17.2	10.2	9.4	7.7	1.47	1.74	2.08
1	0	15.3	16	16.6	8.5	9.4	8.6	1.75	1.64	1.73
	3	15.1	15.8	16	7.8	8.4	7.6	1.87	1.81	1.78
	6	16.2	16.3	16.7	9.2	7.5	6.7	1.68	1.96	2.23
2	0	15.9	16.4	17	10.5	9.3	8.8	1.5	1.7	1.87
	3	16	16.2	17.2	11.2	9.1	8	1.47	1.73	2.09
	6	16	16.8	17.9	7.9	7.4	6.1	1.97	2.22	2.58
3	0	15.6	16.3	17.5	7.9	7.8	9.2	1.9	2.01	2.22
	3	16	16	16.9	NA	6.1	4.9	NA	2.64	3.71
	6	16.9	16.7	15.9	7.2	6.4	4.4	2.2	2.56	3.26
4	0	16	16.2	17	7.7	5.7	4.5	2.13	2.76	2.56
	3	15.7	16.1	17.8	NA	NA	NA	NA	NA	NA
	6	NA	19.6	20.8	NA	NA	NA	NA	NA	NA
	LSD	2.0 (P=0.014)			3.0 (P=0.096)			0.51 (P=0.150)		

NA = samples could not be obtained due to chilling injury

Table 4.7.3 Classes of volatile organic compounds (VOCs) most detected by SPME/GC-MS analysis of peaches at different maturity stages. The identification code (ID) for each compound, the chemical formula and the retention time (RT) are reported.

Name	***ID***	***Formula***	***RT***
Aldehydes			
(E)-2-Hexenal	Ald_1	$C_6H_{10}O$	10.34
(E,E)-2,4-hexadienal	Ald_2	C_6H_8O	16.39
(Z)-3-Hexenal	Ald_3	$C_6H_{10}O$	8.05
Hexanal	Ald_4	$C_6H_{12}O$	6.23
Pentanal	Ald_5	$C_5H_{10}O$	3.87
Furfural	Ald_6	$C_5H_4O_2$	18.43
Alcohols			
(Z)-3-hexen-1-ol	Alc_1	$C_6H_{12}O$	16.00
2-Ethylhexan-1-ol	Alc_2	$C_8H_{18}O_2$	19.52
1-Pentanol	Alc_3	$C_5H_{12}O$	11.99
Esters			
Ethyl acetate	Est_1	$C_4H_8O_2$	2.55
Methyl acetate	Est_2	$C_3H_6O_2$	2.06
Methyl butanoate	Est_3	$C_5H_{10}O_2$	24.63
(Z)-3-hexenyl acetate	Est_4	$C_8H_{14}O_2$	14.07
Lactones			
6-Amyl-α-pyrone	Lac_1	$C_{10}H_{14}O_2$	37.31
γ-Decalactone	Lac_2	$C_{10}H_{18}O_2$	37.61
δ-Decalactone	Lac_3	$C_{10}H_{18}O_2$	36.48
γ-Hexalactone	Lac_4	$C_6H_{10}O_2$	25.24
γ-Octalactone	Lac_5	$C_8H_{14}O_2$	30.86
5-ethyl-(5H)-furan-2-one	Lac_6	$C_6H_8O_2$	22.34

4.8 Figures

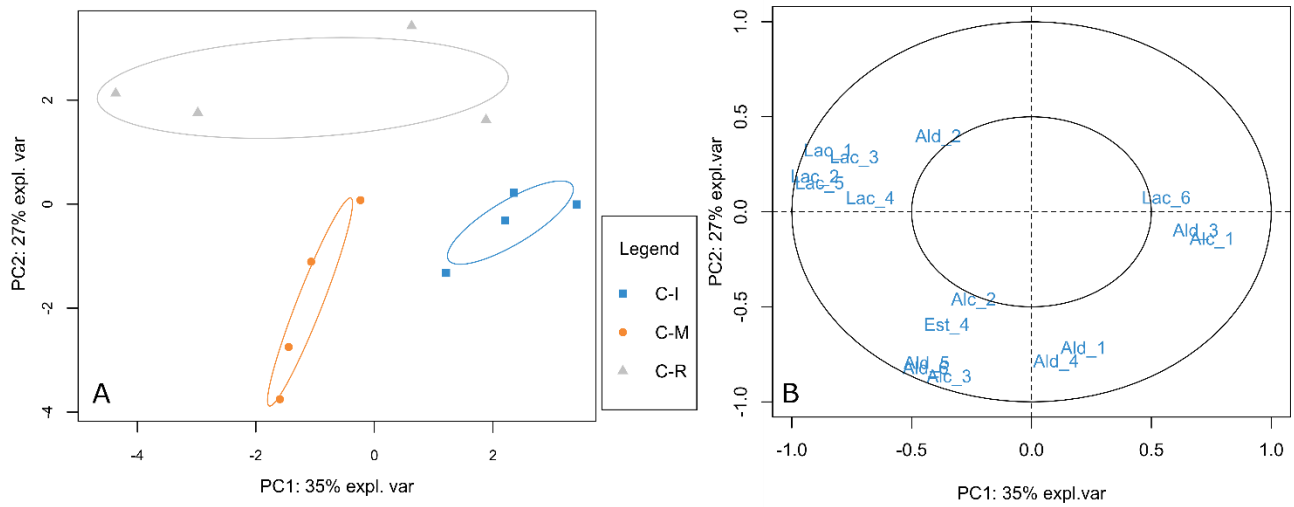

Figure 4.8.1 (A) Two-dimensional principal component analysis (PCA), elaborated using the VOCs quantified by the GC-MS at harvest for the three maturity classes (C-I: immature; C-M: mature; C-R: ripen. The ellipse confidence level is set at 0.5 (50 % region). **(B)** Variable plot projecting the variables (VOCs) identified at harvest. VOCs ID is reported in Table 1. Two circumferences of radius 1 and 0.5 are plotted to report the correlation structure of the variables.

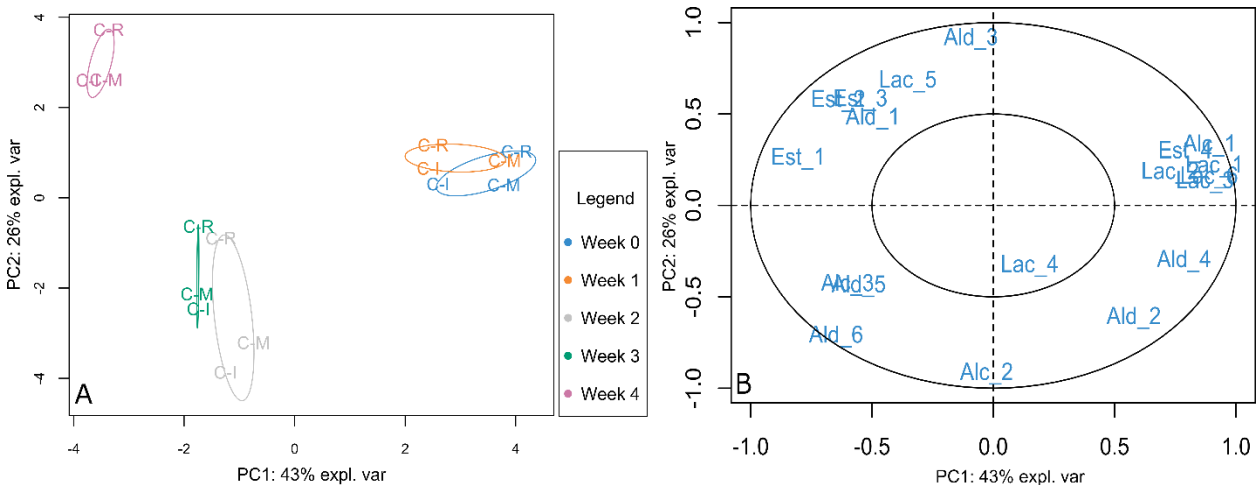

Figure 4.8.2 (A) Two-dimensional principal component analysis (PCA), elaborated using the VOCs quantified by the GC-MS at day 0 of simulated shelf life for the three maturity classes (C-I: immature; C-M: mature; C-R: ripen. The ellipse confidence level is set at 0.5 (50 % region). **(B)** Variable plot projecting the variables (VOCs) identified at day 0 of simulated shelf life. VOCs ID is reported in Table 1. Two circumferences of radius 1 and 0.5 are plotted to report the correlation structure of the variables

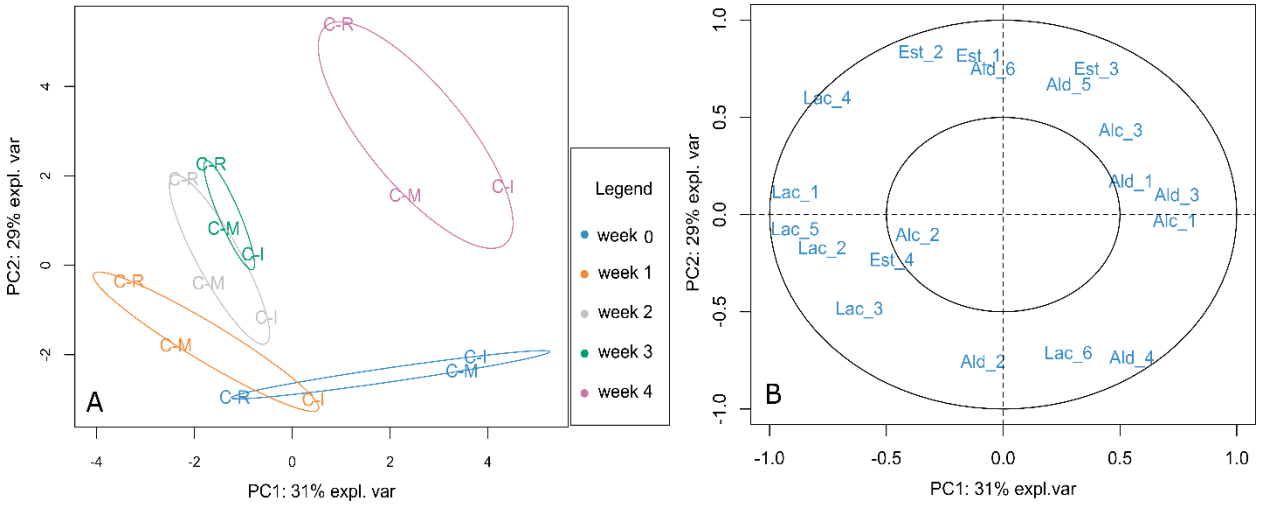

Figure 4.8.3 (A) Two-dimensional principal component analysis (PCA), elaborated using the VOCs quantified by the GC-MS at day 6 of simulated shelf life for the three maturity classes (C-I: immature; C-M: mature; C-R: ripen. The ellipse confidence level is set at 0.5 (50 % region). **(B)** Variable plot projecting the variables (VOCs) identified at day 6 of simulated shelf life. VOCs ID is reported in Table 1. Two circumferences of radius 1 and 0.5 are plotted to report the correlation structure of the variables.

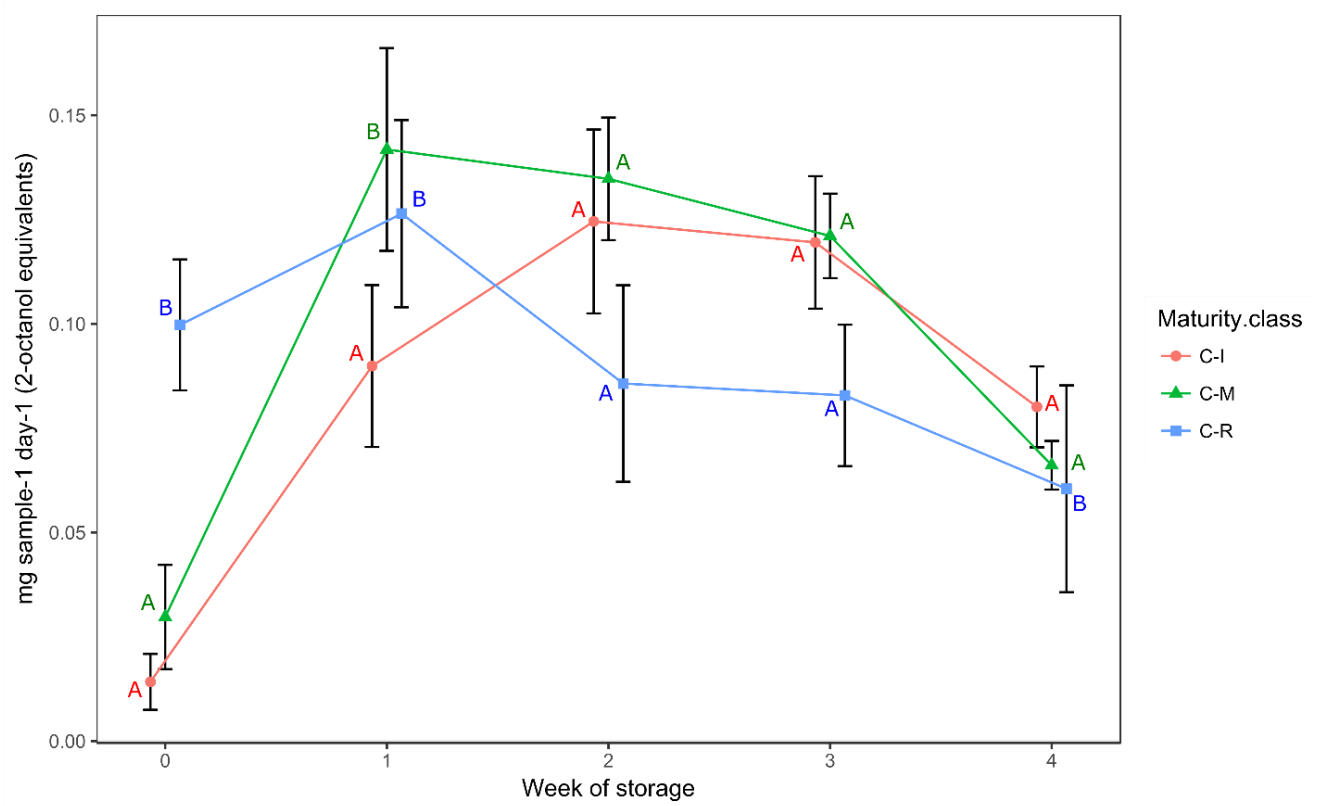

Figure 4.8.4 Daily emission rates of total lactones, measured during shelf life from fruit in three maturity classes (C-I: immature; C-M: mature; C-R: ripen) as a function of cold storage duration. For each time point, data marked with different letters are significantly different ($P < 0.05$) according to ANCOVA followed by Fisher's LSD test. Bars represent ± SE

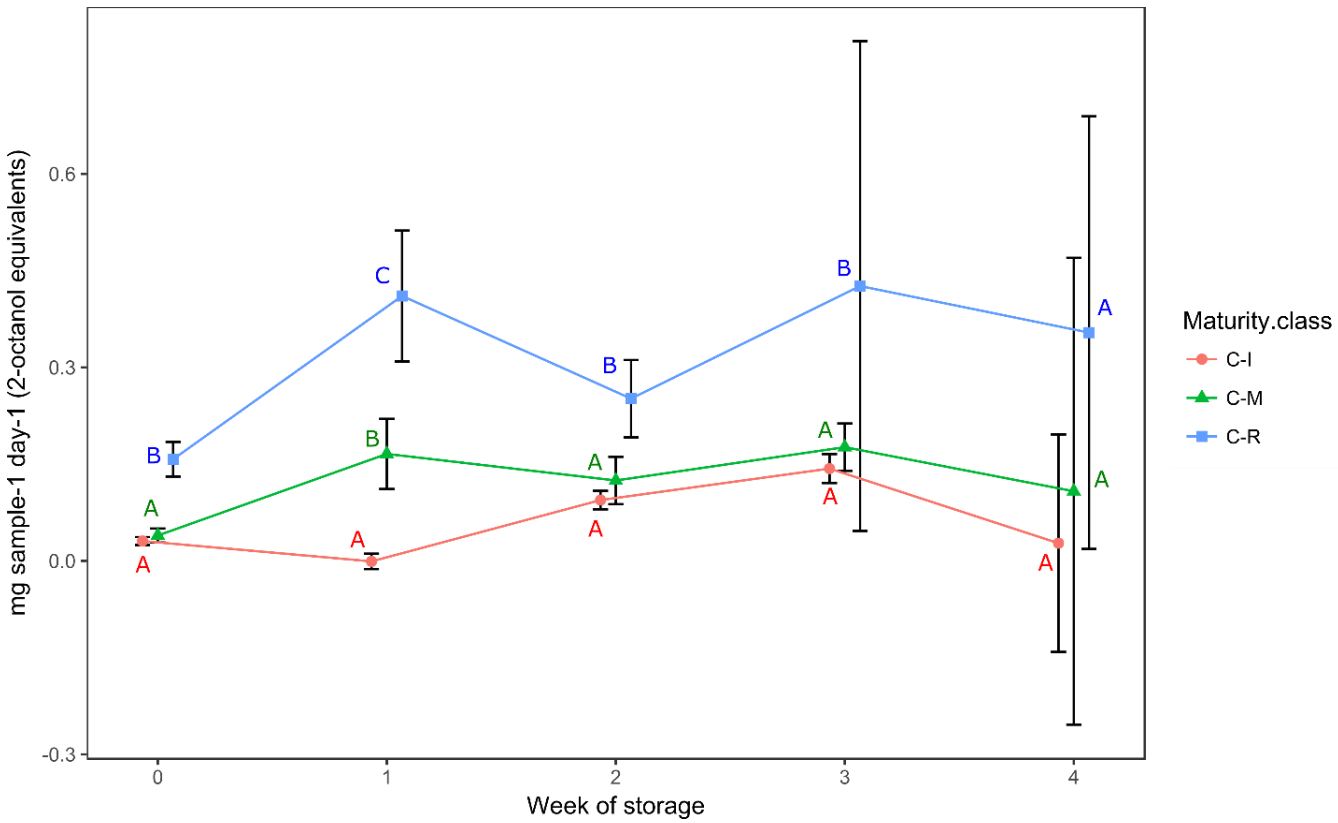

Figure 4.8.5 Daily emission rates of total esters, measured during shelf life from fruit in three maturity classes (C-I: immature; C-M: mature; C-R: ripen) as a function of cold storage duration. For each time point, data marked with different letters are significantly different ($P < 0.05$) according to ANCOVA followed by Fisher's LSD test. Bars represent ± SE

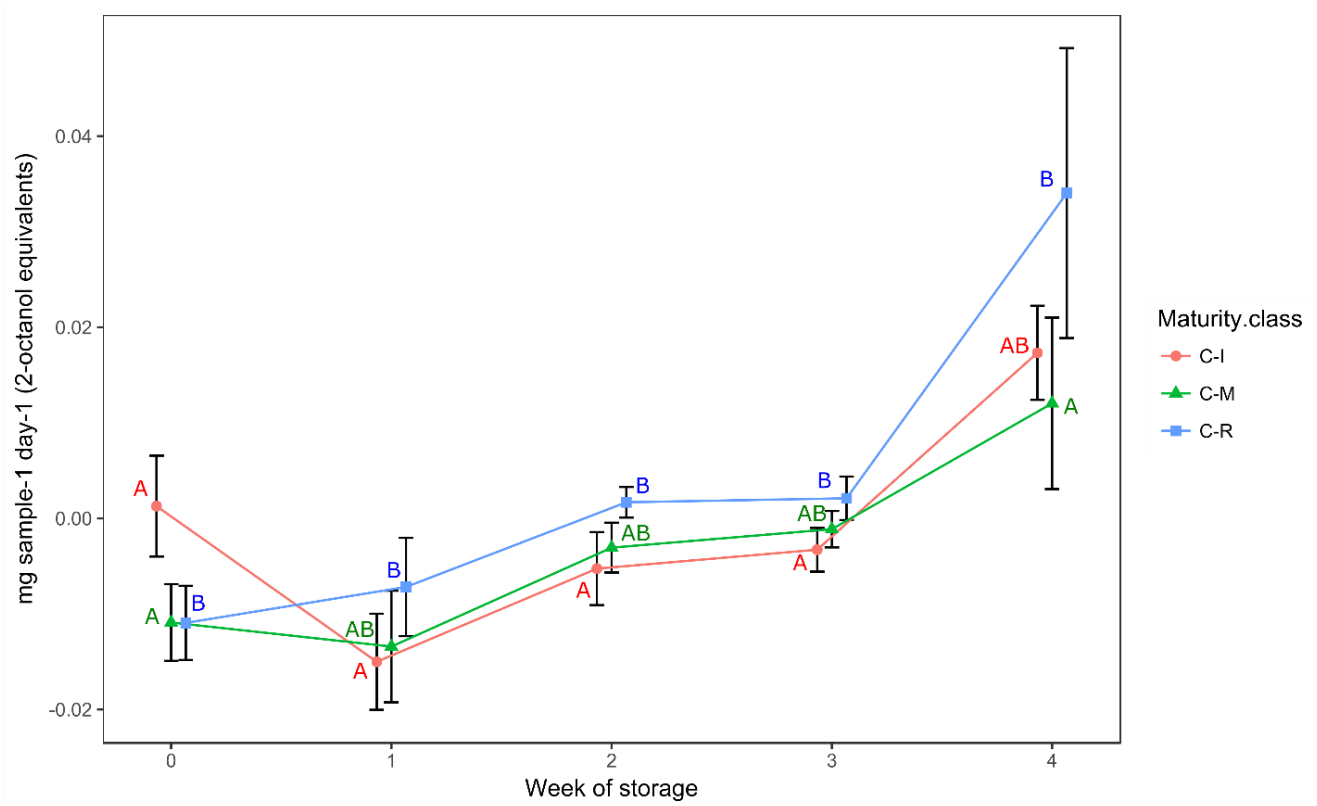

Figure 4.8.6 Daily emission rates of total alcohols, measured during shelf life from fruit in three maturity classes (C-I: immature; C-M: mature; C-R: ripen) as a function of cold storage duration. For each time point, data marked with different letters are significantly different ($P < 0.05$) according to ANCOVA followed by Fisher's LSD test. Bars represent ± SE

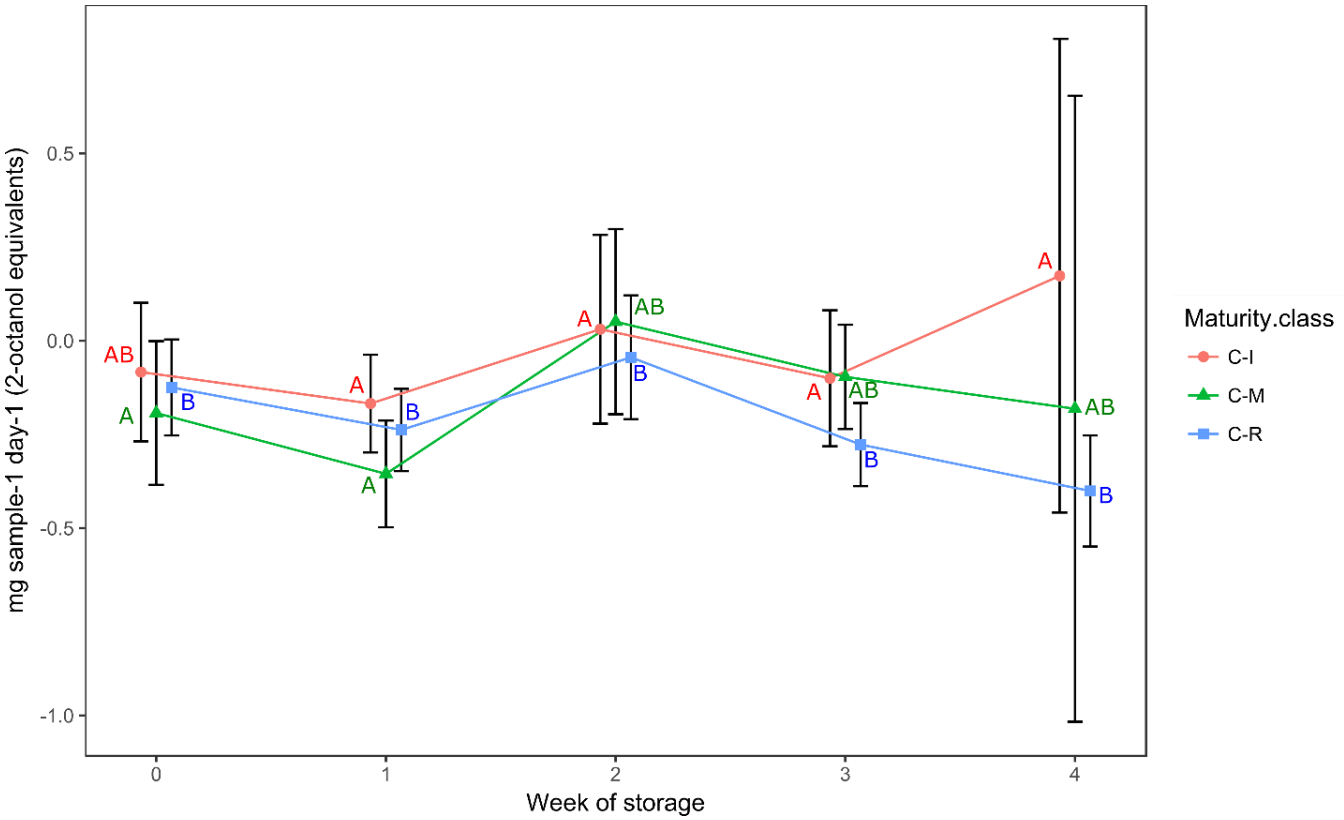

Figure 4.8.7 Daily emission rates of total aldehydes, measured during shelf life from fruit in three maturity classes (C-I: immature; C-M: mature; C-R: ripen) as a function of cold storage duration. For each time point, data marked with different letters are significantly different ($P < 0.05$) according to ANCOVA followed by Fisher's LSD test. Bars represent ± SE

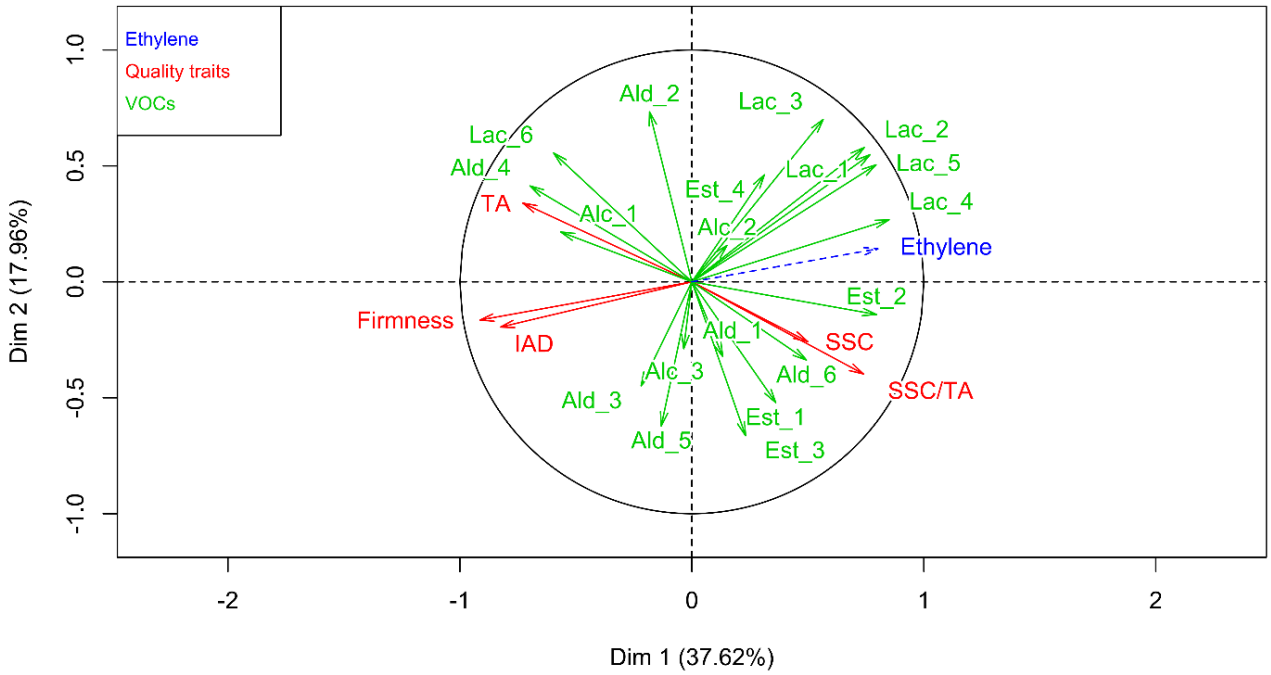

Figure 4.8.8 Multiple Factor Analysis. Variable plot projecting the different groups of variables (VOCs, quality traits, ethylene). VOCs ID is reported in Table 1.

5. Effect of ripening and storage on VOCs development of fresh-cut nectarines

Abstract

The offer of fresh-cut peaches and nectarines is a valid alternative for stone fruit commercialization, and matches the increasing demand of ready-to-eat product on the market.

However, achieving high quality fresh-cut peaches and nectarines represents a technological challenge for the industry. Fruit aroma has a great impact on the product marketability, and fresh-cut fruit is highly perishable. Thus, there is a need to investigate the development of this quality trait after processing.

The aim of this study was to explore the effect of fruit processing (slicing, coring and packing) on VOCs synthesis and evolution over time in relation to different maturity stages of the fruit at harvest. To do so, 3 cultivars of nectarines ('August Red', 'Western Red' and 'Morsiani 60') were selected and minimally processed in an industrial line and stored for 5 days at 5°C. The aroma evolution was assessed every day in both intact and processed nectarines by PTR-ToF-MS and SPME-GC-MS. Chromatic parameters were monitored to determine the degree of surface browning, which is a major impediment for the commercialization of the fresh-cut produce.

Results suggested that the VOCs profile of fresh-cut nectarines differed substantially from the intact fruit immediately after processing. Slicing induced an increase of VOCs composing the aroma profile of the nectarines (i.e. esters, alcohols, terpenes, lactones). In addition, such variation was cultivar-dependent.

Surface browning did not occur during storage in any of the cultivars tested. Storage induced a significant evolution of the VOCs profile of processed nectarines, inducing an increase of pleasant aromas (such as esters and some terpenes), as well as a conspicuous increase of off-flavour volatiles such as ethanol and acetaldehyde deriving from fermentations.

The utilization of smart labels to indicate the excessive presence of off-flavour volatiles in the fruit packaging is discussed.

Keywords: Aroma, SPME-GC-MS, minimal processing, cold storage, PTR-ToF-MS

5.1 Introduction

The offer of fresh-cut fruit on the market has increased over the last years (Denoya et al., 2015), in response to the increasing demand of convenience and ready-to-use products more aligned with the modern life-style (Denoya et al., 2017). On the other hand, the consumption of peaches and nectarines has faced a significant decrease over the last decades, in response of the poor flavour characteristics perceived by consumers (Bruhn et al., 1991). Thus, fresh-cutting may represent a valuable alternative to improve peach and nectarine marketability. However, achieving high quality fresh-cut peaches and nectarines still represents a technological challenge for the industry.

The fresh-cut process consists in several mechanical operations, such as cutting, slicing, and coring, that are critical to determine the potential shelf life of the fruit (Soliva-Fortuny and Martín-Belloso, 2003). These operations induce the disruption of the cell compartmentalization bringing enzymes and substrates in contact. Furthermore, wound stress, caused by cutting and slicing, may accelerate the progression of fruit maturity and senescence, caused by an increase of ethylene emission (Varoquaux and Wiley, 2017). Possible consequences are the increased fruit perishability, flesh softening and surface browning (Artés and Gómez, 2006). The latter aspect was shown as one of the major impediment for the successful commercialization of the fresh-cut fruit (Eissa et al., 2006; Toivonen and Delaquis, 2006).

Several studies were performed to extend shelf life of processed peaches and nectarines. These studies were mostly focused on the suitability for processing of different cultivars, heat treatments (Koukounaras et al., 2008), the application of edible coatings (Pizato et al., 2013), the inactivation of enzymatic activities by high pressure processing (Denoya et al., 2016, 2015), low temperature storage, and modified atmosphere packaging (MAP) (Koukounaras et al., 2008).

However, no thorough investigation has been conducted so far on some of the factors determining the consumer acceptability of the fresh-cut products, such as the development of the volatile aromas and the insurgence of off-flavour odours during storage.

The aroma of peaches and nectarines is considered a key component in determining consumer satisfaction (Bruhn, 1994; Lavilla et al., 2002; Wang et al., 2009), and relies on the complex interaction of several classes of VOCs, including esters, C6 aldehydes, terpenes, alcohols, and lactones (Eduardo et al., 2010; Horvat and Chapman, 1990; Jia et al., 2005; Lavilla et al., 2002; J. A. Robertson et al., 1990; Visai and Vanoli, 1997; Wang et al., 2009). The latter molecular class is reported to include some of the major contributors of the peach and nectarine aroma (Horvat and Chapman, 1990; Jia et al., 2005; Lavilla et al., 2002; J. A. Robertson et al., 1990; Visai and Vanoli, 1997). However, peach and nectarine aroma may deteriorate with the insurgence of off-flavour

compounds, mainly induced by fermentative metabolism. Packed fruit may easily ferment when the level of O_2 is below an optimal concentration (Solomos, 1994), thus inducing the synthesis of ethanol and acetic acid. Therefore, a complete characterization of the VOCs dynamics is important to enhance the perceived quality of fresh-cut peaches and nectarines.

In the present study, the evolution of VOCs from fresh cut nectarines during refrigerated storage was investigated by PTR-ToF-MS and SPME-GC-MS technologies. The aim was to explore the effect of fruit processing (slicing, coring and packing) on VOCs development in postharvest according to the maturity stage of the fruit at harvest.

5.2 Material and methods

5.2.1 Plant material and maturity stage definition

Nectarines (*Prunus persica*, L. Batch) from three cultivars - 'Western Red' (WR), 'August Red' (AR) and 'Morsiani 60' (M60) - were collected from a commercial packhouse located in Faenza, Emilia Romagna, Italy.

Fruit of each cultivar was sorted in homogenous maturity classes (table 5.6.1), analytically identified by DA-Meter (TR, Forli, Italy), a VIS-spectrometer which allows the non-destructive monitoring of the fruit maturation by measuring chlorophyll-a content in the fruit flesh (Ziosi et al., 2008). Maturity stages were expressed as Index of Absorbance Difference (I_{AD}) ranging from 0.0 to 2.0, with the lower values indicating a more advanced fruit maturity (Bonora et al., 2014, 2013; Ziosi et al., 2008).

5.2.2 Experimental design

50 nectarines per each maturity class were collected and sorted into two batches of 25 fruit each. The first batch was fresh-cut processed, whilst the second was maintained intact. After processing both fresh-cut and intact nectarines were stored at 5°C for 5 days to simulate the refrigerated storage. In this period, five biological replicates for both intact and processed fruit were taken daily for the assessment of quality traits and VOCs analyses.

5.2.3 Fruit processing

Prior to processing, the fruit was washed and dipped for 2 minutes in a solution of water and peracetic acid to eliminate contaminants on the skin. The nectarines were then processed in an industrial line commercially used to produce fresh-cut pome and stone fruit. The slicing occurred by pushing the fruit longitudinally with a pneumatic plunger through a sharp corer, producing eight symmetrical slices of homogeneous thickness. The fruit core was automatically discarded whilst the slices, transported by a conveyor belt, were soaked for 1 minute in an antioxidant solution (2.5 g/l ascorbic, 2.5 g/l sodium ascorbate) to prevent surface browning. The slices where then automatically packed into commercial plastic boxes heat-welded with a micro-perforated plastic film, allowing gas exchange. Intact and fresh-cut fruit was then maintained at 5°C until analysis, the first assessment (T0) being performed within 8 hours from processing.

5.2.4 Surface browning and quality determinations

Surface browning of the nectarine slices was evaluated with a Minolta CR-400 chromameter (Konica Minolta, Japan), using the L*a*b* indexes (Drogoudi et al., 2016). At each assessment, intact fruit were sliced and L*a*b* measured in the fruit flesh. Chroma and °Hue were then derived from the above mentioned chromatic parameters (Drogoudi et al., 2016). Surface browning was estimated as browning index (BI), a parameter closely related to PPO activity (Denoya et al., 2017) and calculated as following (Mohammad et al., 2008):

$$BI = 100 \times \frac{(x - 0.31)}{0.172}$$

where x = (a* + 1.75L*) / (5.645L* + a* - 3.012b*)

5.2.5 Sample preparation for VOCs analysis

On each day after processing (T0-5), intact and fresh-cut nectarines (including the skin), were sampled, immediately frozen in liquid nitrogen and ground with a stainless-steel analytical mill (IKA, Germany). For PTR-ToF-MS analysis, one gram of powdered frozen fruit of each biological replicate was transferred into a 20-mL glass vial, sealed with 18 mm PTFE/silicon septum (Agilent

technologies). PTR-ToF-MS analysis were performed on 5 biological replicates per each maturity class at each day of assessment. SPME-GC-MS were carried out on 1 sample per each maturity class and consisted of a mixture (0.2 g) of the abovementioned biological replicates and carried out at T0, T2, T4 and T5. For both type of analysis, to each vial, 1 ml of antioxidant solution (400 g/l of sodium chloride, 5 g/L of ascorbic acid, and 5 g/L of citric acid) was added to prevent tissue oxidation (Farneti et al., 2014). Samples were kept at -80°C until analysis.

5.2.6 VOCs analysis by SPME-GC-MS

The vials were equilibrated at 40°C for 10 min with constant stirring. Solid-phase microextraction fiber (DVB/CAR/PDMS, Supelco, Bellefonte, PA, USA) was exposed for 30 min in the vial headspace. The compounds adsorbed by HS-SPME were analyzed with a GC interfaced with a mass detector operating in electron ionization (EI) mode (internal ionization source; 70 eV) with a scan range of *m/z* 33 to 350 (GC Clarus 500, PerkinElmer, Norwalk CT, USA). Separation was carried out in an HP-INNOWax fused silica capillary column (30 m, 0.32-mm ID, 0.5-µm film thickness; Agilent Technologies, Palo Alto, CA, USA). The initial GC oven temperature was 40°C rising to 220°C at 4°C min^{-1}, the temperature of 220°C was maintained for 1 min, then increased at 10°C min^{-1} until it reached 250°C, which was maintained for 1 min. The carrier gas was helium at a constant column flow rate of 1.5 ml min^{-1}. Semi quantitative data were expressed as area units. Compound identification was based on mass spectra matching with the standard NIST/EPA/NIH (NIST 14) and Wiley 7th Mass Spectral Libraries, and linear retention indices (LRI) compared with the literature. LRI were calculated under the same chromatographic conditions after injection of a C7–C30 n-alkane series (Supelco).

5.2.7 VOCs analysis by PTR-ToF-MS

VOC measurements of nectarine flesh were performed in five replicates with a commercial PTR-ToF-MS 8000 apparatus (Ionicon Analytik GmbH, Innsbruck, Austria). The drift tube conditions were the following: 110°C drift tube temperature, 2.30 mbar drift pressure, 550 V drift voltage. This leads to an E/N ratio of about 140 Townsend (Td) (E corresponding to the electric field strength and N to the gas number density; 1 Td = 10^{-17} Vcm^2). The sampling time per channel of ToF acquisition was 0.1 ns, amounting to 350,000 channels for a mass spectrum ranging up to *m/z* = 400. Every single

spectrum is the sum of about 28.600 acquisitions lasting 35 μs each, resulting in a time resolution of 1 s. Sample measurements were performed in 60 cycles resulting in an analysis time of 60 s/sample. Each measurement was conducted automatically after 20 min of sample incubation at 40°C by using an adapted GC autosampler (MPS Multipurpose Sampler, GERSTEL) and it lasted for around 2 min. During the measurements, 100 sccm of zero air was continuously injected into the vial, through a needle heated to 40°C, and the outflow going through a second heated needle was delivered via Teflon fittings to the PTR-ToF-MS. Zero air was flushed continuously through the vial, to prevent depressurization during gas aspiration.

The analysis of PTR-ToF–MS spectral data proceeded as follows. Count losses due to the ion detector dead time were corrected off-line via a methodology based on Poisson statistics (Titzmann et al., 2010). To reach a good mass accuracy (up to 0.001 Th), internal calibration was performed according to a procedure described by Cappellin et al. (2011b). Noise reduction, baseline removal and peak intensity extraction were performed according to Cappellin et al. (2011a), using modified Gaussians to fit the peaks. Absolute headspace VOC concentrations expressed in ppbv (parts per billion by volume) were calculated from peak intensities according to Cappellin et al. (2012).

5.2.8 Data analysis

The detection of the array of masses identified by PTR-ToF-MS was reduced by applying noise and correlation coefficient thresholds. In the first case, peaks not significantly different from blank samples were removed (Farneti et al., 2015). With regard to correlation coefficient thresholds, peaks having over 99 % correlation were excluded as putative isotopes of monoisotopic masses (Farneti et al., 2017).

Data analysis was performed with R.3.3.3 software using internal functions and external packages such as “Agricolae” for ANOVA and “mixOmics”, “ChemometricswithR”, “heatmap3” for multivariate statistical analysis and “ggplot2” for graphic representations.

5.3 Results and discussion

5.3.1 Quality traits affected by processing and cold storage

Fruit processing decreased L* values in the three nectarine cultivars, most evidently in the three maturity classes of AR (fig 5.7.1). This variation was maintained over time during postharvest storage and indicates a decrease in colour brightness in response to fruit processing. Higher values of a* (associated with a higher red colour degree of the fruit flesh) were induced by fruit processing in AR and WR, but not in M60 (fig 5.7.2). Higher values of b* resulted from intact fruit of WR and M60 and, at least in the last time points, in AR, suggesting a lower yellow intensity of the flesh of fresh-cut fruit (fig 5.7.3). The chroma index (fig 5.7.4), representing colour saturation (Bolin and Huxsoll, 1989), is largely affected by b*. Thus, the cv AR presents a slight discoloration of the fruit flesh over time and/or after fresh-cut processing (Allegra et al., 2015; Koukounaras et al., 2008). Lower °h values were generally measured in C-R samples of both intact and fresh-cut fruit (data not shown).
No significant surface browning, emerged during the days of cold storage (fig 5.7.5). Similarly, Giné Bordonaba et al. (2014) did not find any significant browning in 5 fresh-cut cultivars of nectarines stored at 5° C for 12 days with a similar antioxidant solution application. Browning Index is closely related to polyphenol oxidase activity (Denoya et al., 2017), by which phenols are oxidised to quinones in the presence of oxygen (Eissa et al., 2006). In our experimental conditions, an antioxidant treatment was applied to nectarine slices after cutting to minimise tissue oxidation. Besides, the dipping of fruit slices may also remove from the fruit surface the enzymes released during cutting and slicing processes (Soliva-Fortuny and Martín-Belloso, 2003).

5.3.2 Nectarine VOC profiling

The characterisation of nectarine VOCs profiles by direct injection allowed the identification of most volatile compounds responsible for nectarine aroma (table 5.6.2), as recently described in literature by PTR-ToF-MS analysis (Bianchi et al., 2017). The PTR-ToF-MS setting and data elaboration adopted in this study allowed the detection of 112 fragments, 60 of which could be chemically identified based on literature references, chemical standards, and correlation with SPME-GC-MS analysis.
Gas chromatographic analysis was carried out by SPME-GC-MS to support the identification of the molecular masses detected by PTR-ToF-MS. The analysis of both intact and processed fruit assessed in the three nectarine cultivars allowed the detection of 75 VOCs (table 5.6.3), one of which could

not be identified. Alcohols formed the most numerous group of compounds (16). Other compounds detected were classified as esters (13), aldehydes (11), monoterpenes (9), acids (7), ketones (6), lactones (5), hydrocarbons (2), methylphenols (2), isothiocyanates (1), norisoprenoids (1) and sesquiterpenes (1).

In intact fruit, aldehydes (mainly Hexanal, Pentenal, and 2-Hexenal) were the most abundant VOCs group, based on relative concentration, amounting to 50.6 % (WR), 69.9 % (AR) and 92.2 % (M60) of total VOCs emissions. Monoterpenes, especially Linalool, were the second most abundant group, accounting for 21.2 % of the total volatile emissions of WR, 2.5 % for AR and 1.6 % for M60. The relative emission of esters (including Hexyl Acetate, Isoamyl Acetate and Butyl Acetate) ranged from 9.9 % of the total volatiles in AR to 0.9 % for M60. Alcohols (for the most 1-Pentanol and Ho-trienol) accounted for about 7.2 % of the total VOC profile of AR and 7.7 % for WR whilst only 1.6 % of M60. The highest fraction of Lactones was composed by γ-Hexalactone and γ- Decalactone and represented 4.7 % of the VOC profile of WR, 1.2 % for AR and 1 % of M60. Ketones concentration (for the most 1-Octen-3-One and 6-Methyl-5-Hepten-2-One) represented 3.3 % of the of WR volatiles, 2.8 % of AR and 0.8 % of M60. Isothiocyanate Cyclohexane (Isothiocyanates) was mostly present in AR accounting for 2.5 % of the VOC profile and 1.8 % in WR and 0.6 % in M60. Sesquiterpenes, only represented by Nerolidol, were mostly present in WR, accounting for 1.8 % of the VOC profile while 0.03 % in AR and no detection occurred in M60. Hydrocarbons such as Toluene and Styrene accounted for 1.9 % of the total volatiles of AR, 1.3 % for WR and 0.45 % for M60. Acids (for the most Isovaleric Acid and Pentanoic Acid) accounted for 0.9 % of the VOC profile for WR and AR whilst 0.36 % for M60. β-Damascenone (Norisoprenoids) accounted for 0.1 % of the VOC profile in WR, 0.04 % in AR, and 0.03 % in M60.

VOC screening by PTR-ToF-MS allowed the detection of additional compounds not detected by SPME-GC-MS analysis. Among them, Ethanol (*m/z* 47.049) and Methanol (*m/z* 33.033) represented the highest fraction of the detected alcohols whilst among the aldehydes, Acetaldehyde (*m/z* 45.0329) was the most represented in the three cultivars. Ketones such as Acetone (*m/z* 59.0492) and sulfuric compounds (indicated by *m/z* 63.0391 and tentatively identified as Dimethyl Sulfide and/or Ethanethiol) were also detected in the three nectarine cultivars.

5.3.3 Effect of fresh-cut processing on VOCs

The fruit VOCs profile of each nectarine cultivar was significantly modified by the fruit processing, as described by the principal component analysis (PCA) carried out using either PTR-ToF-MS (fig 5.7.6 A) or SPME-GC-MS (fig 5.7.6 B). In both cases, the first two principal components accounted

for about 80% of total variance. Differences of VOCs between intact and processed fruit were mostly described by the first principal component, whilst differences between cultivars separated the samples on the second principal component. This variation was led by a higher concentration of several VOCs composing the volatile profile as shown in the heatmaps of figure (5.7.6 C, D).

The vertical hierarchical dendrogram of the heatmaps revealed that VOCs were grouped into different clusters mainly in relation to their abundance as expressed as Log transformed values of the ratio between the VOC concentration after and before processing. The abundance of some volatiles was enhanced, reduced or remained unvaried in response to fruit processing in a different manner for the different cultivars.

The concentration of masses related to monoterpenes *m/z* 137.1348 (i.e. (-)-Limonene, (+-)-Limonene Linalool, trans-Carveol, 4-Terpineol, Geraniol and β-Myrcene), *m/z* 93.0698 and *m/z* 95.0868 (monoterpene fragments) increased after processing. This increase was evident in processed fruit (fig 5.7.6 A, E) and significant in the AR cultivar (table 5.6.2).

This may be a consequence of mechanical wounding of the fruit, as it enhances a diverse array of enzymatic pathways, associated in with the generation of volatiles (Toivonen, 1997), such as the mevalonic acid and methylerythritol phosphate pathways for the production of isopentenyl diphosphate and dimethylallyl diphosphate, as substrates for the activity of the terpene synthases enzyme (Forney, 2016).

Different trends of variation in aldehyde emissions were found in the three cv after processing. However, a common feature was the increase of C6-aldehydes indicated by *m/z* 99.0807 (2-Hexenal and (2E)-Hexenal) and *m/z* 101.0962 (Hexanal) detected by PTR-ToF-MS (table 5.6.2) and identified by SPME-GC-MS.

An increase of C6-Aldehydes is generally associated with tissue disruption (Varoquaux, 2002) as a typical response to mechanical injury, and driven by the lipoxygenase (LOX) activity (Deza-Durand and Petersen, 2011). Furthermore, C6-Aldehydes are part of the signalling network resulting in the activation of plant defences triggered by mechanical damages in plant tissues (Matsui, 2006).

C6-aldehydes can be further converted into the associated C6-alcohols through the action of alcohol dehydrogenase (Forney, 2016). Consequently, C6-alcohols (*m/z* 83.0862) identified by SPME-GC-MS as (E)-3-Hexen-1-ol, (Z)-3-Hexen-1-ol, (Z)-2-Hexen-1-ol increased after processing. This increase was significant in M60 and not in AR and WR cultivars (table 5.6.2).

Among the alcohols, also Methanol (*m/z* 33.0336) was significantly found higher in processed fruit of M60 (table 5.6.2) and may originate from the enhanced degradation of the pectin in the cell wall (Fall and Benson, 1996) due to cell disruption.

The emission of Ethanol (*m/z* 47.0491) significantly increased in processed fruit of the three cultivars suggesting a common response in emitting Ethanol in response to cell disruption (table 5.6.2). This increase in Ethanol was followed by a significant increase of Ethyl acetate (*m/z* 61.0285) suggesting its conversion into the related ester by the action of the alcohol acyltransferase (Balbontín et al., 2010).

Tissue disruption also enhanced the formation of esters including *m/z* 89.0597 and *m/z* 131.1076 tentatively identified as Amyl Acetate by PTR-ToF-MS (table 5.6.2) and Ethyl Crotonate, Amyl Acetate, Hexanoic Acid Ethyl Ester, Octanoic Acid Ethyl Ester, Hexyl Acetate detected by SMPE-GC.MS (table 5.6.3). These esters are related to positive odour notes and therefore contributing to the pleasant aroma of nectarines.

Green-odour esters such as (*m/z* 143,1093) identified as cis-3-Hexenyl Acetate and trans-2-Hexenyl Acetate were also enhanced in response to fresh-cut processing. Cis-3-Hexenyl Acetate was significantly increased in AR (table 5.6.2) while 2-Hexenyl Acetate increase was evident in WR and AR (table 5.6.3)

Lactones (i.e. γ-Hexalactone, γ-Octalactone, γ-Decalactone, δ-Decalactone, γ-Undecalactone) identified by SPME-GC-MS, were also increased after fruit processing in the three cultivars (table 2; fig 2 B, F). Lactones are associated with pleasant and fruity notes (Rizzolo et al., 2006; Zhang et al., 2011). Therefore, their increase after fruit processing is a desirable trait, likely to positively affect the aroma of the processed nectarines.

5.3.4 Effect of storage on fresh-cut and intact fruit VOCs

Principal component analysis was carried out on the PTR-ToF-MS data to describe the relative effect of cultivar-dependent features, fruit processing, maturity stage and duration of storage on VOCs emission (fig 5.7.7 A). Over 82 % of the total variability was described by the first two principal components. The first principal component (corresponding to 65.2% of the total variance) discriminated VOCs emission between intact and processed fruit, whilst differences between cultivars were evidenced on the second principal component (12.17% of the total variance). For the processed samples, a storage time-dependent drift was also found, whereas maturity class did not substantially influence the VOCs profiles. As per the loading plot (fig 5.7.7 B), both fruit processing and storage time correlated with the same set of molecular fragments, suggesting that the biochemical processes triggered by fruit slicing kept on during storage.

Starting from 3 days in storage, some changes in aroma composition were explainable by molecular fragments related to fermentative metabolism such as *m/z* 47.049 (Ethanol) (fig 5.7.8) and *m/z* 45.0329 (Acetaldehyde) (fig 5.7.9). Their accumulation during maturation can be associated with the synthesis of other aroma volatiles such as acetate esters and ethyl esters (Ke et al., 1994; Larsen and Watkins, 1995), as shown by the parallel increase of molecular fragments *m/z* 89.0597 or *m/z* 75.0440 (Ethyl acetate) (fig 5.7.10) and *m/z* 61.0285 (ester fragment). Esters are generally associated with fruity and floral aromas and therefore this increase may contribute positively to the pleasant aroma of the nectarines.

However, the accumulation of these compounds is common during ripening and can be enhanced by several factors, including chilling injury, temperature (Purvis, 1997) and fermentation, consequent to the fruit exposure to low concentration of O_2 (Pesis, 2005). In our experimental conditions, the packaging process of fruit slices may have induced a depletion of O_2 or an accumulation of CO_2, resulting in the accumulation of fermentative metabolites. The off-flavour sensations are generally associated with the accumulation of fermentative metabolites, such as ethanol and acetaldehyde (Hagenmaier, 2002; Porat et al., 2008), hence fresh-cut nectarines may have incurred in aroma spoilage during the last days of refrigerated storage. In fact, other off-flavour compounds increased starting from storage day 3 in processed fruit, such as sulphur compounds (tentatively identified as Dimethyl Sulphide or Ethanethiol, *m/z* 63.0391) (fig 5.7.11) and C5 acids (Isovaleric Acid or Pentanoic Acid, *m/z* 103.0759). Methanol (*m/z* 33.0336) may originate from the degradation of pectin in the cell walls (Fall and Benson, 1996) occurring during fruit softening (Ortiz et al., 2010) and was found to increase in both intact and fresh-cut fruit during cold storage (fig. 5.7.12).

The variation of the PC1 was also determined by the increase of an array of other volatile compounds such as *m/z* 87.0442 (Butyrolactone), *m/z* 73.0650 (Butanal or Isobutyraldehyde), *m/z* 79.0364 (Acetic acid cluser), *m/z* 43.0174 (common frangment) and *m/z* 65.0577 (not identified).

On the second principal component of variance, a clear discrimination of AR from the two other cultivars is evident. The abundance of molecular fragments attributed to terpenoid compounds such as *m/z* 137.138 (i.e. (-)-Limonene, (+)-Limonene and β-Myrcene), *m/z* 95.0868, *m/z* 81.0704, *m/z* 93.0698 (i.e. terpene fragments), may explain such difference.

However, processed AR samples tend to cluster with the other cvs at late storage times, suggesting that other compounds (including fermentative and off-flavour products) may become predominant.

5.4 Conclusions

Fresh-cut processing induced a major variation in the volatile profile of the nectarines. Although the genetic differences of the cultivars affected the behaviour of the fruit, common responses to processing were the immediate release of VOCs associated with positive sensations, and the production of off-flavour volatiles over time in cold storage. The maturity of the fruit at harvest did not induce major differences in the aroma profile of the fruit either intact or processed.

Given that visual appearance of the fruit wedges did not variate substantially as the browning index remained stable over time, the aroma profile of the processed fruit is possibly one of the key aspects of fresh-cut nectarine marketability. In this scenario, the discrepancy between a visually perceived freshness of the product at purchase, and the perception of off-flavours at eating may reduce the likelihood of repurchase by consumers. Thus, smart packaging solutions sensitive to some off-flavour metabolite such as ethanol may be adopted during production, to ensure high quality standards on the market.

5.5 References

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5.6 Tables

Table 5.6.1: Cultivars and maturity classes segregation by function of the I_{AD}

Cultivar	I_{AD} group identified	Maturity	ID class
August red	0.9- 1.2	Immature	C-I
	0.5-0.8	Commercial maturity	C-M
	0.2-0.4	Ripe	C-R
Western red	0.6-0.9	Commercial maturity	C-M
	0.2-0.4	Ripe	C-R
Morsiani 60	0.3-0.5	Ripe	C-R

Table 5.6.2: Volatile compounds detected by PTR-ToF-MS. Values are reported as concentration (ppbv). * indicates compounds identified by SPME-GC-MS and [a] indicates compounds identified by revision of Bianchi et al., 2017. Letters indicate significant differences between cultivars and intact and processed fruit according to ANOVA and Tukey HSD (P< 0.005).

m/z	Formula	Tentative identification	Western Red		August Red		Morsiani 60	
			Intact	Processed	Intact	Processed	Intact	Processed
ms27.0264	$C_2H_3^+$	Common fragment	0,21 bc	0,43 ab	0,17 bc	0,41 ab	0,08 c	0,62 a
ms27.0345		n.i.	0,03 cd	0,19 bc	0,07 bcd	0,23 ab	0,03 d	0,39 a
ms27.0430		n.i.	0,05 b	0,09 ab	0,05 b	0,12 ab	0,02 b	0,17 a
ms28.0185		n.i.	0,38 a	0,35 a	0,52 a	0,62 a	0,32 a	0,46 a
ms28.0313	$C_2H_4^+$	Ethylene	0,15 b	0,30 ab	0,26 ab	0,38 a	0,12 b	0,37 a
ms29.0396	$C_2H_5^+$	Ethanol fragment	3,74 c	50,30 ab	1,72 c	36,99 b	2,38 c	79,78 a
ms31.0181	CH_2OH^+	Formaldehyde	3,39 bc	5,02 ab	3,15 bc	4,87 b	2,27 c	6,82 a
ms33.0336	CH_4OH^+	Methanol	207,91 bc	375,24 ab	255,99 bc	326,32 ab	98,86 c	465,21 a
ms34.9952	H_2SH^+	Hydrogen sulfide	5,37 a	3,51 a	3,57 a	5,56 a	2,90 a	5,27 a
ms39.0220	$C_3H_3^+$	Common fragment	9,97 ab	8,19 ab	6,94 b	6,67 b	3,49 b	15,30 a
ms41.0386	$C_3H_5^+$	Common fragment	36,29 a	32,65 ab	26,70 ab	32,93 ab	11,04 b	44,81 a
ms42.0102	$C_2H_2O^+$	n.i.	0,10 bc	0,25 ab	0,14 bc	0,25 ab	0,07 c	0,36 a
ms42.0336	$C_2H_3NH^+$	Acetonitrile	0,72 a	1,24 a	1,24 a	1,25 a	0,70 a	1,42 a
ms43.0174	$C_2H_3O^+$	Common fragment	37,29 c	216,18 a	39,36 bc	82,36 bc	19,56 c	167,93 ab
ms43.0541	$C_3H_7^+$	Common fragment	8,13 ab	10,47 ab	9,68 ab	15,66 a	3,48 b	12,55 a
ms45.0329	$C_2H_4OH^+$	Acetaldehyde	94,46 c	993,58 b	163,24 c	1447,46 b	47,42 c	2276,30 a

ms47.0491	$C_2H_6OH^+$	Ethanol	19,47 c	231,60 ab	6,20 c	197,10 b	10,21 c	381,13 a
ms49.0109	CH_4SH^+	Methanethiol	0,90 a	0,37 b	0,67 ab	1,03 a	0,37 b	0,54 ab
ms51.0229		n.i.	0,32 a	0,41 a	0,20 a	0,24 a	0,17 a	0,52 a
ms53.0389	$C_4H_5^+$	n.i.	1,87 ab	1,49 ab	1,82 ab	1,76 ab	1,03 b	3,78 a
ms55.0168		n.i.	0,07 a	0,34 a	0,23 a	0,17 a	0,08 a	0,35 a
ms55.0544	$C_4H_7^+$	Common fragment	47,47 ab	33,05 ab	50,07 ab	52,88 ab	16,43 b	94,95 a
ms57.0336	$C_3H_4OH^+$	Common fragment	13,17 ab	9,01 b	35,79 ab	17,69 ab	19,83 ab	66,73 a
ms57.0702	$C_4H_9^+$	1-Butanol*, high alcohol fragment	11,80 ab	29,52 a	5,65 b	14,64 ab	2,32 b	16,99 ab
ms59.0492	$C_3H_6OH^+$	Acetone	61,98 a	82,16 a	72,26 a	84,73 a	40,46 a	85,12 a
ms60.0216	$C_2H_4O_2^+$	n.i.	0,02 bc	0,11 a	0,02 bc	0,05 abc	0,01 c	0,09 ab
ms61.0285	$C_2H_4O_2H^+$	Fragment of Acetic acid*, Acetoin*, Ethyl Acetate, common ester fragment	28,73 b	320,96 a	27,95 b	80,04 b	18,84 b	182,42 ab
ms63.0086		n.i.	0,69 a	0,56 a	1,78 a	5,85 a	0,25 a	0,72 a
ms63.0391	$C_2H_6SH^+$	Dimethyl sulfide, Ethanethiol	0,69 c	5,98 bc	1,26 c	11,87 a	0,31 c	11,67 ab
ms65.0193		n.i.	2,90 abc	2,40 abc	3,84 ab	4,28 a	0,87 c	1,52 bc
ms65.0577	$C_5H_5^+$	n.i.	0,42 b	5,84 a	0,21 b	7,39 a	0,19 b	9,63 a
ms66.0241		n.i.	0,06 ab	0,08 ab	0,11 ab	0,12 a	0,03 b	0,04 ab
ms67.0548	$C_5H_7^+$	n.i.	1,85 bc	1,34 bc	1,96 bc	4,62 a	0,60 c	2,50 b
ms68.0585	$C_5H_7^+$	n.i.	0,17 bc	0,13 bc	0,18 bc	0,48 a	0,06 c	0,21 b
ms69.0334	$C_4H_4OH^+$	Furan	0,60 ab	0,49 ab	0,57 ab	0,70 a	0,27 b	0,62 ab
ms69.0703	$C_5H_9^+$	Aldehyde fragment	28,61 a	17,02 ab	20,31 ab	23,26 ab	7,32 b	32,90 a
ms70.0414		n.i.	0,05 ab	0,11 a	0,08 ab	0,07 ab	0,03 b	0,10 a
ms71.0493	$C_4H_6OH^+$	Butenal	2,67 a	1,63 ab	2,20 a	2,53 a	0,69 b	2,16 a
ms71.0858	C_5H11^+	3-methyl-1-butanol + 2-methyl-1-butanol*, 3-Pentanol*, 1-Pentanol*	2,57 ab	3,43 a	1,67 ab	2,60 ab	0,64 b	3,96 a
ms73.0286	$C_3H_4O_2H^+$	n.i.	1,68 bc	1,55 cd	2,48 ab	3,01 a	0,79 d	1,65 c
ms73.0650	$C_4H_8OH^+$	Butanal*, isobutyraldehyde	12,94 c	109,16 a	7,69 c	43,44 bc	5,90 c	78,20 ab
ms75.0440	$C_3H_6O_2H^+$	Methyl Acetate*	8,80 ab	19,11 a	4,45 b	8,82 ab	2,13 b	12,75 ab
ms75.0799	$C_4H_{10}OH^+$	2-Methylpropanol [a]	0,05 b	0,22 a	0,02 b	0,09 ab	0,03 b	0,21 a
ms77.0197		n.i.	0,16 a	0,20 a	0,19 a	0,19 a	0,14 a	0,21 a
ms77.0489		n.i.	0,23 a	0,28 a	0,19 a	0,25 a	0,16 a	0,26 a

ms77.0728		n.i.	0,21 a	0,21 a	0,22 a	0,24 a	0,20 a	0,26 a
ms79.0364		Acetic acid cluster	0,02 b	1,39 a	0,04 b	0,49 ab	0,05 b	0,81 ab
ms79.0546	$C_6H_7^+$	Benzene	5,44 a	4,91 a	4,91 a	3,82 a	3,01 a	4,38 a
ms80.0589		n.i.	0,37 ab	0,36 ab	0,48 a	0,51 a	0,22 b	0,41 ab
ms80.9901		n.i.	15,53 ab	13,00 ab	20,06 ab	23,97 a	4,32 b	7,53 ab
ms81.0704	$C_6H_9^+$	Fragment of aldehydes (Hexenals); fragment of terpenes (Linalool)	8,94 b	12,20 b	38,19 b	130,90 a	6,89 b	32,59 b
ms83.0494	$C_5H_6OH^+$	Methylfuran	1,46 a	1,02 ab	1,05 ab	1,03 ab	0,49 b	1,50 a
ms83.0862	$C_6H_{11}^+$	(E)-3-Hexen-1-ol, (Z)-3-Hexen-1-ol, (Z)-2-Hexen-1-ol*, Hexanal,2-Hexanone	36,01 ab	21,06 ab	38,75 ab	42,12 ab	12,04 b	72,63 a
ms84.0536	$C_5H_6OH^+$	n.i.	0,14 ab	0,10 ab	0,13 ab	0,13 ab	0,06 b	0,17 a
ms85.0652	$C_5H_8OH^+$	2-Pentenal [a]	4,76 a	2,19 a	4,02 a	4,23 a	1,63 a	4,91 a
ms87.0442	$C_4H_6O_2H^+$	Butyrolactone	1,57 ab	1,51 ab	1,48 ab	1,94 a	0,59 b	1,54 ab
ms87.0802	$C_5H_{10}OH^+$	2-methyl butanal+3-methyl butanal, Pentanal*	5,06 ab	3,65 ab	3,99 ab	5,22 a	2,04 b	6,51 a
ms88.0798		n.i.	0,51 a	0,60 a	0,89 a	0,99 a	0,52 a	0,96 a
ms89.0597	$C_4H_8O_2H^+$	Ethyl Acetate*, Butanoic Acid*	2,27 b	79,97 a	1,13 b	13,68 b	2,40 b	39,72 ab
ms91.0680	$C_7H_7^+$	Benzyl Alcohol	0,45 bc	1,12 a	0,64 abc	0,98 ab	0,27 c	1,05 ab
ms93.0368	$C_3H_8OSH^+$	n.i.	1,73 ab	1,63 ab	2,29 a	2,47 a	0,91 b	2,61 a
ms93.0698	$C_7H_9^+$	Toluene*, Monoterpene fragment	8,55 abc	13,49 ab	4,81 bc	16,60 a	2,21 c	15,41 a
ms93.0889		n.i.	0,22 a	0,43 a	0,15 a	0,40 a	0,08 a	0,34 a
ms95.0183		n.i.	0,51 ab	0,48 ab	0,67 a	0,72 a	0,28 b	0,71 a
ms95.0868	$C_7H_{11}^+$	2-Heptenal*, Monoterpene fragment	2,88 b	2,63 b	3,62 b	18,68 a	0,65 b	3,81 b
ms97.0288	$C_5H_4O_2H^+$	Furfural	1,00 ab	0,85 ab	0,81 ab	0,94 ab	0,43 b	1,42 a
ms97.0657	$C_6H_8OH^+$	2,4-Hexadienal	0,82 ab	0,49 bc	1,08 a	1,00 ab	0,27 c	0,72 abc
ms97.1023	$C_7H_{13}^+$	Heptanal, fragment	1,27 a	0,78 b	0,91 ab	1,19 a	0,24 c	0,70 b
ms99.0807	$C_6H_{10}OH^+$	2-Hexenal*, (2E)-Hexenal	4,31 ab	2,94 b	14,73 ab	7,95 ab	6,63 ab	22,59 a
ms101.0607	$C_5H_8O_2H^+$	2,3-Pentanedione	0,59 a	0,38 ab	0,56 a	0,61 a	0,22 b	0,52 a

ms101.0962	$C_6H_{12}OH^+$	Hexanal*	5,14 ab	2,77 ab	5,60 ab	5,22 ab	1,71 b	10,28 a
ms103.0759	$C_5H_{10}O_2H^+$	Isovaleric Acid*, Pentanoic Acid*	0,43 bc	0,77 ab	0,47 abc	0,76 ab	0,17 c	0,90 a
ms105.0501	$C_4H_8O_3H^+$	n.i.	0,02 a	0,02 a	0,03 a	0,05 a	0,02 a	0,02 a
ms105.0719	C_8H_9+	Styrene*	0,39 ab	0,39 ab	0,45 ab	0,77 a	0,24 b	0,48 ab
ms107.0809	$C_8H_{10}H^+$	Ethyl Benzene, Xylene	3,57 a	3,33 a	7,31 a	6,09 a	1,11 a	3,02 a
ms109.0692	$C_7H_8OH^+$	4-Methyl Phenol*, 3-Methyl Phenol*	0,08 a	0,08 a	0,12 a	0,12 a	0,09 a	0,08 a
ms109.1030	$C_8H_{13}^+$	n.i.	1,86 a	1,21 ab	1,79 a	1,89 a	0,43 b	1,29 a
ms113.0246		n.i.	0,19 c	0,18 c	0,27 ab	0,31 a	0,13 c	0,20 bc
ms113.0603	$C_6H_8O_2H^+$	Sorbic acid	0,20 bc	0,16 cd	0,30 ab	0,36 a	0,09 d	0,20 bc
ms113.0969	$C_7H_{12}OH^+$	Heptenal	1,12 a	0,64 ab	0,61 ab	0,89 a	0,18 b	0,65 ab
ms115.0763	$C_6H_{10}O_2H^+$	Ethyl Crotonate (Ethyl (2E) -2-butenoate) *, 5-Ethyldihydro-2(3H)-Furanone*	0,38 a	0,29 ab	0,29 ab	0,31 a	0,12 b	0,37 a
ms115.1131	$C_7H_{14}OH^+$	Γ-Hexalactone*,Heptanone, Heptanal	0,37 ab	0,25 b	0,28 b	0,47 a	0,07 c	0,22 bc
ms117.0917	$C_6H_{12}O_2H^+$	Isobutyl Acetate*, Butyl Acetate*, Hexanoic Acid*	0,41 bc	1,12 a	0,51 bc	0,94 ab	0,24 c	0,72 abc
ms119.0875	$C_9H_{11}^+$	n.i.	0,14 bc	0,16 abc	0,15 abc	0,24 a	0,11 c	0,22 ab
ms121.0669	$C_8H_8OH^+$	Benzeneacetaldehyde [a]	0,15 bc	0,15 bc	0,24 a	0,25 a	0,10 c	0,19 ab
ms121.1035	$C_9H_{13}^+$	n.i.	0,40 b	0,40 b	0,40 b	0,99 a	0,23 b	0,41 b
ms123.0832	$C_8H_{10}OH^+$	n.i.	0,08 ab	0,05 bc	0,10 a	0,10 a	0,03 c	0,08 abc
ms123.1191	$C_9H_{15}^+$	Nonenal	0,31 ab	0,25 b	0,32 ab	0,37 a	0,12 c	0,27 ab
ms125.0982	$C_8H_{12}OH^+$	n.i.	0,60 a	0,38 a	0,44 a	0,51 a	0,11 b	0,41 a

ms127.1135	$C_8H_{14}OH^+$	1-octen-3-one*, 6-Methyl-5-Hepten-2-one*, (E)-2-Octenal*	1,62 a	0,90 ab	1,10 a	1,28 a	0,25 b	1,05 a
ms129.0915	$C_7H_{12}O_2H^+$	γ-Heptalactone [a]	0,15 ab	0,16 ab	0,22 a	0,25 a	0,09 b	0,20 a
ms129.1293	$C_8H_{16}OH^+$	2-octanone, Octanal*, 1-Octen-3-ol*	0,51 a	0,39 ab	0,40 a	0,56 a	0,11 b	0,35 ab
ms131.1076	$C_7H_{14}O_2H^+$	Isoamyl Acetate+Ethyl Benzene*, Amyl Acetate*, Heptanoic Acid*	0,20 c	0,31 bc	0,42 b	0,60 a	0,14 c	0,42 b
ms135.1140	$C_{10}H_{15}^+$	HO-Trienol*, trans-Carveol*	0,23 b	0,26 b	0,33 b	0,76 a	0,16 b	0,31 b
ms137.1348	$C_{10}H_{17}^+$	(-) -Limonene*, (+-) -Limonene*, β-Myrcene*, Linalool*, 4-Terpineol*, Geraniol*	1,56 b	5,27 b	10,01 b	80,04 a	0,92 b	9,59 b
ms141.0916	$C_8H_{13}O_2^+$	n.i.	0,05 c	0,05 c	0,11 ab	0,12 a	0,02 c	0,06 bc
ms141.1290	$C_9H_{16}OH^+$	2-Nonenal [a]	0,16 ab	0,14 bc	0,18 ab	0,20 a	0,07 c	0,17 ab
ms143.1093	$C_8H_{14}O_2H^+$	cis-3-Hexenyl Acetate*, 2,5-Octanedione*, trans-2-Hexenyl Acetate*, 5-Butyldihydro-2(3H)-Furanone*	0,41 bc	0,39 bc	0,47 b	0,81 a	0,14 c	0,40 bc
ms145.1246	$C_8H_{16}O_2H^+$	Ethyl Hexanoate*, Hexyl Acetate*, 2-Ethyl Hexanoic Acid*	0,15 c	0,29 c	0,32 bc	0,61 a	0,14 c	0,48 ab
ms149.0510		n.i.	0,39 a	0,32 a	0,36 a	0,47 a	0,24 a	0,32 a
ms149.1322	$C_{11}H_{17}^+$	n.i.	0,03 b	0,05 ab	0,06 a	0,06 ab	0,04 ab	0,06 ab
ms153.1306	$C_{10}H_{16}OH^+$	HO-Trienol*, Epoxylinalool*, 2,4-Decadienal, 2,6-dimethyl-3,7-octadiene-2,6-diol*	0,14 bc	0,13 bc	0,16 abc	0,23 a	0,08 c	0,17 ab

ms155.1039	$C_9H_{14}O_2H^+$	n.i.	0,30 b	0,31 b	0,61 a	0,66 a	0,19 c	0,28 bc
ms155.1457	$C_{10}H_{18}OH^+$	Linalool*, 4-Terpineol*	0,10 abc	0,08 bc	0,19 ab	0,20 a	0,06 c	0,13 abc
ms157.1248	$C_9H_{16}O_2H^+$	n.i.	0,14 bc	0,14 bc	0,25 a	0,27 a	0,07 c	0,17 b
ms159.1418	$C_9H_{18}O_2H^+$	n.i.	0,22 c	0,22 c	0,37 ab	0,40 a	0,22 c	0,29 bc
ms163.0970		n.i.	0,13 ab	0,09 ab	0,13 ab	0,20 a	0,05 b	0,09 ab
ms163.1523	$C_{12}H_{19}^+$	n.i.	0,01 b	0,02 ab	0,03 ab	0,04 a	0,02 b	0,02 ab
ms167.0572		n.i.	0,13 ab	0,12 ab	0,16 ab	0,21 a	0,07 b	0,12 ab
ms169.1645		n.i.	0,04 b	0,04 b	0,09 a	0,11 a	0,03 b	0,04 b
ms173.1573	$C_{10}H_{20}O_2H^+$	Butanoic Acid Hexyl Ester*, Octanoic Acid Ethyl Ester*, Decanoic Acid	0,17 a	0,14 a	0,10 a	0,13 a	0,07 a	0,13 a
ms177.1108	$C_{13}H_{21}^+$	n.i.	0,02 abc	0,02 abc	0,03 ab	0,03 a	0,01 c	0,01 bc

Table 5.6.3: Volatile compounds detected by SPME-GC-MS. Values are reported as %.

Name						Western Red		August Red		Morsiani 60	
	ID	Formula	RT	KI Calc	KI NIST	Intact	Processed	Intact	Processed	Intact	Processed
ACIDS											
Acetic Acid	Ac_1	$C_2H_4O_2$	20	1562	1449	0.11	0	0.19	0.02	0.05	0.07
Isovaleric Acid	Ac_2	$C_5H_{10}O_2$	25	1748	1666	0.23	0.23	0.25	0.12	0.16	0.25
Butanoic Acid	Ac_3	$C_4H_80_2$	27	1821	1625	0.07	0.05	0.04	0.01	0.01	0.01
Pentanoic Acid	Ac_4	$C_5H_{10}O_2$	30	1922	1733	0.3	0.29	0.2	0.07	0.1	0.12
2-Ethyl Hexanoic Acid	Ac_5	$C_8H_{16}O_2$	32	2003	1960	0	0	0.01	0	0	0
Hexanoic Acid	Ac_6	$C_6H_{12}O_2$	33	2025	1846	0.09	0.05	0.07	0.02	0.02	0.02
Heptanoic Acid	Ac_7	$C_7H_{14}O_2$	2198	1950	138157	0.09	0.04	0.11	0.02	0.02	0.02
Total (%)						***0.89***	***0.66***	***0.87***	***0.27***	***0.36***	***0.49***
ALCOHOLS											
3-Pentanol	Al_1	$C_5H_{12}0$	6	1125	1110	0.18	0.09	0.19	0.01	0.04	0.03
1-Butanol	Al_2	C_4H_9OH	7	1162	1142	0.23	0.09	0.04	0.01	0.02	0.02
2+3-Methyl-1-Butanol	Al_3	$C_5H_{12}O$	9	1225	1208+1209	0.12	0.27	0.09	0.05	0.06	0.12
1-Pentanol	Al_4	$C_5H_{12}O$	10	1266	1250	2.59	2.04	1.43	0.28	0.36	0.17
Hexanol	Al_5	$C_6H_{14}0$	14	1366	1355	0.41	0.46	0.43	0.19	0.11	0.34
3-Octanol	Al_6	$C_8H_{18}0$	15	1404	1393	0	0.29	0.01	0	0	0
(Z)-2-Hexen-1-ol	Al_7	$C_6H_{12}0$	15	1417	1416	0.04	0.19	0.06	0.16	0.04	0.08
1-Octen-3-ol	Al_8	$C_8H_{16}0$	17	1460	1450	0.57	0.39	0.41	0.12	0.18	0.11
1-(2-Methoxy-1-methylethoxy)-2-propanol	Al_9	$C_7H_{16}O_3$	18	1486	1478	0.05	0.05	0.43	0	0.02	0.05
2-Ethyl-1-Hexanol	Al_10	$C_8H_{18}0$	18	1497	1491	0.34	0.49	0.49	0.24	0.09	0.2
1-(2-methoxypropoxy)-2-propanol	Al_11	$C_7H_{16}O_3$	19	1526	1532	0.31	0.21	1.5	0.1	0.09	0.2
1-Octanol	Al_12	$C_8H_{18}0$	20	1565	1557	0.37	0.43	0.34	0.2	0.15	0.08
HO Trienol 29957-43-5	Al_13	$C_{10}H_{16}0$	22	1616	1613	1.38	1.31	0.71	0.19	0.17	0.09
alpha,alpha-dimethyl-benzenemethanol	Al_14	$C_9H_{12}0$	26	1763	1773	0.49	0.32	0.55	0.11	0.14	0.09
2,6-dimethyl-3,7-octadiene-2,6-diol	Al_15	$C_{10}H_{18}O_2$	31	1953	1945	0.1	0.05	0.04	0.02	0.01	0
Phenol	Al_16	C_6H_60	32	2010	2000	0.5	0.33	0.5	0.09	0.12	0.09
Total (%)						***7.68***	***7***	***7.2***	***1.77***	***1.6***	***1.69***

ALDEHYDES											
Butanal	Ad_1	C_4H_80	2	902	877	0.52	2.26	0.33	0.14	0.12	0.35
Pentanal	Ad_2	$C_5H_{10}0$	3	980	979	11.83	6.99	8.89	1.13	2	0.81
Hexanal	Ad_3	$C_6H_{12}0$	5	1094	1083	18.33	7.11	22.78	6.98	28.14	29.42
2-Hexenal	Ad_4	$C_6H_{10}0$	9	1214	1213	0.2	0	0.18	0.1	0.53	0.41
(2E)-Hexenal	Ad_5	$C_6H_{10}0$	9	1230	1216	5.43	9.8	29.3	19.19	55.09	49.52
Octanal	Ad_6	$C_8H_{16}0$	12	1300	1289	2.69	1.82	1.82	0.35	1.48	0.41
2-Heptenal	Ad_7	$C_7H_{12}0$	13	1330	1323	4.13	2.38	2.57	0.28	1.09	0.59
Nonanal	Ad_8	$C_9H_{18}O$	15	1399	1391	3.59	2.24	1.37	0.34	2.02	0.66
(E)-2-Octenal	Ad_9	$C_8H_{14}0$	16	1432	1429	2.73	1.44	1.73	0.42	0.76	0.36
Decanal	Ad_10	$C_{10}H_{20}0$	18	1500	1498	0.73	0.71	0.36	0.13	0.76	0.24
Benzaldehyde	Ad_11	C_7H_60	19	1522	1520	0.42	0.3	0.6	0.28	0.25	0.49
Total (%)						***50.61***	***35.03***	***69.95***	***29.36***	***92.26***	***83.25***
ESTERS											
Ethyl Acetate	E_1	$C_4H_80_2$	2	893	888	0.22	4.53	0.38	0.19	0.13	0.81
Isobutyl Acetate	E_2	$C_6H_{12}O_2$	4	1019	1012	0.15	2.55	0.16	0.25	0.08	0.35
Butyl Acetate	E_3	$C_6H_{12}O_2$	5	1084	1074	1.07	1.98	2.22	0.28	0.19	0.46
Isoamyl Acetate + Ethyl Benzene	E_4		6	1135	1122+1129	1.36	0.74	2.29	0.21	0.25	0.33
Ethyl Crotonate	E_5	$C_6H_{10}O_2$	8	1179	1160	0	0	0.01	0.25	0	0
Amyl Acetate	E_6	$C_7H_{14}O_2$	8	1190	1176	0.08	0.99	0.15	0.05	0	0.11
Hexanoic Acid Ethyl Ester	E_7	$C_8H_{16}O_2$	10	1247	1233	0	0.07	0.01	0.53	0	0
Hexyl Acetate	E_8	$C_8H_{16}O_2$	11	1286	1272	2.26	4.02	4.22	2.6	0.23	4.23
cis-3-Hexenyl Acetate	E_9	$C_8H_{14}O_2$	13	1329	1315	0.14	3.36	0.21	2.79	0.02	0.32
trans-2-Hexenyl Acetate	E_10	$C_8H_{14}O_2$	13	1346	1333	0	2.55	0.09	2.74	0	0.39
Butanoic Acid Hexyl Ester	E_11	$C_{10}H_{20}O_2$	16	1421	1414	0.05	0.01	0.1	0.02	0	0
Octanoic Acid Ethyl Ester	E_12	$C_{10}H_{20}O_2$	16	1441	1435	0	0.65	0.01	0.07	0	0.21
Benzyl Acetate	E_13	$C_9H_{10}O_2$	25	1731	1720	0.16	0.15	0.13	0.02	0	0
Total (%)						***5.51***	***21.61***	***9.97***	***9.99***	***0.9***	***7.21***
HYDROCARBONS											
Toluene	H_1	C_7H_8	4	1045	1042	0.95	0.56	1.29	0.43	0.26	0.34
Styrene	H_2	C_8H_8	11	1269	1261	0.37	0.17	0.59	0.21	0.2	0.29
Total (%)						***1.31***	***0.72***	***1.89***	***0.64***	***0.45***	***0.63***

ISOTHIOCYANATES											
Isothiocyanato Cyclohexane	I_1	$C_7H_{11}NS$	23	1661	1667	1.83	1.14	2.53	0.47	0.66	0.43
Total (%)						***1.83***	***1.14***	***2.53***	***0.47***	***0.66***	***0.43***
KETONES											
Acetoin	K_1	$C_4H_8O_2$	11	1295	1284	0	0.08	0.01	0.01	0	0.02
1-octen-3-one	K_2	$C_8H_{14}O$	12	1312	1300	1.15	0.71	0.67	0.16	0.35	0.15
2,5-Octanedione	K_3	$C_8H_{14}O_2$	13	1339	1319	0.95	0.63	0.81	0.15	0.24	0.12
6-Methyl-5-Hepten-2-one	K_4	$C_8H_{14}O$	13	1347	1338	1.06	0	1.26	0	0.22	0.08
2-Undecanone	K_5	$C_{11}H_{22}O$	21	1598	1598	0	0.11	0.01	0.01	0	0.02
6-pentyl-2H-Pyran-2-one	K_6	$C_{10}H_{14}O_2$	2139	2171	630290	0.16	0.18	0.05	0.02	0	0
Total (%)						***3.32***	***1.7***	***2.8***	***0.36***	***0.81***	***0.39***
LACTONES											
γ-Hexalactone	L_1	$C_6H_{10}O_2$	24	1696	1694	3.5	4.59	0.69	0.39	0.93	0.69
γ-Octalactone	L_2	$C_8H_{14}O_2$	30	1907	1910	0.09	0.09	0.01	0.01	0	0.02
γ-Decalactone	L_3	$C_{10}H_{18}O_2$	2112	2138	2286887	0.63	0.64	0.31	0.12	0.06	0.09
δ-Decalactone	L_4	$C_{10}H_{18}O_2$	2151	2194	575447	0.13	0.16	0.07	0.03	0.01	0.02
γ-Undecalactone	L_5	$C_{11}H_{20}O_2$	2300	2259	669608	0.33	0.19	0.11	0.03	0.01	0.01
Total (%)						***4.69***	***5.67***	***1.19***	***0.58***	***1.01***	***0.83***
METHYLPHENOLS											
4-Methyl Phenol	Mp_1	C_7H_8O	34	2073	2080	0.18	0.11	0.17	0.03	0.04	0.04
3-Methyl Phenol	Mp_2	C_7H_8O	2079	2091	1905313	0.73	0.53	0.77	0.14	0.22	0.17
Total (%)						***0.91***	***0.64***	***0.94***	***0.17***	***0.26***	***0.2***
MONOTERPENES											
β-Myrcene	Mt_1	$C_{10}H_{16}$	7	1175	1161	0.06	0.07	0.01	0.35	0	0.03
(-)-Limonene	Mt_2	$C_{10}H_{16}$	8	1206	1199	0.1	5.44	0.42	26.11	0.11	2.19
(+-)-Limonene	Mt_3	$C_{10}H_{16}$	8	1208	1200	0.07	7.23	0.66	26.6	0.15	1.92
Linalool	Mt_4	$C_{10}H_{18}O$	20	1555	1547	20.65	11.05	0.99	3.06	1.21	0.73
4-Terpineol	Mt_5	$C_{10}H_{18}O$	21	1601	1602	0	0.04	0.01	0.07	0	0.01
Carvone	Mt_6	$C_{10}H_{14}O$	25	1727	1740	0	0.12	0.01	0.04	0	0.02
Epoxylinalool	Mt_7	$C_{10}H_{18}O_2$	26	1767	1721	0.05	0.1	0.02	0.04	0.01	0.01

trans-Carveol	Mt_8	$C_{10}H_{16}O$	28	1836	1845	0	0	0.01	0.02	0	0
Geraniol	Mt_9	$C_{10}H_{18}O$	28	1852	1847	0.31	0.26	0.33	0.09	0.08	0.07
Total (%)						***21.26***	***24.32***	***2.46***	***56.37***	***1.57***	***4.99***
NORISOPRENOIDS											
β-Damascenone	N_1	$C_{13}H_{18}O$	27	1814	1823	0.1	0.03	0.04	0.01	0.03	0.02
Total (%)						***0.1***	***0.03***	***0.04***	***0.01***	***0.03***	***0.02***
SESQUITERPENES											
Neroridol	S_1	$C_{15}H_{26}O$	33	2035	2034	1.83	1.48	0.03	0.01	0	0
Total (%)						**1.83**	**1.48**	**0.03**	**0.01**	**0**	**0**
UNKNOWN											
Unknown	U_1	Unknown	16	1429	1432	0.09	0	0.18	0	0	0
Total (%)						**0.09**	**0**	**0.18**	**0**	**0**	**0**

5.7 Figures

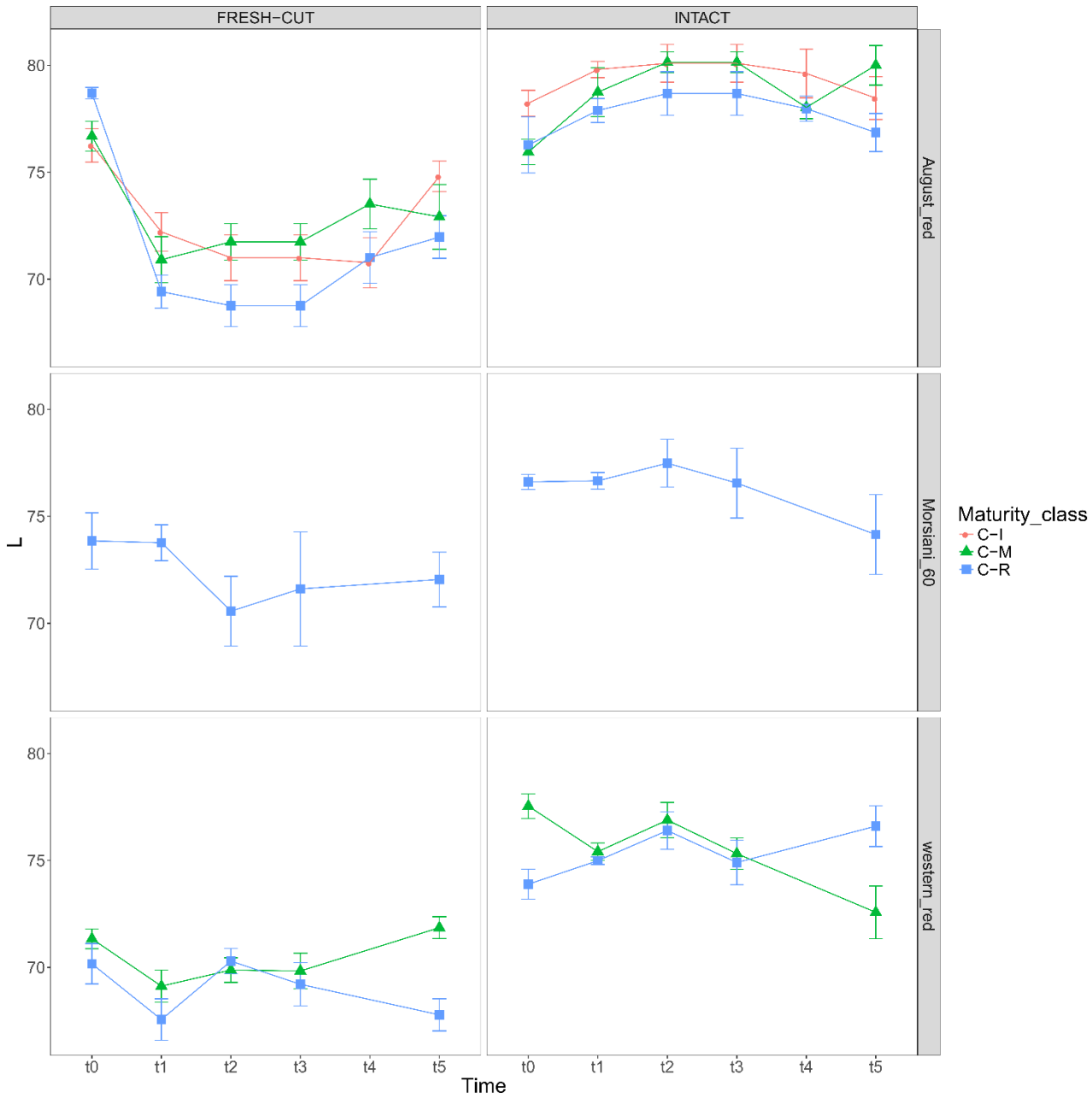

Figure 5.7.1: chromatic parameter (L*) evolution over time during refrigerated storage of both intact and fresh-cut nectarines for the different maturity classes identified.

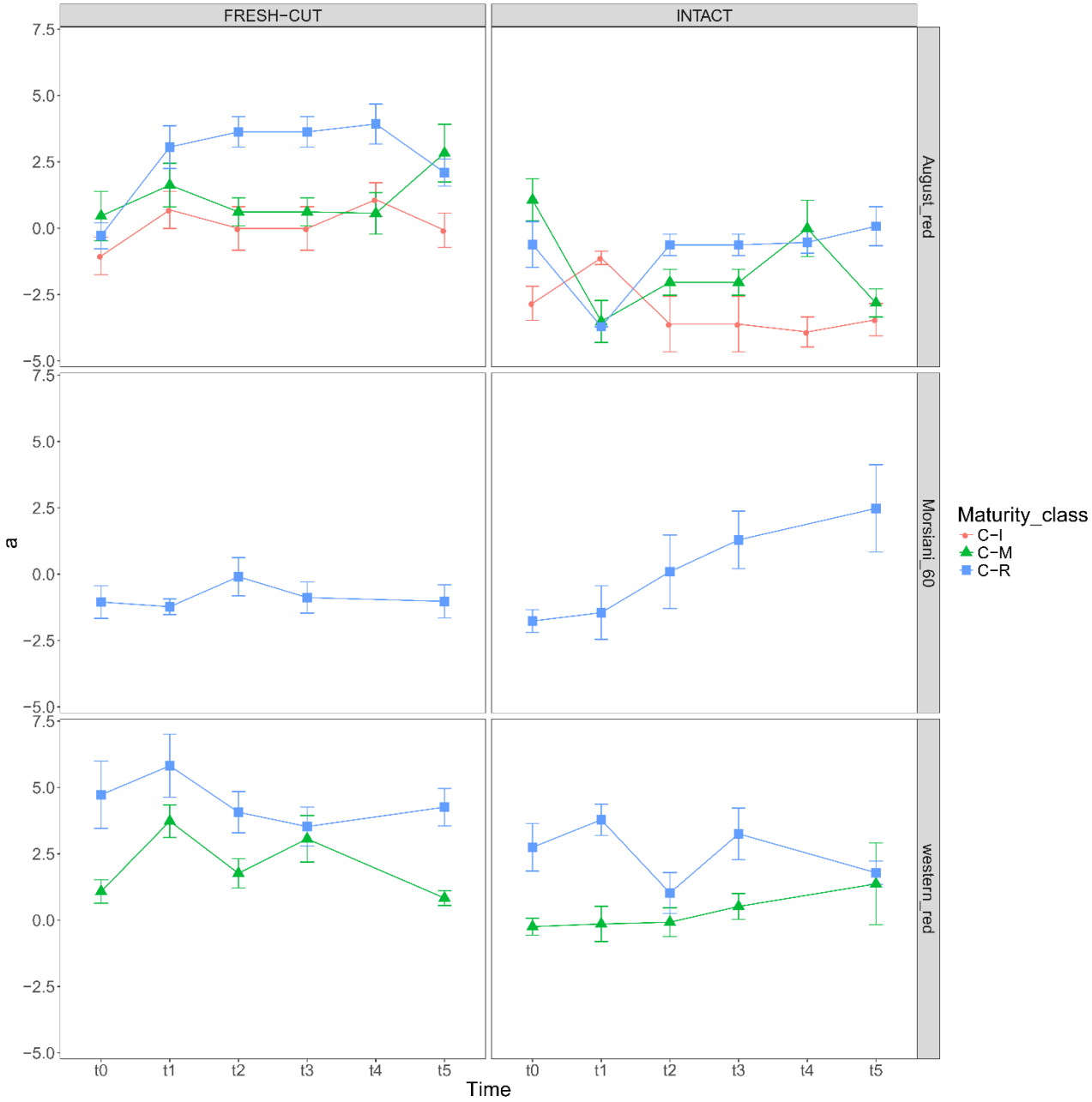

Figure 5.7.2: chromatic parameter (a*) evolution over time during refrigerated storage of both intact and fresh-cut nectarines for the different maturity classes identified.

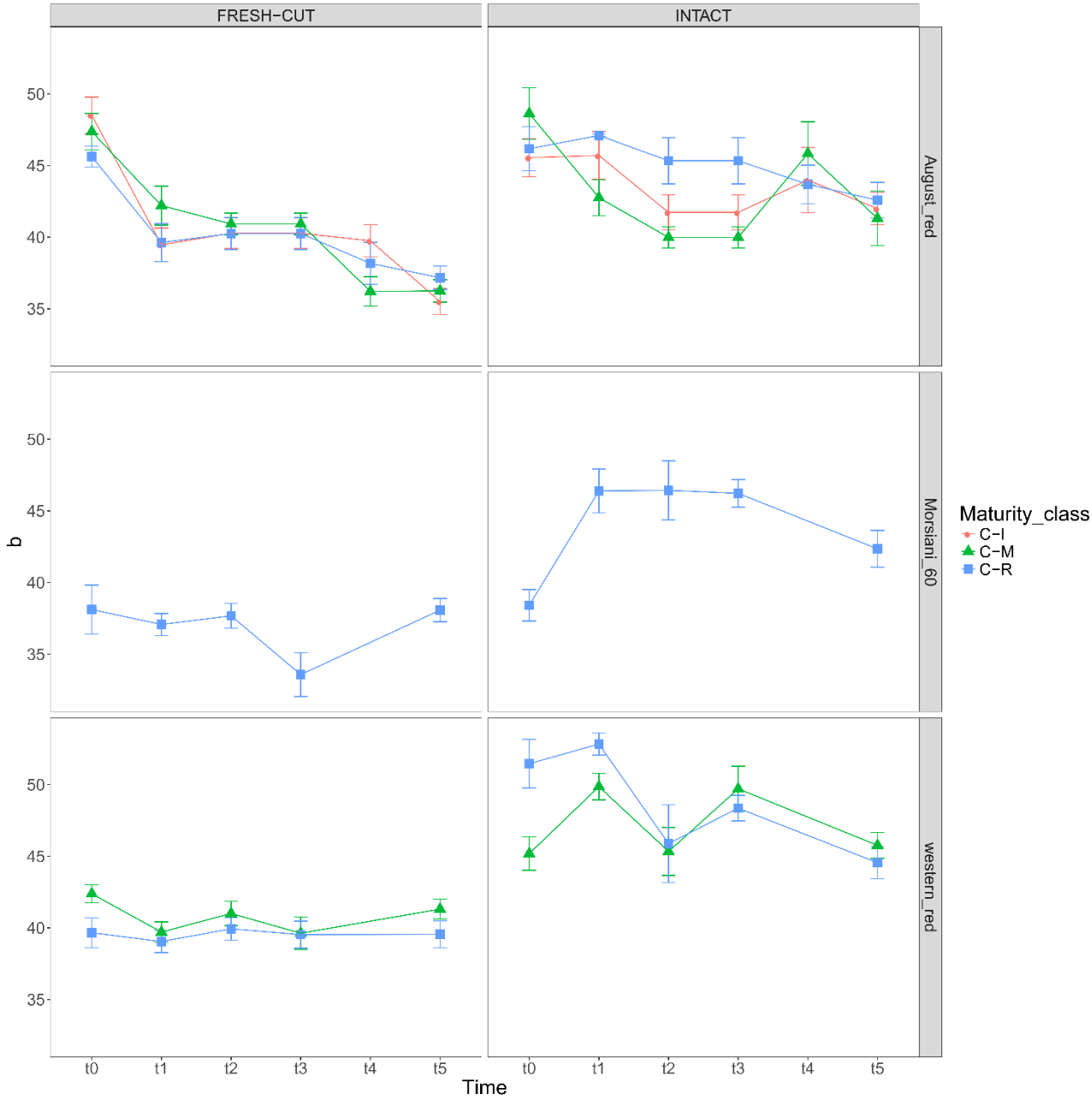

Figure 5.7.3: chromatic parameter (b*) evolution over time during refrigerated storage of both intact and fresh-cut nectarines for the different maturity classes identified.

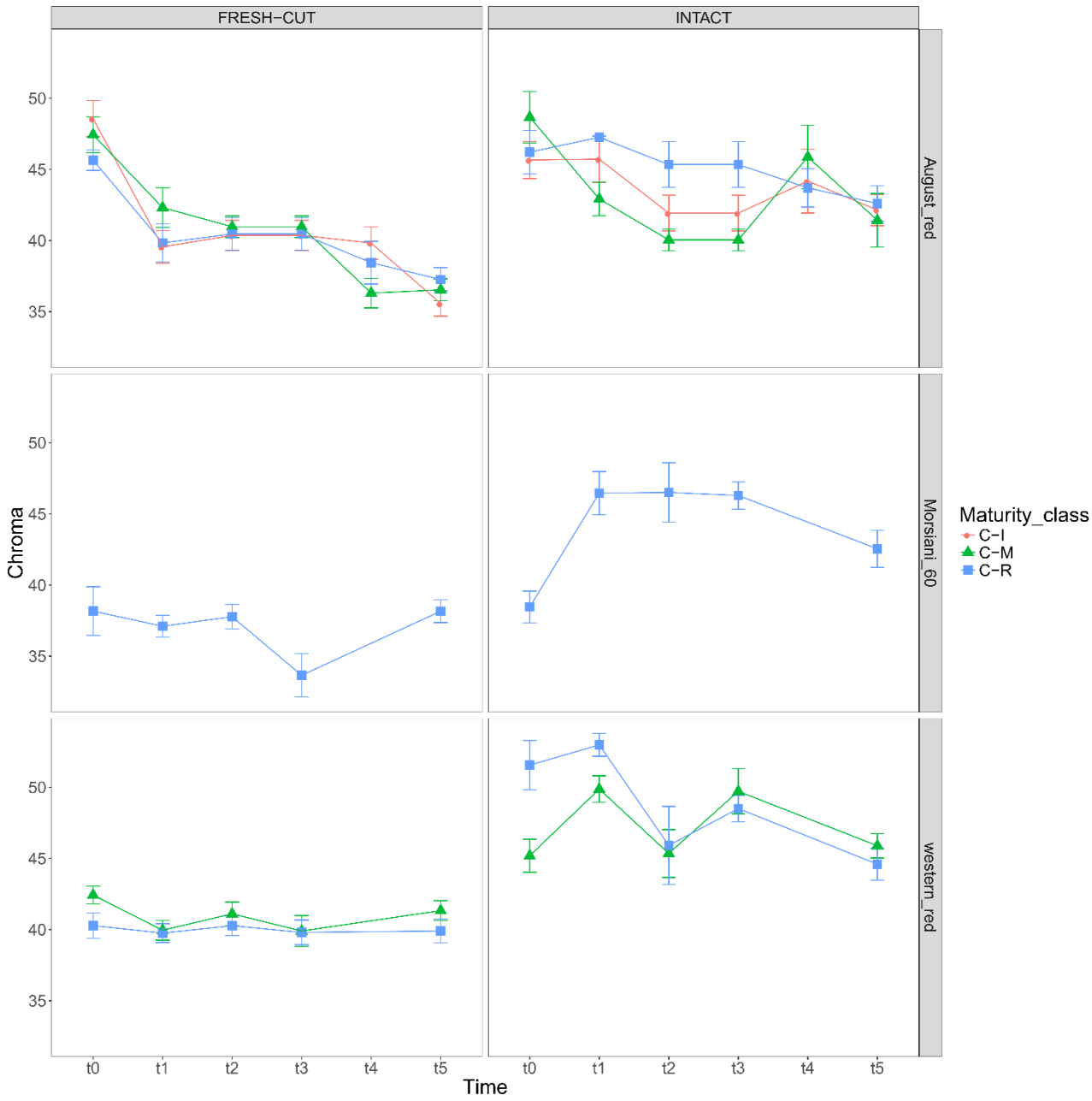

Figure 5.7.4: Chroma evolution over time during refrigerated storage of both intact and fresh-cut nectarines for the different maturity classes identified.

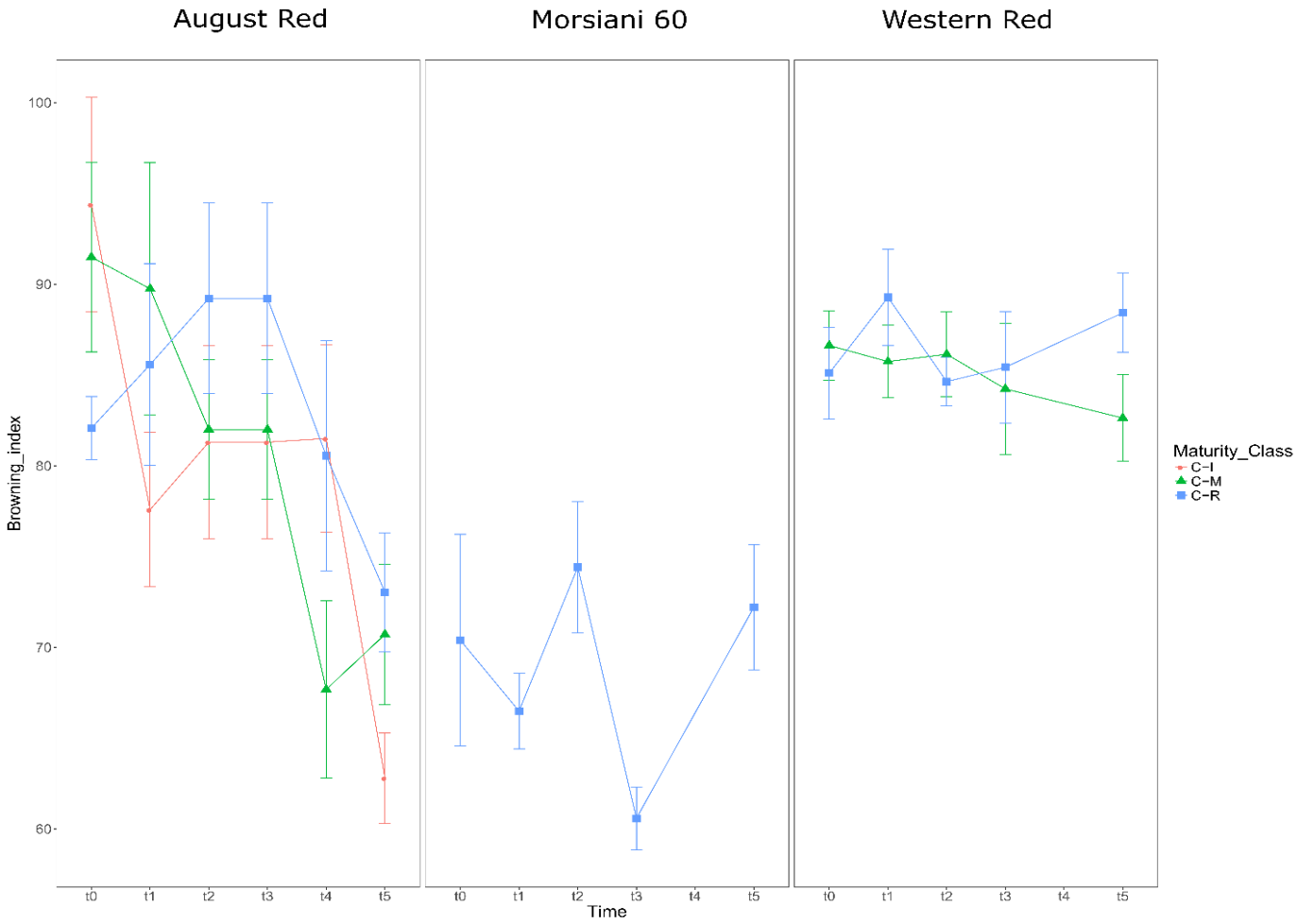

Figure 5.7.5: browning index (BI) evolution over time during refrigerated storage of both intact and fresh-cut nectarines for the different maturity classes identified.

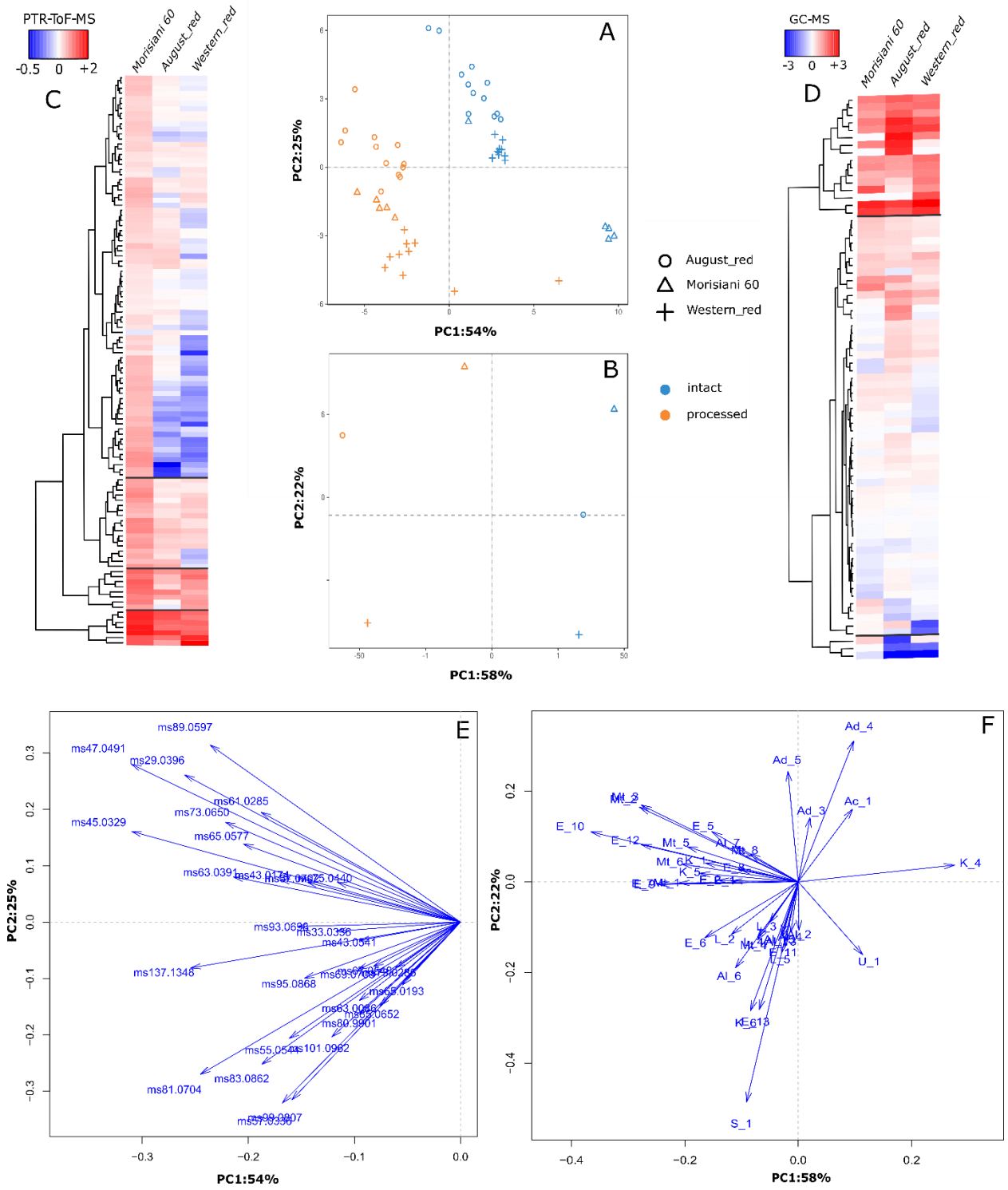

Figure 5.7.6 Analysis of the nectarines VOC profile assessed by PTR-ToF-MS and SPME-GC-MS: Principal component analysis of variance performed on PTR-ToF-MS **(A)** and SPME-GC-MS **(B)** data, respectively, obtained by intact and freshly processed fruit. Plot **(C)** and **(D)** represent the heatmaps and hierarchical dendrograms of the VOCS detected with PTR-ToF-MS and SPME-GC-MS, respectively. Heatmaps reports VOCs as Log transformed values. **(E)** and **(F)** are the loading plots of the PCA performed by PTR-ToF-MS and SPME-GC-MS data. The compound codes correspond to the IDs indicated in table 3.

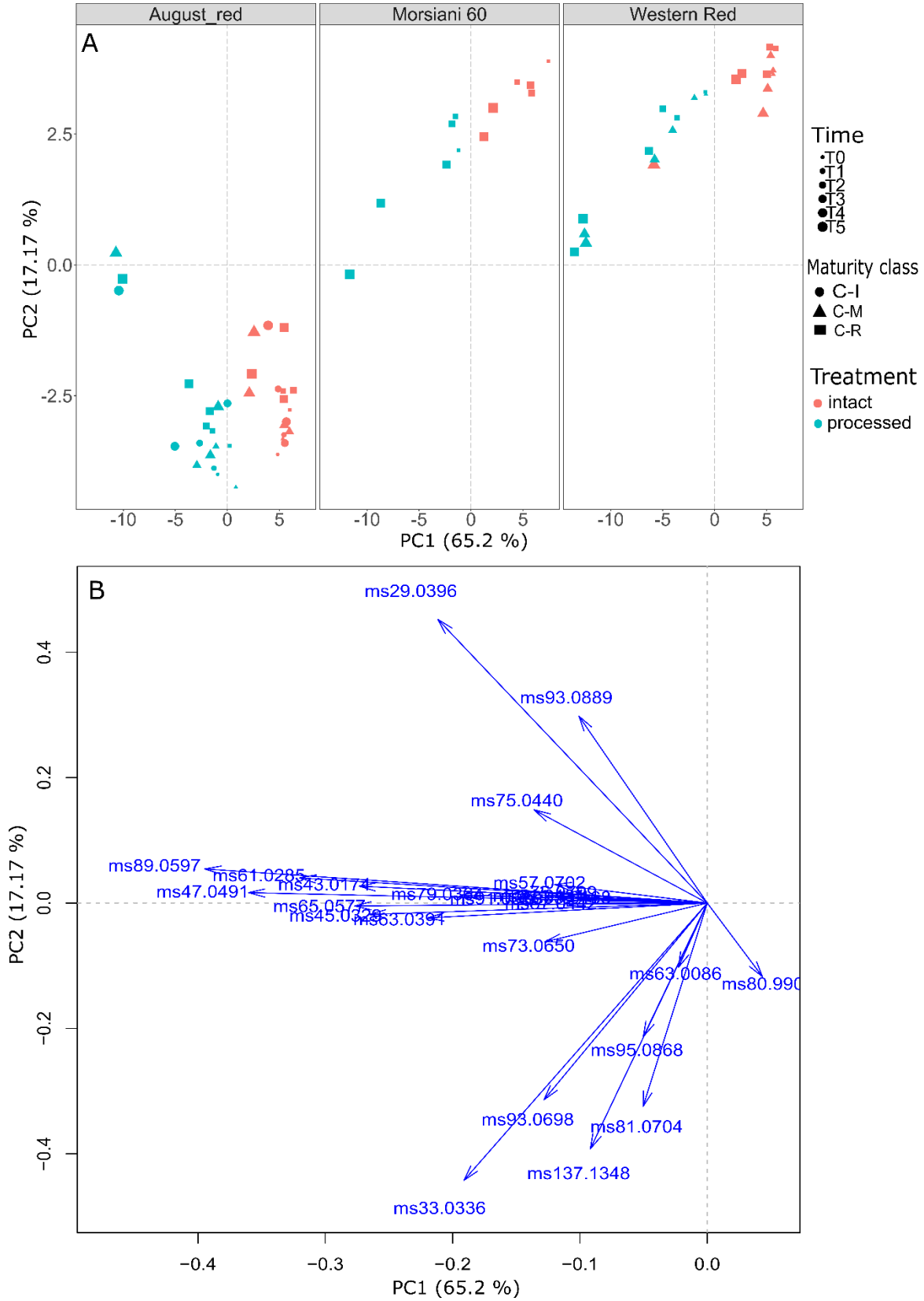

Figure 5.7.7 Multivariate Analysis of the nectarines VOC profile during storage. (A) Principal Component analysis performed with PTR-ToF-MS data measured in intact and processed fruit the during storage- **(B)** Loading plot of the correlation of each variable to the principal components.

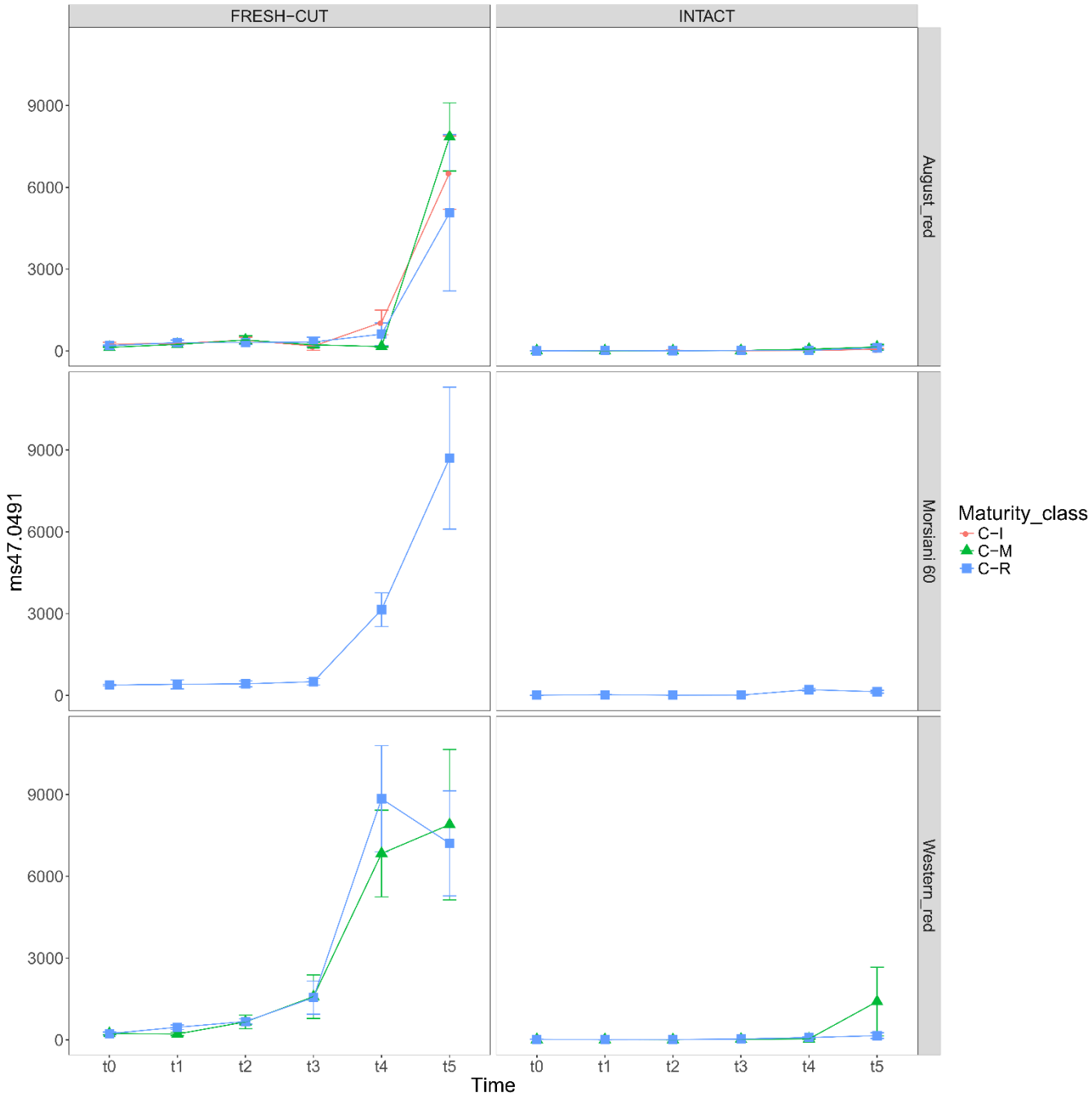

Figure 5.7.8: concentration (ppbv) trends of Ethanol (*m/z* 47.0491) during cold storage from intact and fresh-cut fruit of the maturity classes identified (C-I: immature; C-M: mature; C-R: ripe). Bars represent (± standard error).

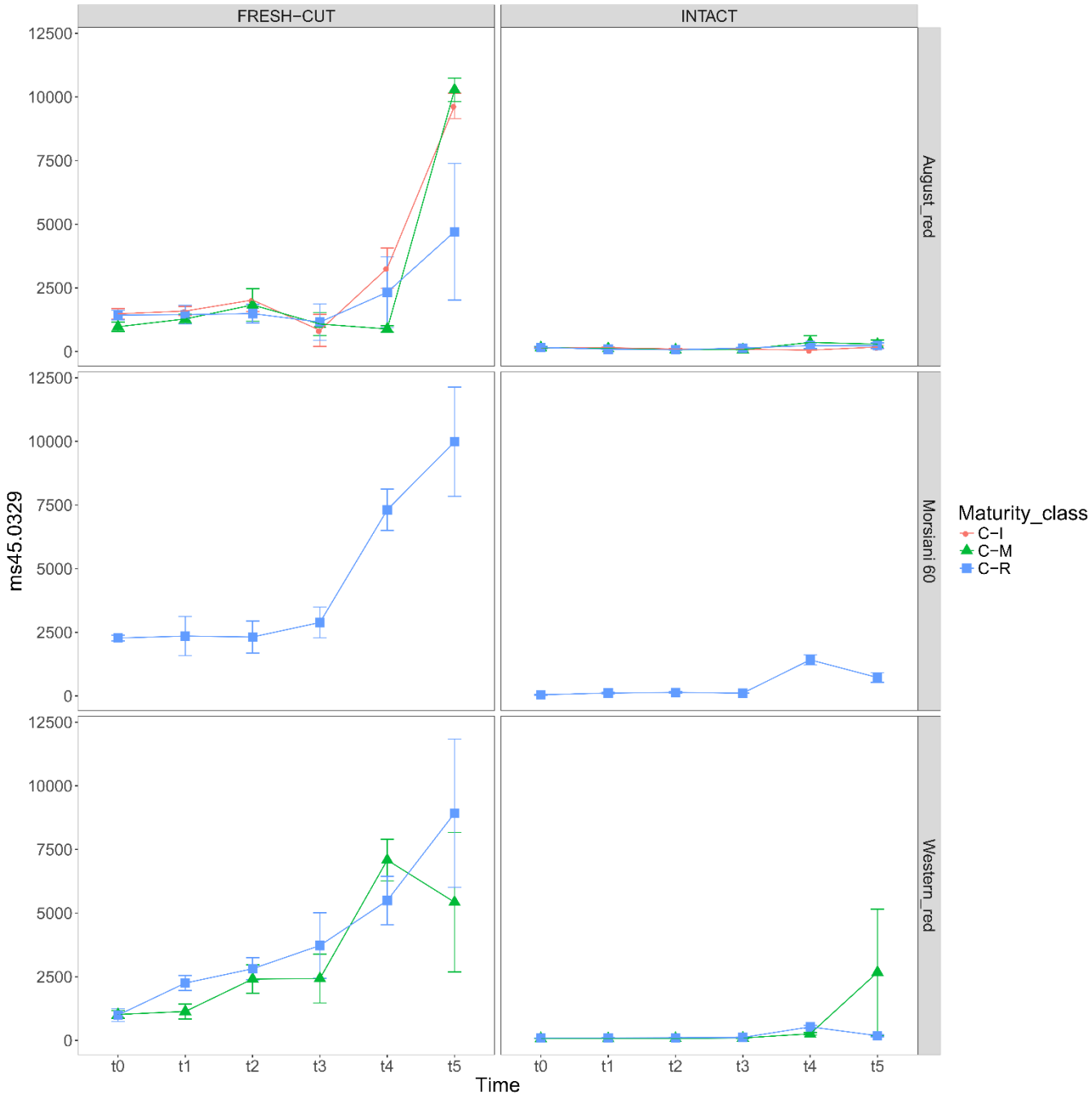

Figure 5.7.9: concentration (ppbv) trends of Acetaldehyde (*m/z* 45.0329) during cold storage from intact and fresh-cut fruit of the maturity classes identified (C-I: immature; C-M: mature; C-R: ripe). Bars represent (± standard error).

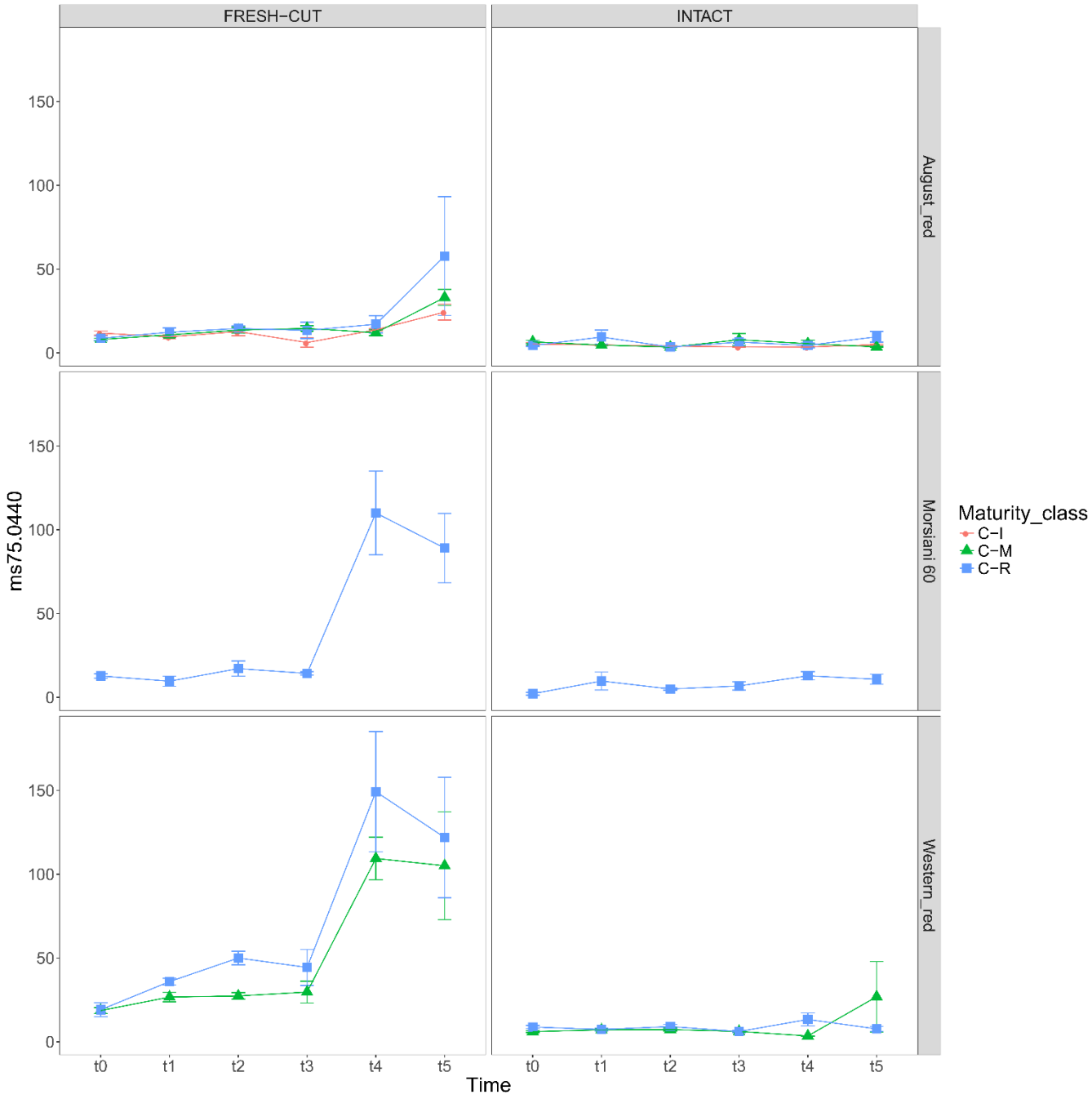

Figure 5.7.10: concentration (ppbv) trends of Ethyl Acetate (*m/z* 75.0440) during cold storage from intact and fresh-cut fruit of the maturity classes identified (C-I: immature; C-M: mature; C-R: ripe). Bars represent (± standard error).

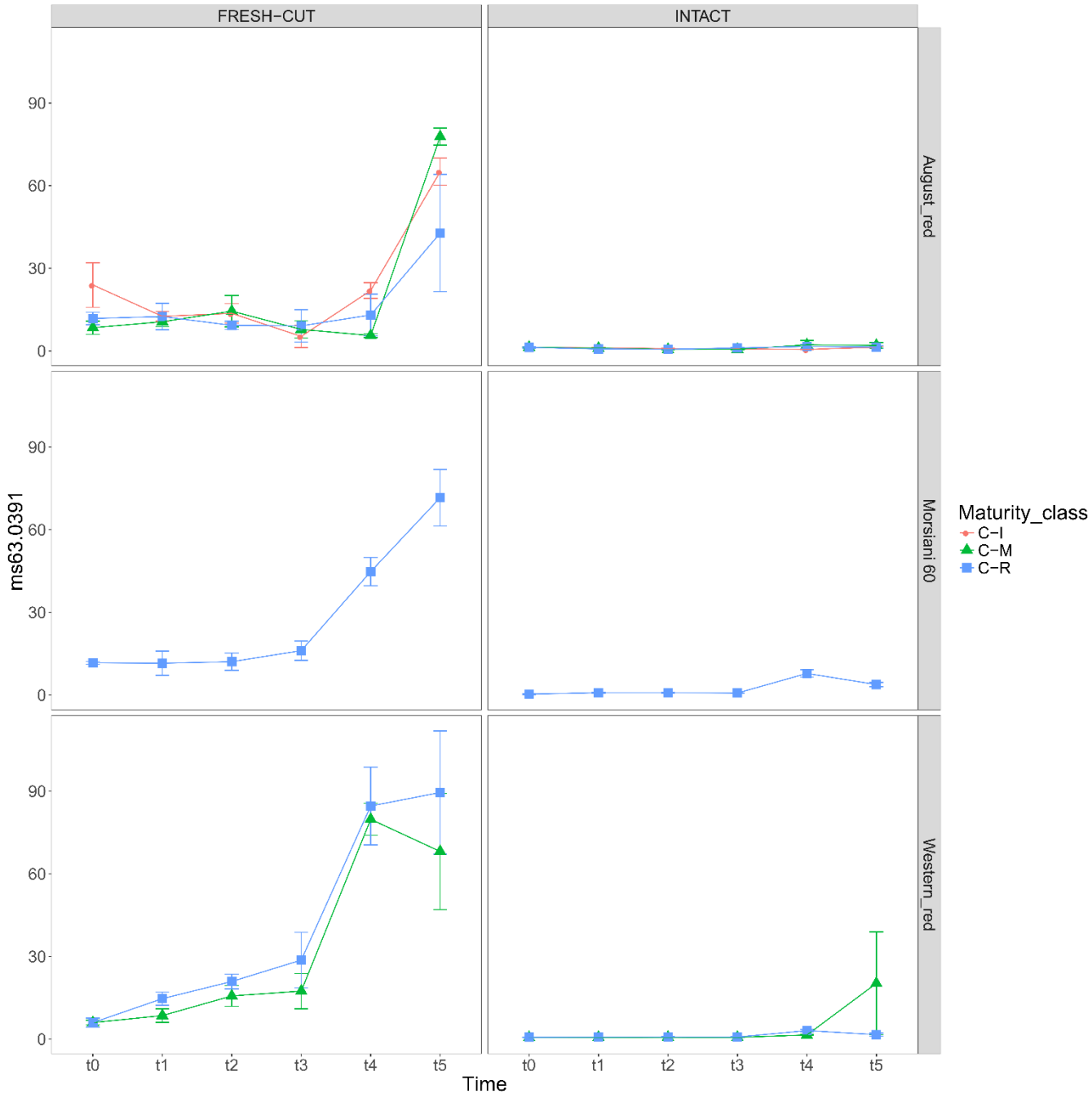

Figure 5.7.11: concentration (ppbv) trends of Sulphur compounds (*m/z* 63.0391) during cold storage from intact and fresh-cut fruit of the maturity classes identified (C-I: immature; C-M: mature; C-R: ripe). Bars represent (± standard error).

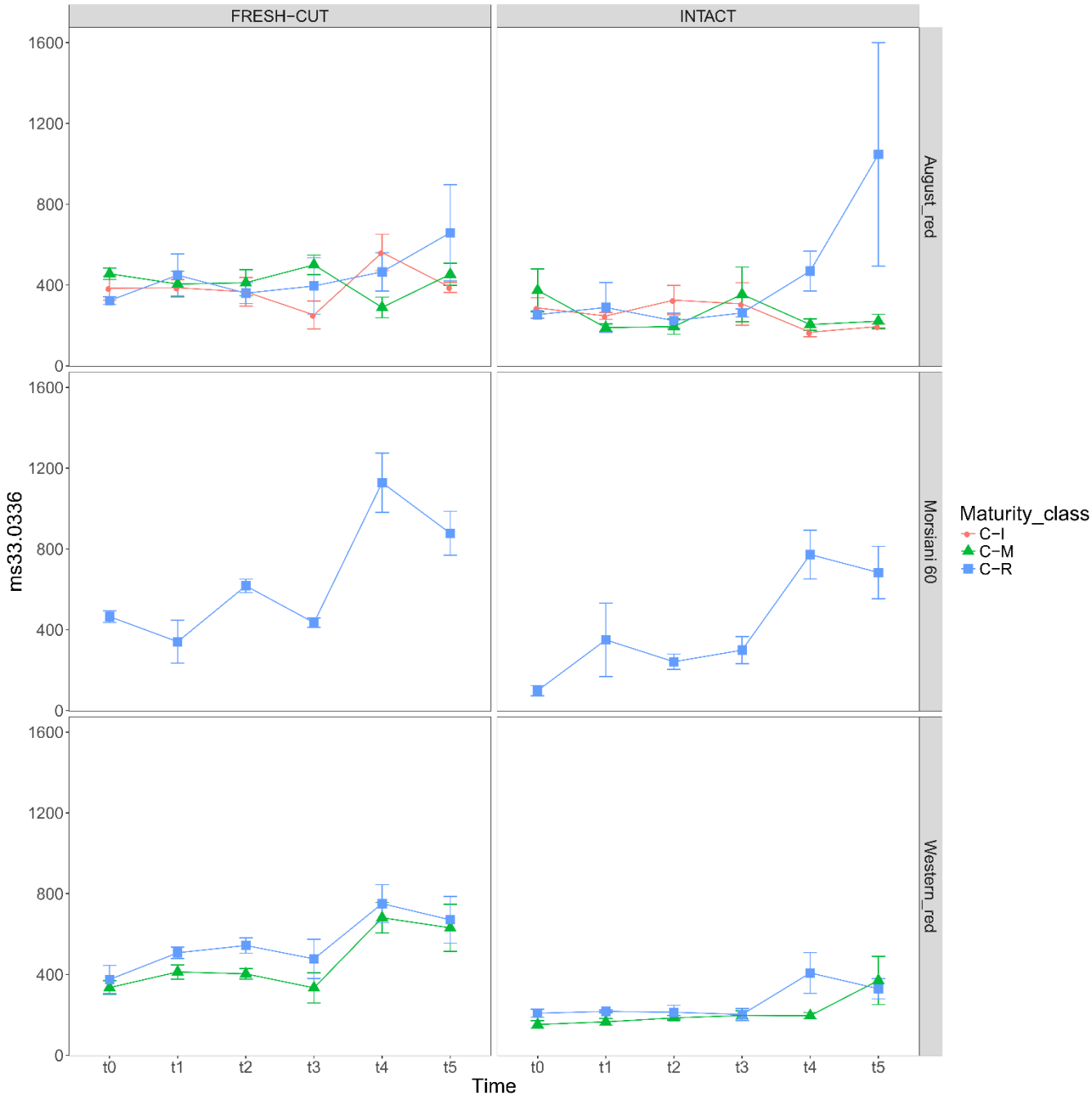

Figure 5.7.12: concentration (ppbv) trends of Methanol (*m/z* 33.0336) during cold storage from intact and fresh-cut fruit of the maturity classes identified (C-I: immature; C-M: mature; C-R: ripe). Bars represent (± standard error).

6. General conclusions

The present work has contributed to unravel the complex relationships between peach and nectarine flavour, the fruit maturity stage at harvest and different postharvest and storage conditions.

Fruit harvested at different maturities presented clear differences of quality traits and VOCs responsible for the flavour. When fruit were submitted to cold storage the volatile profile of the nectarines was generally enhanced, reducing the differences present at harvest and determining small variations in the VOCs emission between the maturity classes. This may reflect a positive contribution of cold storage in improving peach quality in case of fruit harvested prior the physiological maturity. Ethylene emission was significantly enhanced in fruit submitted to cold temperatures and therefore enhanced the emission of the VOCs during shelf life irrespective of the maturity stage of the fruit.

However, harvest maturity represents a key component in determining the evolution of peach quality, as fruit collected at the physiological maturity were generally characterized by better flavour properties even prior cold storage.

Cold storage tended to fasten the reduction of firmness when fruit were subsequently submitted to shelf life at ambient temperatures, which represent a commercialization limit as fruit became overly soft after only few days. Finally, prolonged cold storage may induce storage disorders such as chilling injury, which represent a conspicuous storage impediment, especially if the cultivar is exported to far-off destinations.

This work has also investigated the VOC profile of different nectarine cultivars in response to fresh cut processing as tool to improve the marketability of peaches and nectarines.

Fresh-cut processing, induced a major variation in the volatile profile of the nectarines, enhancing the emission of the major groups of aroma VOCs. Although the genetic differences of the cultivars affected the behaviour of the fruit, common responses to processing were the immediate release of VOCs associated with positive sensations, and the production of off-flavour volatiles over time in cold storage. Also in this case, the maturity of the fruit at harvest did not induce major differences in the aroma profile of the fruit either intact or processed.

The visual appearance of the processed fruit did not variate during cold storage, therefore the aroma profile of the processed fruit becomes one of the key aspects of fresh-cut nectarine marketability. The discrepancy between the visually perceived freshness of the product at purchase, and the perception of off-flavours at eating may reduce the consumers likelihood of repurchase. The application of smart packaging solutions must be adopted during storage, to grant high quality standards on the market.