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The influence of volatile organic compound release, texture and microstructure on the perception of apple flavour

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Abstract

Aroma and texture are known to influence the overall perception of apple flavour and are subconsciously evaluated by consumers during consumption. Aroma is derived from the volatile organic compounds (VOCs) released from the apple both before (orthonasal) and during consumption (retronasal). The degree to which the VOC impacts upon the flavour profile is affected by the mechanical breakdown of the apple flesh during mastication. The characteristics of apple texture, microstructure and breakdown are cultivar specific. This thesis aimed to understand the cultivar specific differences of apple texture, microstructure and VOC release in relation to their role in overall flavour perception and to explore the correlations between sensory derived attributes and instrumental derived parameters.

In vitro (orthonasal) and *in vivo* (retronasal) measurements of VOC release were carried out using either a proton transfer reaction quadrupole mass spectrometer (PTR-QUAD-MS) or a proton transfer reaction time of flight mass spectrometer (PTR-ToF-MS). Both instrumental techniques separated cultivars based on their *in vitro* headspace volatile organic compound (VOC) profiles of either intact or cut apple portions. However, PTR-ToF-MS was found to be superior to PTR-QUAD-MS due to its higher sensitivity. The *in vivo* nosespace method involving the measurements of dynamic VOC release from the nasal cavity, during swallowing as panellists consumed apple portions, resulted in improved data and temporal resolution, as well as an increase in the number of detectable nosespace VOCs when the PTR-ToF-MS was used. Ester related VOCs were predominantly detected in the *in vivo* nosespace measurements followed by carbonyl and alcohol related compounds. Measured nosespace parameters showed potential for use in understanding differences in the mechanical breakdown of apple flesh during mastication; with for example juicy cultivars inducing a faster time to first swallow (T_{swal}). Panellists also displayed longer consumption times (T_{con}) when consuming firm samples, as opposed to softer cultivars. In a preliminary study using the PTR-QUAD-MS, the time to maximum VOC intensity (T_{max}) was found to be shorter for soft apple flesh owing to their faster breakdown during mastication and their resulting increase in surface area. However, the PTR-ToF-MS study which used more cultivars suggested

that T_{max} could also be shorter for firm juicy cultivars, owing to the occurrence of more swallowing events during mastication as juice was released.

The feasibility of being able to differentiate between cultivars using solely instrumental techniques was investigated by comparing instrumentally derived results to those gained from descriptive sensory analysis. Sensory odour/ flavour data was compared to VOC profiles obtained from the PTR-ToF-MS, while sensory texture was compared to mechanical and acoustic textural parameters. Both analytical and sensory techniques characterised the cultivars similarly, indicating the potential of solely instrumental techniques to separate apple cultivars based on their VOC profiles and textural properties. Different VOC groups (terpenes; esters and their associated fragments; butanoate esters) were associated to specific sensory attributes. For example, butanoate esters (m/z 103, 117, 131) were positively correlated to lemon flavour and odour whereas hexyl acetate (m/z 145) was positively correlated to overall apple odour signifying its importance towards base apple flavour. Physico-chemical properties such as titratable acidity (TA) were positively correlated to sour taste which could be considered in prediction models. Sweet taste was negatively correlated to TA but was not correlated to soluble solids content (SSC). However, a positive correlation between sweet taste and fruity ester related compounds (m/z 43, 61, 145) was observed indicating a possible aroma-taste interaction. Instrumentally derived texture parameters were well correlated to sensory descriptions. However, the former also provided additional information that could differentiate between cultivars that were firm but not crunchy.

As the behaviour of apple flesh breakdown during mastication was shown to affect perceived texture, X-ray micro-computer tomographic (µ-CT) scanning was used to investigate microstructural differences that could affect apple texture. Morphological properties of apple parenchyma such as connectivity and porosity were associated with VOC release, whereas anisotropy was associated with firm texture. Porosity was negatively correlated to firm texture however, this was cultivar specific owing to the fact that porosity is a summation of all intercellular spaces within a volume; it does not take into account the variation in size and distribution which has been shown to affect texture. The use of an ethylene inhibitor, 1-

methylcyclopropene (1-MCP) retained the apple texture at a cost of lower VOC emission. However, its efficacy favoured cultivars of high ethylene concentration (Golden Delicious) as opposed to low ethylene emitting cultivars (Fuji). Therefore, it is important to understand the effects of 1-MCP on specific cultivars.

This study demonstrated the viable use of PTR-MS and texture measurements to understand the interrelationships between texture and aroma release during consumption. These techniques were shown to characterise apple cultivars similarly to sensory measurements. Moreover, μ-CT scanning proved to be a powerful tool to visualise the role of microstructure in mechanical texture. Overall, these analyses showed that apple cultivars are distinctly different and suggest that such information could be used to compile a database of phenotypes, such as the cultivar specific VOC and texture characteristics. This database could be used as a reference to create high quality apples through careful selection of specific traits in order to create customised apples targeted towards consumer specific needs.

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Publications and conference presentations

Publications:

- Ting Valentina JL, Soukoulis Christos, Silcock Patrick, Cappellin Luca, Romano Andrea, Aprea Eugenio, Bremer Phil J, Märk Tilmann D., Gasperi Flavia, Biasioli Franco. In Vitro and In Vivo Flavor Release from Intact and Fresh-Cut Apple in Relation with Genetic, Textural, and Physicochemical Parameters. Journal of Food Science, 77 (11): C1226 - 1233. **[Accepted: 16th July 2012]**

- Ting Valentina JL, Silcock P., Bremer P. J., Biasioli F.. X-Ray Micro-Computer Tomographic Method to Characterize Different Apple Cultivars. Journal of Food Science, 78 (11): E1735 – 1742. **[Accepted: 1st September 2013]**

- Farneti B., Khomenko I., Cappellin L., Ting V., Romano A., Biasioli F., Costa G., Costa F. 2014. Comprehensive VOC profiling of an apple germplasm collection by PTR-ToF-MS. Metabolomics: 1-13. **[Accepted: 16th October 2014]**
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- Ting Valentina JL, Romano A., Silcock P., Bremer P.J., Corollaro M.L., Soukoulis C., Cappellin L., Gasperi F., Biasioli F. Apple flavour: Linking sensory perception to volatile release and textural properties. Journal of Sensory Studies. **[Submitted: December 2014]**

Conference poster presentations:

- Valentina Ting, Christos Soukoulis, Luca Cappellin, Eugenio Aprea, Samuel Heenan, Patrick Silcock, Flavia Gasperi and Franco Biasioli. In-vitro and in-vivo on-line monitoring of flavour compound release during consumption of fruits from different apple cultivars. **Presented at: 2nd MS Food Day; 19-21 October 2011; Trieste, Italy.**

- Valentina JL Ting, Christos Soukoulis, Eugenio Aprea, Andrea Romano, Patrick Silcock, Phil Bremer, Luca Cappellin, Flavia Gasperi, Franco Biasioli. In-vitro and in-vivo volatile organic compound (VOC) release from intact and fresh-cut apple cultivars in relation to their genetic, textural and physicochemical properties. **Presented at: NZIFST conference 2012; 26-28 June 2012; Hamilton, New Zealand. Selected for 3minute poster competition. Awarded 1st prize for poster presentation**

- Valentina JL Ting, Christos Soukoulis, Eugenio Aprea, Andrea Romano, Patrick Silcock, Phil Bremer, Luca Cappellin, Flavia Gasperi and Franco Biasioli. In-vitro and in-vivo flavour release from intact and fresh-cut apple in relation with textural and physicochemical parameters. **Presented at: 2nd International Conference on Food Oral Processing – Physics, Physiology and Psychology of Eating; 1-5 June 2012; Beaune, France [Not in attendance, presented by Romano A.]**

- V.J.L. Ting, C. Soukoulis, P. Silcock, P. Bremer, F. Gasperi, F. Biasioli et al. In-vitro and In-vivo Flavour Release From Intact and Fresh-cut Apple In Relation with Textural and Physico-chemical Parameters. **Presented at: 5th European Conference on Sensory and Consumer Research; 9-12 September 2012; Bern, Switzerland [Not in attendance, presented by Romano A.]**

- Ting V. JL, Soukoulis C., Aprea E., Romano A., Silcock P., Bremer P., Cappellin L., Gasperi F., and Biasioli F. In-vitro and in-vivo flavour release from intact and fresh cut apple in relation to textural and physicochemical parameters. **Presented at: 6th Rosaceous Genomics Conference; 30th September – 4th October 2012: Mezzocorona, Trento, Italy.**

- Valentina JL Ting, Christos Soukoulis, Eugenio Aprea, Andrea Romano, Patrick Silcock, Phil Bremer, Luca Cappellin, Flavia Gasperi and Franco Biasioli. In-vivo Volatile Organic Compound (VOC) Release from Fresh-Cut Soft and Firm Apple Cultivars. **Presented at: 6th International PTR-MS Conference 2013: 3rd – 8th February 2013; Universitätszentrum Obergurgl, Obergurgl; Austria. Awarded 3rd prize for poster presentation.**

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List of abbreviations and definitions of terms

µ-CT	Micro-computer tomography
1-MCP	1-Methylcyclopropene
ACC	1-Aminocyclopropane carboxylic acid
ACO	ACC oxidase
AED	Acoustic envelope detector
ANOVA	Analysis of variance
AUC	Area under the curve
BBD	Braeburn browning disorder
CA	Controlled atmosphere
CCD	Charge coupled device
CoA	Coenzyme-A
cps	Counts per second
DIMS	Direct injection mass spectrometry
DMAP	Dimethylallyl diphosphate
DMC	Dry matter concentration
FEM	Fondazione Edmund Mach
Fl.	Flavour
FPP	Farnesyl diphosphate
GA-3P	Glyceraldehyde-3-Phosphate
GGDP	Geranylgeranyl diphosphate

GGP	Geranyl diphosphate
HS	Headspace
IEC	Internal ethylene concentration
IFM	Individual factor map
I_{max}	Maximum intensity
IPP	Isopentenyl diphosphate
IS	Intercellular space
LOX	Lipoxygenase
m/z	Mass to charge ratio
MCP	Multichannel plate
MFA	Multi factor analysis
MVA	Mevalonic acid
MW	Molecular weight
NASE	Nosespace air sampling extension
NS	Nosespace
N_{swals}	Number of swallows
Od.	Odour
PCA	Principal component analysis
PLS	Partial least square
ppbv	Parts per billion by volume
pptv	Parts per trillion by volume
PTR-QUAD-MS	Proton transfer reaction quadrupole mass spectrometer

PTR-ToF-MS	Proton transfer reaction time of flight mass spectrometer
REV	Representative elemental volume
RH	Relative humidity
Rv	Regression vector
SAM	S-adenosyl-L-methionine
sccm	Standard cm^3 per minute
SD	Standard deviation
sec	Seconds
SEM	Scanning Electron Microscopy
SF	Smart Fresh™
SSC	Soluble solids content
TA	Titratable acidity
T_{con}	Time for total consumption
Td	Townsend
TDS	Temporal dominance of sensations
T_{max}	Time to maximum intensity
T_{swal}	Time to first swallow
VOC	Volatile organic compound
w/ w	weight per weight

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Chapter 1: Introduction

1.1 Introduction

Apples, *Malus x domestica* Borkh are climacteric fruits with an annual production of 63 million tonnes worldwide (FAS, 2012). With more than 7, 500 different cultivars to choose from a wide selection of unique taste, texture and aroma combinations are available to consumers. Although an extensive knowledge of apple aroma and texture is available, there is a limited understanding on the perception of apple flavour.

Consumers generally prefer apple cultivars which have a firm texture and a balanced sweet/ sour taste (Harker and others, 2008). Consumers appear to give little consideration to an apples aroma despite the apple's unique aroma composition along with taste being what differentiates its flavour from other cultivars (Jaeger and others, 1998). During apple consumption, aroma and taste attributes are experienced together leading to the perception of flavour. Flavour is perceived through a combined sensation of taste, aroma and chemical sensations evoked during consumption (ASTM, 2009). However, the perception of flavour can be influenced by flavour – texture interactions as the release of aroma during consumption is dependent on how quickly the food is broken down (Crocker, 1945; Delwiche, 2004). Although the influence of texture on flavour release has been investigated using model gel systems (Mestres and others, 2005; Leclercq and Blancher, 2012), studies on flavour release of unprocessed food systems are scarce. This presents the need to study interactions between flavour and texture in a real food system such as apples.

Apples have been extensively studied using descriptive sensory analysis however, apple aroma/ flavour descriptors are commonly described using generic terms such as 'overall apple flavour' (Altisent and others, 2011). This is because humans perceive aromas/ flavours as a single aromatic odorant (Livermore and Laing, 1998; Jinks and Laing, 1999) though analytically, apple aroma is a convolution of >300 volatile organic compounds (VOCs) (Yahia, 1994; Dixon, 2000). Furthermore the extent that an apple's texture influences flavour perception in sensory studies is unknown and challenging to monitor. However, these limitations open up new

avenues for investigations into flavour release using techniques such as *in vivo* nosespace analysis.

In vivo nosespace analysis is the measurement of expired air from the nose upon swallowing and is representative of the odorants perceived during eating (Land, 1996). This 'air' can be acquired using direct injection mass spectrometry (DIMS) technologies that enable real time monitoring of important VOCs. *In vivo* nosespace analysis can be carried out easily without the need for laborious panel training (Murray and others, 2001). When *in vivo* nosespace analysis is complemented by *in vitro* headspace and textural measurements, the investigation of flavour – texture interactions in apples is possible.

The influence of texture on flavour is dependent on the rate of sample breakdown during eating which for apples, is dependent on their microstructure. Earlier investigations on apple microstructure in relation to texture reported a positive correlation between porosity and a soft, mealy texture (Harker and Hallett, 1992; Allan-Wojtas and others, 2003). The development of texture softening due to a loss of cellular turgidity is associated with ripening (Rizzolo and others, 2010). Traditionally, apple microstructure has been measured using light or scanning electron microscopy (SEM). However, these techniques are unable to consider the depth or volume of porous regions. This limitation can be overcome by the use of X-ray micro-computer tomographic (μ-CT) scanners that acquire 3-D images enabling the study of the morphological properties of apples such as intercellular space size, anisotropy and porosity (Mendoza and others, 2007; Mendoza and others, 2010).

The importance of aroma, texture and taste attributes in the perception of apple flavour have been highlighted however, the effects of ripening on these attributes need to be acknowledged. As climacteric fruits, apples continue to ripen after harvest increasing VOC concentrations, texture softening and sweet taste (Blankenship and Dole, 2003). As consumer acceptability is strongly dependent on texture, postharvest treatments have focused on impeding apple ripening to retain apple firmness. The most common treatments used are cold storage and the application of an ethylene inhibitor. Although these techniques are beneficial in maintaining texture, they can impede VOC biosynthesis (DeEll and others, 2001; Johnston and others, 2002). The

efficacy of these postharvest treatments on specific apple cultivars is still debatable and requires further investigation.

This thesis will investigate cultivar differences in aroma and texture in relation to *in vivo* nosespace and *in vitro* headspace flavour release; and the effects of ethylene inhibition on apple microstructure, texture and VOC production. The overall aim is to determine cultivar specific characteristics in aroma and texture, and their influence on flavour perception. This will be explored through the following objectives:

- To explore the relationship between texture and VOC release in apples during consumption using *in vivo* and *in vitro* analysis. This will be covered in Chapters 3 and 4.
- To use descriptive sensory analysis to differentiate cultivars and investigate the feasibility of correlating experimentally derived parameters to specific cultivar dependent sensory attributes. This will be elaborated in Chapter 5.
- To investigate the effects of microstructure on texture and determine the difference in VOC release, textural and morphological properties of untreated apples and apples subjected to ethylene inhibitor treatment. This will be discussed in Chapters 6 and 7.

Chapter 2: Literature Review

In this chapter, the importance of apple texture, taste and aroma in relation to eating quality will be discussed. Figure 2.1 illustrates the main topics covered in this review. To begin, topics related to the eating quality of apples and how flavour is perceived will be elaborated. Next, to understand what determines apple flavour, characteristics such as volatile organic compounds (VOCs), texture and taste will be discussed. Lastly, this review covers how changes in VOCs, texture and taste over time can be controlled with the use of postharvest treatments. This literature review aims to identify gaps within the current knowledge.

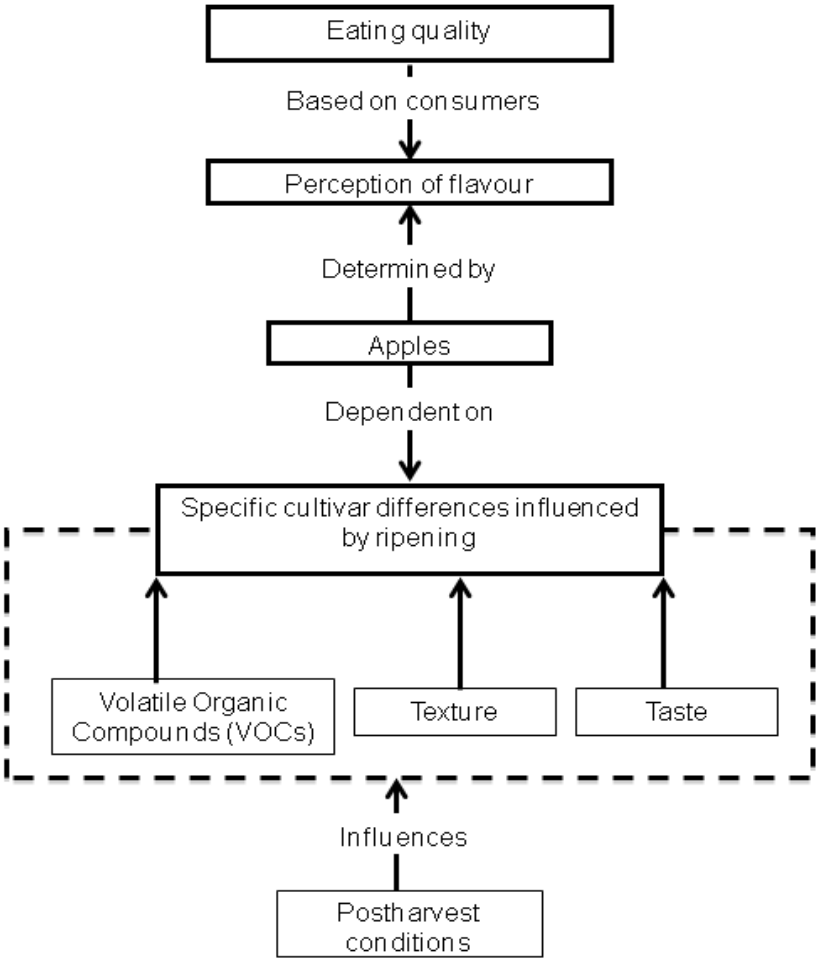

Figure 2.1: Schematic diagram of the main topics covered in the literature review

Apple texture, taste and aroma are important quality characteristics that drive consumer preference (Daillant-Spinnler and others, 1996; Jaeger and others, 1998; Harker and others, 2003). Consequently, changes in texture, taste and aroma during ripening may cause consumers to accept or reject specific apple cultivars. In this section, specific apple texture, taste and aroma traits that consumers prefer will be discussed. In terms of texture, consumers prefer firm, crisp and crunchy apples associating these terms with the freshness of the product (Fillion and Kilcast, 2002; Szczesniak, 2002). However, during ripening, softening of the fruit can occur resulting in a mealy, floury or grainy texture that may cause consumers to reject the fruit (Andani and others, 2001). Therefore, the structural and mechanical aspects of apple texture can play a major role in shaping consumer preference. This will be elaborated later in this review (Section 2.3.5).

Sweet and sour tastes are dominant attributes in apples and are determined by the ratio of available sugars (sucrose, fructose, glucose) and acids (malic acid) (Yahia, 1994). The available sugars and acids are instrumentally measured as Brix˚/soluble solids content (SSC) and titratable acidity (TA) respectively. Compared to apple texture in which firmness is almost universally preferred, consumer preference for apple taste can be segmented into those that prefer sweet, hard apples or sour, juicy apples (Daillant-Spinnler and others, 1996; Harker and others, 2008). This segmentation may be due to the different sugar and acid ratios between cultivars. For example, Granny Smith contains a low sugar: acid ratio and is appreciated for its sour flavour while high sugar: acid ratio cultivars such as Red and Golden Delicious are enjoyed for their sweet taste. Interestingly, the acceptance of sweet or sour fruit only increases when the fruit is firm, with acceptance for apples that were unacceptably soft regardless of SSC or TA values remaining unchanged (Harker and others, 2008).

Although texture and taste attributes in apples are important, it is the specific VOCs representing aroma that distinctly characterises an apple cultivar from other varieties (Jaeger and others, 1998). The perception of apple aroma is the result of a convolution of mainly esters, aldehydes, alcohols and ketones (Dimick, 1983) (the biosynthesis of these compounds will be covered later Section 2.3.3). The study of apple aroma dates back to 1920 when apple aromas were concentrated from fresh apples through steam distillation. The resulting apple concentrate was found to be predominantly made up of acetaldehyde and esters of formic, acetic and hexanoic acids (Power and Chesnut, 1920). Technological advancements such as the introduction of gas chromatography mass spectrometry (GC-MS) and GC-olfactometry (GC-O) have increased the list of known VOCs and aromas recognised in apples. Some examples of important VOCs and their aroma descriptors include hexenal (grassy/ green aroma), butyl acetate (sweet fruity aroma), 2-methylbutyl acetate (overall fruity apple aroma), ethyl butanoate (fruity pineapple odour), hexyl acetate (fruity pear aroma), α-farnesene (green, herbaceous) and hexyl hexanoate (apple/ cucumber aroma) to name a few (Karlsen and others, 1999; Plotto and others, 1999; Mehinagic and others, 2006). A number of these VOCs are often perceived by consumers and a higher preference towards cultivars with fruity VOCs has been reported (Daillant-Spinnler and others, 1996; Jaeger and others, 1998).

In this section, the eating qualities of apples were discussed. Consumers generally prefer firm, crisp, juicy apples which have a fruity aroma and a balanced sweet-sour taste. During apple consumption, taste and aroma attributes are perceived simultaneously creating the perception of flavour. The interaction between taste, aroma and texture will be addressed in the following section.

2.2 Flavour perception

Flavour is a perception resulting from a combined stimulation of the ortho and retronasal olfaction, taste and chemesthetic receptors within the oral cavity. In other

words, flavour perception includes the combined effects of tastes sensations, VOCs and chemical feelings evoked by the product introduced to the oral cavity (ASTM, 2009). Although flavour perception has been defined as combined stimulations within the nasal and oral cavity, other factors such as touch, auditory and visual cues can also influence flavour perception by consumers when making food choices (Zampini and Spence, 2012). For apples, the expectations of flavour begin as a visual inspection of the fresh produce followed by orthonasal aroma. Once these attributes have been observed, consumers form expectations around an apple's flavour and texture.

The acts of eating and perceiving flavour are innate behaviours, which are used almost unconsciously in daily life. Due to their complexity, the understanding of these behaviours is incomplete despite numerous studies. This is because the three sensory modalities (texture, taste and aroma) that make up flavour interact with each other differently. From this point forward 'flavour' refers to retronasal aroma, which is the air enriched with VOCs from the oral cavity and expired through the nasal cavity during swallowing. In contrast, 'aroma' refers to orthonasal aroma, which is the aroma perceived by smelling. Previous investigations on taste - aroma interactions showed that the sense of taste and aroma are often perceived together and are difficult to separate (Delwiche, 2004; Auvray and Spence, 2008). For example, vanilla aroma has been consistently reported as sweet smelling although sweetness is sensed by the stimulation of taste (Stevenson and Boakes, 2004). Moreover, the presence of an aroma may heighten the perception of a taste if the taste - aroma pairs are commonly encountered together. For example, when sucrose was added to a fruit juice, aroma ratings of 'berry-like', 'fruity' and 'sweet odour' increased as well as perceived sweetness. On the other hand, 'green' and 'vinegar' ratings decreased (von Sydow and others, 1974). Flavour - texture interactions have been extensively researched. In general, a solution of high viscosity such as that of hydrocolloid thickeners (Cook and others, 2003; Boland and others, 2006) or yogurt (Kora and others, 2003; Saint-Eve and others, 2006) leads to a decrease in both taste and flavour (Delwiche, 2004). In addition, the release of flavour during swallowing is assumed to be controlled by texture as the accessibility of the VOCs from the food product is dependent on the time needed to breakdown the food matrix (Crocker, 1945).

Previous studies have shown how the perception of flavour can be influenced by taste-aroma and aroma-texture interactions. Therefore, to study the perception of apple flavour, the use of sensory studies is of great importance. However, apple aroma as a single modality is a complex convolution of VOCs. Furthermore, consumers perceive this convolution of VOCs as apple aroma by processing these VOCs and expressing it as a single entity, as if it was a single odorant. Studies to understand the capability of trained panellists to discriminate and identify odorants within a mixture concluded the panellists were only able to identify up to four odorants within a mixture (Livermore and Laing, 1998; Jinks and Laing, 1999). This could be why there is currently a lack of apple flavour descriptors and it is commonly described using a single generic attribute such as "apple flavour" (Altisent and others, 2011). Efficient instrumental technologies have the possibility of carrying out in-depth investigations on apple flavour and could ultimately reduce the cost associated with using human participants and, increase throughput, enabling the measurement of more samples objectively with variation arising solely from the sample itself. Aprea and others (2012) investigated the potential of predicting perceived sensory flavours based on GC-MS measurements of apples. They reported that odour properties were better predicted than flavour, perhaps due to underlying texture - aroma interactions as previously discussed. Therefore, a possible way to understand how a range of consumers perceive flavour and to differentiate/characterise complex products such as fresh apples without the need for intensive training could be through the use of *in vivo* nosespace studies.

2.2.1 *In vivo* nosespace

In vivo nosespace studies involve the measurement of VOCs expired through the nose after swallowing and are considered to be a feasible representation of the retronasal olfaction perceived by a consumer under normal eating conditions (Land, 1996; Bojanowski and Hummel, 2012). To understand how *in vivo* measurements are carried out, the pathways that food takes following consumption has to be considered. A schematic diagram of food pathways illustrating the steps for bolus formation and swallowing is shown in Figure 2.2. Food is first introduced into the oral cavity. The food is then masticated until a bolus is formed for swallowing.

Consequently, a build-up of VOCs within the headspace of the oral cavity occurs due to the increased surface area of the food products during mastication. During swallowing, the epiglottis closes off the trachea preventing food or drink from entering the lungs. The air which was previously within the oral cavity, containing the VOCs from the broken down food is now expelled through the nose. This VOC enriched air produces a retronasal element of flavour to the food (Land, 1996). The released air also known as 'swallow breath' enables a consumer to perceive the flavour of the product.

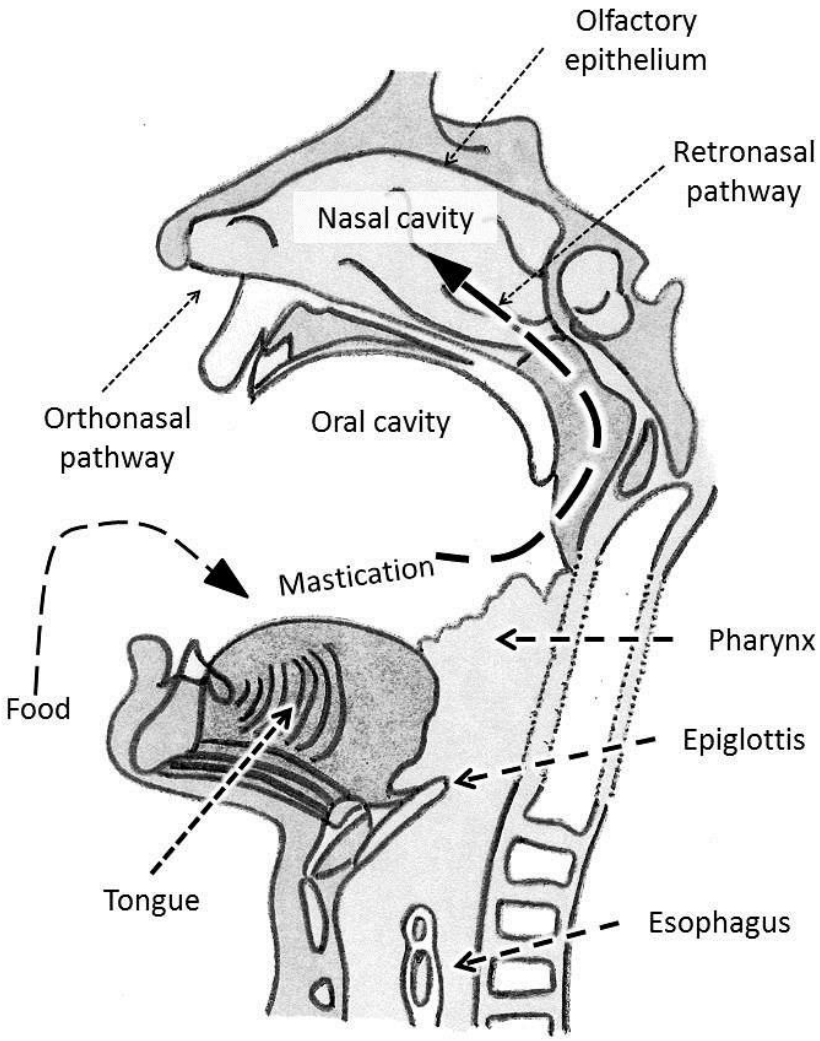

Figure 2.2: Schematic diagram of ortho and retronasal cavity depicting food introduction, mastication and flow of VOCs entering initially through the nose during orthonasal breathing, followed by mastication and the release of VOCs in the oral cavity which exits the nose through the retronasal pathway after swallowing (Adapted from Benjamin (2012)).

During *in vivo* nosespace measurements, there are many factors affecting the swallowing and mastication behaviour of a panellist such as the rheology or texture of the food product which strongly determines the way foods are broken down in the mouth (Buettner and Beauchamp, 2010; Bornhorst and Singh, 2012). For example, a dry biscuit is masticated longer compared to a juicy orange segment in the oral cavity before bolus formation. Moreover, inter-individual variation in oral physiology arising from factors such as dentition, salivation, chewing force, mucosa, and swallowing rate can vary depending on the matrix used (Buettner and Beauchamp, 2010). Considering these factors, the variability that may arise from sensory and *in vivo* measurements is not entirely unexpected. As Romano and others (2014) have shown, variation between panellists was far larger than variation between samples tested. Leclercq and Blancher (2012) have attempted to minimise this variation by comparing an imposed and free mastication profile, but found no beneficial contributions from using the former. Despite the variations, the use of a free mastication profile is still considered to be beneficial to understand the flavour perceived by individual consumers regardless of their physiological differences.

In vivo nosespace has conventionally been measured by concentrating the 'swallow breath' onto a Tenax trap before eluting it into a GC-MS. Some products studied using this technique include fresh strawberries (Ingham and others, 1995) and strong mints (Linforth and Taylor, 1993). Although GC-MS is an indispensable benchmark for the analytical identification and quantification of VOCs, the requirement for sample pre-concentrations limits the monitoring of temporal changes in VOCs in real time (Dewulf and others, 2002). Currently, direct-injection mass spectrometry (DIMS) technologies have been adopted to carry out rapid monitoring and quantification of VOCs. DIMS technologies are able to perform on-line monitoring of VOCs in concentrations down to parts per trillion per volume (pptv) without the need for sample pre-concentrations (Biasioli and others, 2011b). This enables the flavour release pattern of important VOCs such as 2-methylbutyl acetate, which impart overall fruity apple aroma, to be monitored in real time during *in vivo* nosespace measurements. Some examples of the available DIMS instruments include atmospheric-pressure chemical ionization mass spectrometry (APCI-MS), MS e-noses, proton transfer reaction mass spectrometry (PTR-MS) and selected ion-flow-

tube mass spectrometry (SIFT-MS). More information on these techniques will be discussed later in Section 2.3.4.

The data collected from the use of DIMS technologies can be used to understand the flavour release pattern of specific VOCs in different foods by plotting the breath patterns which will be referred to as nosespace profiles throughout this thesis (Figure 2.3). These nosespace profile plots enable important nosespace parameters such as Area under the Curve, AUC; maximum intensity, I_{max}; and time to maximum intensity, T_{max}, to be extracted. Furthermore, other consumption parameters related to texture that could be monitored without the need for invasive techniques include time of consumption, T_{con}; time to first swallow, T_{swal} and number of swallows, N_{swals}. All this information can be obtained without the need for extensive training as compared to conventional descriptive sensory analysis (Murray and others, 2001).

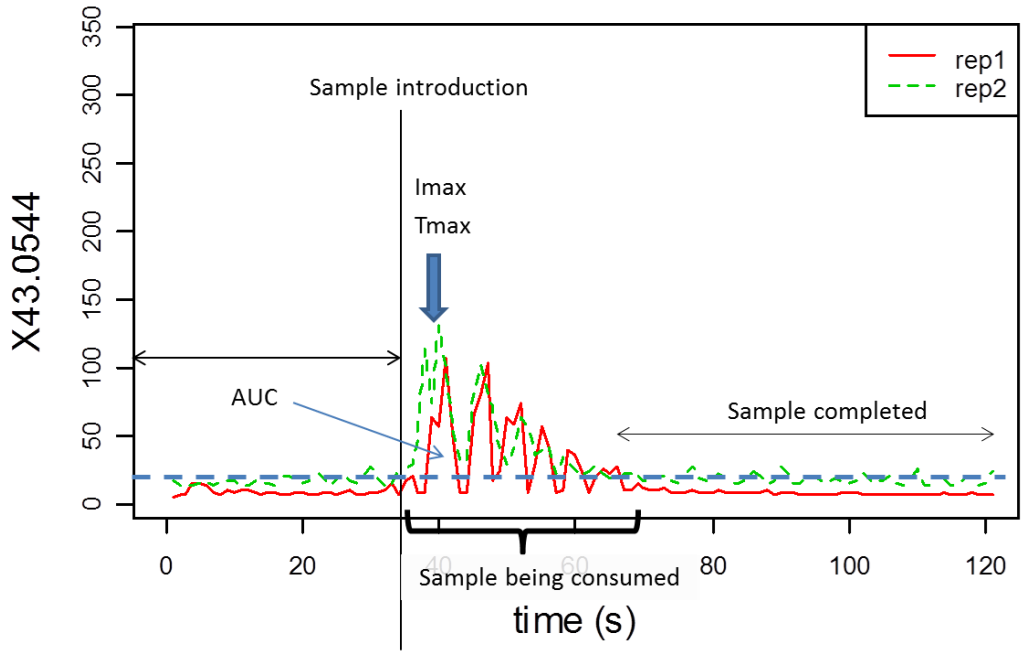

Figure 2.3: An example of an in vivo nosespace profile monitoring an ester fragment (m/z 43.0544) during apple consumption.

In the previous section, it was discussed how consumers choose to consume specific apple cultivars based on their texture and flavour. Although consumers can

describe their liking for texture, describing flavour is challenging. Not only is flavour perceived as a combination of taste, texture and aroma, it can also be influenced by taste-aroma and aroma-texture interactions. Therefore, studying the possible effects of these interactions on the perception of flavour has important implications. Apples were selected as the product of choice in this thesis because different cultivars represented by a diverse combination of taste, texture and aroma are readily available (Daillant-Spinnler and others, 1996). To understand the effects of texture, taste and aroma on the perception of flavour, one must first understand how these modalities are synthesized within an apple.

2.3 Apples

There are more than 7,500 different apple cultivars present in the world (Elzebroek, 2008), each with specific cultivar characteristics that determine their eating quality. This section discusses the properties of apples that can influence the overall eating quality including VOCs, texture and taste. However, in order to understand the biosynthesis of these attributes, it is important to first address the role of ethylene in determining the rate of change in the quality traits of VOC, texture and taste.

2.3.1 Ethylene

Ethylene is a small double bonded two carbon volatile compound that was discovered by the Russian scientist, Dimitry Neljubow in 1901 (Neljubov, 1901). However, it was not until 30 years later, that Gane (1934) found the hormone was naturally produced in plants. Following this discovery, ethylene's significance in plant ripening and development began to be appreciated (Crocker and others, 1935). Ethylene is emitted by higher plants in order to activate and control physiological changes such as plant growth, stress responses, senescence of plant organs and fruit

ripening (Bleecker and Kende, 2000). In general, the production of ethylene is regulated by two systems. System 1 is an autoinhibitory ethylene production system common to both climacteric and non-climacteric fruits for plant growth, fruit development and to aid in stress responses such as mechanical wounding, temperature fluctuations and insect infestation (Yang and Hoffman, 1984). System 2 only operates during the ripening of climacteric fruits in which a large rise in ethylene is stimulated through an autocatalytic response (McMurchie and others, 1972; Alexander and Grierson, 2002).

The biochemical pathway of ethylene biosynthesis has been well documented (Bleecker and Kende, 2000) and is often described in three steps: 1) Ethylene is synthesized using methionine which is first converted to S-adenosyl-L-methionine (SAM) by the SAM synthase enzyme; 2) SAM is transformed into 1-aminocyclopropane-1-carboxylic acid (ACC) by ACC synthase; and finally 3) ACC is converted to ethylene via ACC oxidase (ACO). In step 2, ACC syntase also produces 5-methylthioadenosine which is fed back into the methionine cycle at the cost of 1 ATP molecule per cycle to supply new methionine. This enables ethylene production to be maintained even if intracellular methionine levels are low. During periods in which the plant increases its respiration rate, the higher level of ATP produced in the cell can be used to maintain a steady supply of 5-methylthioadenosine into the methionine cycle to ultimately increase ethylene production.

Apples are climacteric fruits. This means that they continue to ripen postharvest. Ripening in climacteric fruits is characterised by a rise in respiration and a concomitant sharp rise in ethylene production (Barry and Giovannoni, 2007). Ripening induces biological, chemical and structural changes associated with changes in colour, texture, sugar: acid ratios and VOC levels (Bleecker and Kende, 2000). As ripening commences, the fruit become sweeter, produces more VOCs and changes in texture including excessive softening may occur. These changes associated with ethylene production and ripening can lead to a decrease in apple shelf life and consumer perceived quality.

Although the general role of ethylene in fruit has been established, its direct involvement on apple quality attributes such as texture and VOC production is less

well known. Gussman and others (1993) observed that Golden and Red Chief Delicious cultivars had higher internal ethylene concentrations (IEC) (production rates >100µl•kg^{-1}•hour^{-1}) and were softer in texture compared to two cultivars (PA14-238 and D101-110) which had lower IEC (production rates <10µl•kg^{-1}•hour^{-1}), suggesting a cultivar dependent positive correlation between apple texture softening and IEC during storage. To understand the role of ethylene in VOC production, researchers investigated transgenic apples with impaired ethylene production, and reported a significant reduction of ester accumulation but not of aldehydes (Dandekar and others, 2004; Defilippi and others, 2004). Using a genomics approach, the involvement of ethylene in VOC production was later confirmed in another study. It was found that ethylene primarily participated in the final stages of ester VOC production (Schaffer and others, 2007). These studies demonstrated several ways in which ethylene can alter the perceived eating qualities of apples. This aspect will be discussed more in Section 2.4.

In this section, the biosynthesis of ethylene and its influence on VOC production and texture softening of apples has been presented. The following sections will cover apple taste, the biosynthesis of VOCs and apple microstructure as a function of texture.

2.3.2 Apple taste

Sweet and sour taste are important attributes that drive consumer preference towards specific apple cultivars. Although the onset of ripening increases the sugar to acid ratio in apples, the extent of change is dependent on the cultivar (Jan and Rab, 2012). Sensory studies have been widely used to characterise the taste of apples. However, the use of instrumental measurements such as Brix˚/SSC and TA have been recently proposed to be able to be used to predict apple taste. TA has shown to be strongly correlated to sour taste and could be used to predict sour taste in apples (Harker and others, 2002b; Corollaro and others, 2014). In contrast, contradictory results were found for SSC and sweet taste. Corollaro and others (2014) reported that no correlation was found between SSC and sweet taste, whereas Harker and others (2002b) showed SSC was a good predictor of sweet taste. A reason for the

contradictory results of SSC and sweet taste could be due to the fact that apple taste is derived from the balance of sweet and sour derived from a sugar: acid ratio. This means apple cultivars with a higher sugar: acid ratio would be perceived as sweet (Kühn and Thybo, 2001). Another possibility would be the influence of taste-aroma interactions discussed previously in Section 2.2 in which cultivars may be rated sweeter due to the presence of fruity aromas.

2.3.3 Apple aroma

It has been acknowledged that the preference for apple cultivars is dependent on their distinct VOC profiles (Section 2.1). This could be why apple aroma has been extensively reviewed since 1920 (Power and Chesnut, 1920; White, 1950; Dimick, 1983; Yahia, 1994; Sanz and others, 1997; Dixon, 2000; Fellman and others, 2000; Salazar and Orozco, 2011). However, due to the broad range of existing and newly developed cultivars worldwide, coupled with constant changes that occur in the fruit between harvest and its consumption, there is still considerable interest in apple flavour research. Apple fruits exist as living structures that constantly produce more than 300 different volatile organic compounds (VOCs). The main VOCs are esters (90%) followed by alcohols, aldehydes, ketone, phenolics and sesquiterpenes. Distinct aroma differences between apple cultivars are dependent on the compositional abundance of these different VOCs, and these differences drive consumers' preference. For example, Golden Delicious apples which are described as containing fruity odours and emit high concentrations of propyl acetate, hexyl acetate and butyl acetate (Karlsen and others, 1999) whereas Granny Smith which are described as having cut grass and cucumber odours emit high amounts of 3-hexen-1-ol (Aprea and others, 2012). To study the differences in apple VOC profiles, it is important to have an understanding of the biosynthesis of these VOCs.

Biosynthesis of VOCs

The products of VOC biosynthesis in apples can be separated into primary and secondary VOCs. The 'primary' or natural volatiles are compounds produced through controlled enzymatic reactions within an intact apple fruit. 'Secondary' VOCs arise from uncontrolled enzymatic reactions. They are not normally produced

in an intact apple, but are synthesized when the plant is under stress or when its tissues are damaged by cutting, chewing, heating or homogenization (Schreier, 1984). Both primary and secondary volatiles are equally important for the consumer as the former acts as an indicator for fruit ripeness; while the latter determines the perceived flavour during consumption. Secondary VOCs are also important in processed apple products and in juice production.

The formation of primary and secondary VOCs derive from three main substrates: amino acids, carbohydrates and lipids (Sanz and others, 1997), as elaborated concisely in the following sections and summarised in Figure 2.4.

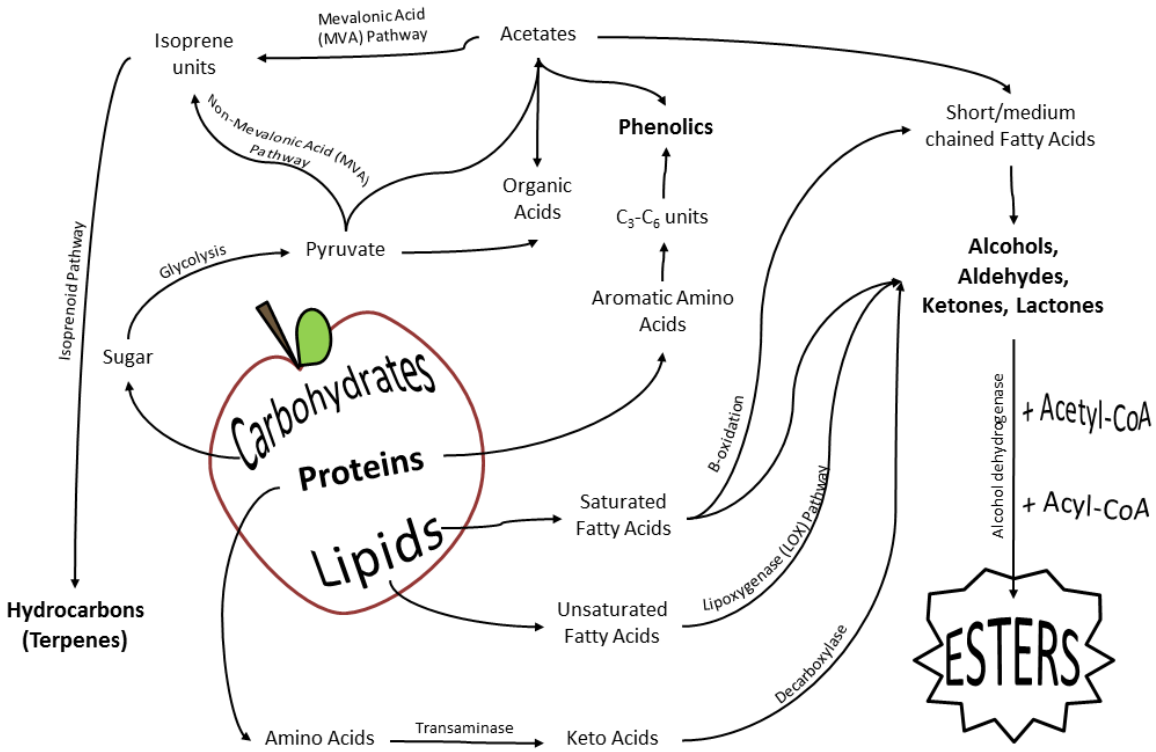

Figure 2.4: Compiled VOC production pathways in apples (adapted from Dimick (1983); Yahia (1994), Sanz and others (1997), and Dixon (2000))

Primary volatile compounds

As mentioned above, primary or natural VOCs are derived from enzymatic metabolism within the plant cells of intact fruit and are generated through

intracellular pathways (Yahia, 1994; Sanz and others, 1997). The available substrates: amino acids, carbohydrates, and lipids are used in various metabolic pathways such as glycolysis, the mevalonic acid (MVA) pathway and the β-oxidation pathway. The first requirement in the biosynthetic pathway of VOC production in apples is the availability of substrates. In the following section, the formation of different VOCs will be discussed based on their substrate requirements and specific pathways.

Amino Acids

In apples, the amount and composition of amino acids is highly regulated during fruit development (Ackermann and others, 1992). Amino acids act as direct precursors in generating primary VOCs which in some cases determine the primary aroma in specific apple cultivars. Branch chained esters, alcohols, carbonyls and acids are generated from three different enzymatic pathways: transaminase, decarboxylase and alcohol dehydrogenase (Figure 2.4) (Sanz and others, 1997).

The direct involvement of amino acids in ester production has been investigated. Rowan and others (1996) demonstrated the role of amino acids in the production of branch chained esters such as ethyl-2-methylbutanoate and hexyl-2-methyl-butanoate, which are important character impact compounds in apples (Mehinagic and others, 2006). These VOCs are characterized as being apple-like, green or fruity and give an impression of ripeness (Paillard, 1990). When either apple peel or whole apple was exposed to radioactive deuterium-labelled isoleucine the labelled deuterium was incorporated into 2-methylbutyl and 2-methylbutanoate ester products confirming the role of isoleucine in VOC production (Rowan and others, 1996). Other reactions that occur involving amino acids such as valine and leucine have been shown to be used in the production of 2-methylpropyl or 2-methylpropanoate; 3-methylbutyl or 3-methylbutanoate and acetate esters respectively (Rowan and others, 1998; Sugimoto, 2008).

Carbohydrates

Although carbohydrates are one of the primary compounds produced during photosynthesis, only a small range of VOCs are produced from them. Terpene VOCs are produced using carbohydrates and biosynthesized through the isoprenoid pathway. In apples, these compounds occur in the form of monoterpenes or

sesquiterpenes and are a result of the conversion of carbohydrate metabolism intermediates, such as Acetyl-Coenzyme A (CoA), pyruvate and Glyceraldehyde-3-Phosphate (GA-3P). These terpenes are a part of the isoprenoid family which have physiological functions such as plant defence, hormonal regulations and electron transport (Sanz and others, 1997).

Sesquiterpenes and monoterpenes are synthesized through two separate pathways. Sesquiterpenes are formed through the Acetyl-CoA/ MVA pathway in the cytosol. Monoterpenes, diterpenes and carotenoids are formed through the GA-3P/ Pyruvate pathway (non-MVA pathway) (Figure 2.4). Although the starting materials are different, both pathways form isopentenyl diphosphate (IPP) (a five-carbon, C_5 molecule) and its isomer dimethylallyl diphosphate (DMAP) (collectively also known as 'isoprene units'). The enzymes belonging to the prenyl transferases family further catalyse IPP and DMAP to form the terpenoid direct precursors, geranyl diphosphate (GGP, C_{10}), farnesyl diphosphate (FPP, C_{15}), and geranylgeranyl diphosphate (GGDP, C_{20}) (Figure 2.3) (Lichtenthaler and others, 1997; Defilippi and others, 2009).

In apples, the acyclic sesquiterpene hydrocarbon, α-farnesene ($C_{15}H_{24}$) is a terpene that has a characteristic green odour. This compound is usually found in higher concentrations in the peels of waxy apple (e.g. Granny Smith) (Mookherjee and others, 1984). Interestingly, α-farnesene has been associated with the physiological defect of superficial scald, which occurs as a scald in specific cultivars (E.g. Red Delicious, Granny Smith) but not in other resistant varieties (e.g. Gala, Empire) (Paliyath and others, 1997; Watkins and others, 2005). The formation of superficial scald starts through the oxidation of α-farnesene which is first seen as a light brown lesion on the skin. As scalding becomes more severe, the cells beneath the lesion start to collapse forming a dark brown sunken lesion (Bain and Mercer, 1963), that spoils the appearance of the apple and decreases its value.

Lipids

Lipids are one of the major precursors of VOC formation in plants via two distinct pathways. β-oxidation is the main metabolic pathway that produces primary VOCs through the intracellular pathways within an intact fruit. While the

lipoxygenase (LOX) pathway accounts for the largest variety of different secondary VOCs that are formed after the disruption of cell tissue through mastication, juicing or crushing (Schreier, 1984). Both pathways produce straight chain esters (Figure 2.4). The following section will focus on these two pathways.

The understanding of the role of lipids as substrates in ester production dates back to a study conducted by Bartley and others (1985) who stated that during ripening, the rate of phospholipid degradation increases thus acting as a probable supply of lipids in VOC synthesis. A study conducted by Song and Bangerth (2003) recovered nine different fatty acids from apples, with five being considered important due to their increase or decrease during fruit development. These were C16:0 (Palmitic acid), C18:0 (Stearic acid), C18:1 (Oleic acid), C18:2 (Linoleic acid) and C18:3 (Linolenic acid). Amongst these, the first two are saturated fatty acids and the others, unsaturated fatty acids. These fatty acids participate in different pathways to produce a range of different VOCs. Nie and others (2005) also reported that free fatty acids increased prior to an increase in VOC and then decreased during the increase of VOC in the post-climacteric stage, and they showed that alcohols formed via the β-oxidation pathway contained the same number of carbons as their aliphatic acids further confirming the role of lipids in VOC production. It is important to note that β-oxidation predominantly acts upon saturated fatty acids whereas the LOX pathway utilizes unsaturated fatty acids (next section) (Figure 2.4).

The role of fatty acids in ester synthesis is integral to providing acetic, butanoic and hexanoic acids (Song and Forney, 2008). These are then reduced into their corresponding alcohols before being transesterified into esters. Even numbered fatty acids produce butanol and hexanol whereas odd numbered fatty acids produce propanol and pentanol (Yahia, 1994). Although β-oxidation of saturated fatty acids predominantly occurs, in some cases α-oxidation occurs whereby hexanoic acid is oxidised to pentyl or pentanoate esters (Rowan and others, 1999).

Secondary volatile compounds

The LOX pathway is the main route of secondary VOC production. VOC formation is caused by but is not limited to cell rupture that occurs during cutting, bruise damage or mastication. The LOX pathway also occurs during ripening. LOX

enzymes and their substrates are separated in different cellular locations during fruit maturation. As the fruit ripens, cell walls degrade and become more permeable enabling the enzymes to travel and reach substrates, thus creating new VOCs. Sanz and others (1997) have reported that although the LOX pathway is highly active when the cells are disrupted, there is a reduced capacity for VOC production under controlled atmosphere (CA) conditions in which the composition of different gases is altered depending on the product needs. This is because many LOX enzymes require oxygen to catalyse conversions of substrates. Further studies observed the importance of oxygen in this pathway as a larger quantity and variety of flavour compounds were found in cut apples compared to intact apples (Guadagni and others, 1971; Defilippi and others, 2005).

As mentioned in the previous section, unsaturated straight chain ester VOCs arise only through the LOX pathway (Rowan and others, 1999). During cell disruption, the LOX pathways are highly active and function to degrade unsaturated C_{18} fatty acids, forming C_6 and or C_9 cleavage products that are highly aromatic. These products are subsequently converted into alcohols, of no longer than six carbon atoms by aldehyde dehydrogenase, which can contribute towards ester synthesis (Rowan and others, 1999; Fellman and others, 2000; Song and Bangerth, 2003). VOC formed from the LOX pathway are important for 'green' notes in apples. The largest ester produced in apples is hexyl hexanoate which is characterized by two C_6 chain units. Although, hexanal has not been found in intact fruit, hexenyl acetate has been found as an apple VOC suggesting some hexanal was used directly in ester biosynthesis (Fellman and others, 2000).

This section has discussed the formation of different VOCs derived from different available substrates. It has reported that both primary and secondary VOCs contribute to eating quality. The differences in VOC abundance and composition determine the characteristic aroma of specific cultivars, such as the green apple odour in Granny Smith apples derived from α-farnesene.

To measure and quantify these VOCs, all studies referred to in this section predominantly utilised GC-MS, which has also be used in earlier *in vivo* nosespace studies (Section 2.2.1). Although GC-MS is the gold standard in headspace analysis,

low time resolutions, requirements of high sample concentration and long operational times are some of its disadvantages (Dewulf and others, 2002). Moreover, the experimental samples needed for the characterization of apple VOCs are relatively large and are limited by time because the samples often need to be measured at the same maturity. These factors render the use of GC-MS impractical. To overcome these limitations, techniques that do not rely on chromatographic separation such as DIMS technologies have been proposed (Biasioli and others, 2011b).

2.3.4 VOC measurements

Direct injection mass spectrometry (DIMS) techniques have become widely used to characterise VOC profiles of fresh apples. A recent review from Biasioli and others (2011b) covered various DIMS systems available in the market today such as MS e-nose, APCI-MS and PTR-MS. MS e-nose has been used to characterise numerous food samples such as the aroma of beer (Vera and others, 2011), licorice roots (Russo and others, 2014) and cumin (Ravi and others, 2013). However MS e-noses cannot measure continuous sample injections and as a result are not viable for *in vivo* nosespace measurements (Biasioli and others, 2011b). APCI-MS can measure samples continuously and have been widely used to study the *in vivo* aroma release during consumption of food products such as cheese (Taylor and others, 2000; Gierczynski and others, 2007; Tarrega and others, 2011; Labouré and others, 2014), yogurt (Saint-Eve and others, 2006) and curry (Hatakeyama and others, 2014). However, the ionizations that occurs in the APCI is complex due to non-specific ionization agents (Biasioli and others, 2011b). The PTR-MS technique is considered to be superior to that of the MS e-nose and APCI-MS due to several advantages such as real time monitoring of VOCs with increased sensitivity with low detection limits (pptv), continuous sampling and controlled ionization using a specific precursor ion source (Biasioli and others, 2011b). PTR-MS has been used in both *in vitro* headspace and *in vivo* nosespace studies for coffee (Romano and others, 2014), dry cured ham (del Pulgar and others, 2011), bread (Heenan and others, 2009), cereal bars (Heenan and others, 2012) and apples (Cappellin and others, 2012a; Soukoulis and others,

2013; Farneti and others, 2014). In this thesis, PTR-MS was used to measure *in vitro* and *in vivo* VOCs from apples.

Proton transfer reaction mass spectrometry

The basis of PTR-MS originated from a combination of the idea of chemical ionisation by Munson and Field (1966) and the 'flowing afterglow' method used to study ion-molecule reaction kinetics in the 1960s (Ferguson and others, 1969). The afterglow method involved injection of a solution into an inert buffer gas containing small amounts of neutral reactants to achieve reactions at thermal or near thermal collision energies. The term afterglow refers to the bright flow created by a discharge of light emitted by electrically excited constituents in the gas, which flows from the source region into the buffer gas, hence the name flowing afterglow. The original concept did not include a primary ion selection procedure prior to reaction, therefore complicating the kinetic analysis (Ferguson and others, 1969). Current PTR-MS instruments have overcome this weakness by using known primary ions/ reactants in which the kinetic analysis of the ion-molecule reaction can be calculated.

In a PTR-MS measurement, water is introduced into the hollow cathode of the ion source to undergo a soft chemical ionisation, producing hydronium ions, H_3O^+, which acts as primary reactants (Figure 2.5). The hydronium ions then flow into the drift tube. VOCs that are going to be analysed are introduced into the drift tube. Here, reactant gas and hydronium ions collide and a proton transfer reaction may occur (Equation 2.1). This soft chemical ionisation is important in reducing the chance of VOC fragmentation (Figure 2.5). The hydronium ion has a low proton affinity (165.2 kcal/mol) and engages in proton transfer reactions with most organic VOCs. This allows measurements to be carried out using ambient air as a carrier or buffer gas without the need to dilute samples during measurements as the proton affinity of water is lower than most VOCs but larger than the elements in ambient air.

$$H_3O^+ + VOC \rightarrow VOC{\bullet}H^+ + H_2O \qquad \text{Equation 2.1}$$

The protonated ion is separated based on: 1) nominal mass by a quadrupole mass analyser (PTR-QUAD-MS) and detected as counts per second (cps) from the secondary electron multiplier; or 2) its exact mass by the time of flight mass

spectrometer (PTR-ToF-MS) detected in cps from the multichannel plate (MCP). The cps is then converted into ppbv using an equation derived from the measured H_3O^+ and product $VOC{\bullet}H^+$ concentrations. Water (Equation 2.1) from the drift tube is released through the pumps. However, during the measurement of moist samples, formation of water clusters ($H_3O^+{\bullet}(H_2O)_n$) may occur. These water clusters can react with the reactant gas and influence the data due to reagent switching.

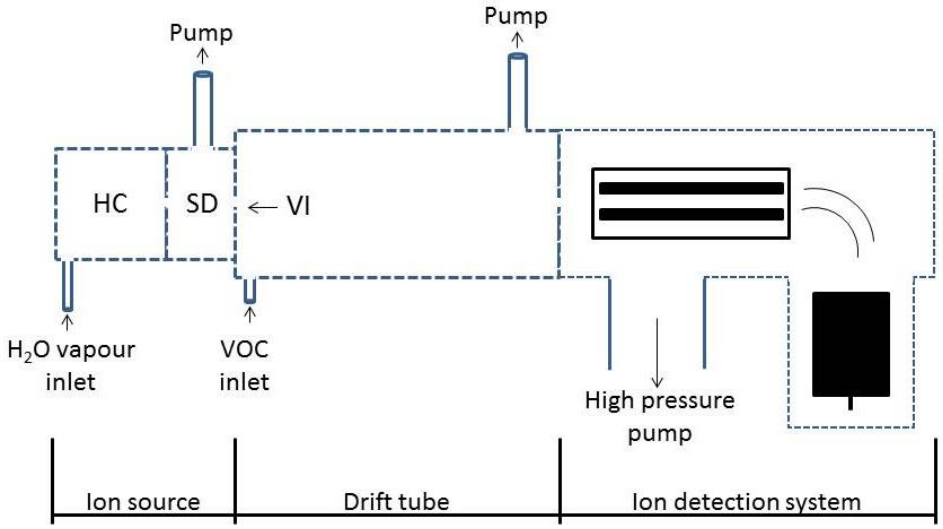

Figure 2.5: Schematic diagram of PTR-QUAD-MS apparatus with quadrupole mass analyser. Abbreviations include: Hollow cathode, HC; Source drift region, SD; and Venturi type inlet, VI (adapted from Lindinger and Jordan (1998)).

To control water cluster formation and VOC fragmentation, several system parameters such as drift voltage, pressure and temperature can be manipulated. Changing these parameters directly influences the ratio of electric field, E and the concentration of neutral particles, N known as the E/N ratio reported in Townsend (Td) units. An increase in E/N ratios enhances hydronium ions and decreases water cluster formation. However it also increases the mean collision energy between ions promoting fragmentation (Lindinger and Jordan, 1998; Tanimoto and others, 2007). Therefore, it is important to work at an E/N ratio that is suitable for the sample of measurement.

In this research, PTR-MS of two variants (PTR-QUAD-MS and PTR-ToF-MS) differing with mass analysers were used. Similarities and differences between these variants are highlighted in Table 2.1.

Table 2.1: A comparison between the PTR-QUAD-MS and PTR-ToF-MS

	PTR-QUAD-MS	PTR-ToF-MS
Types of measurements	On-line monitoring of *in vitro* headspace and *in vivo* nosespace measurements.	
Sample preparation	None or little sample preparation	
Sample injection flow rate	50 – 500 sccm	40 - 500 sccm
Acquisition rate	Measures mass spectrums (m/z21-210) in minutes. Can be quicker when measuring a small selection of VOCs.	Measures mass spectrums (m/z 15-410) in a second. Important for on-line monitoring when VOCs of interest are unknown.
Data acquired	Available software converts cps to ppbv. Only nominal masses acquired and cannot identify isobaric VOCs.	Data undergoes internal calibration and extraction procedure using customised Matlab scripts. VOCs identified up to 3 decimal places enabling the differentiation and simultaneous measurement of isobaric VOCs.

In summary, both PTR-MS machines calculate VOC concentrations without the need for external calibrations. Although major benefits of the PTR-ToF-MS have been highlighted, the quality of data generated from this type of instrument is enormous (thousands of peaks) compared to the PTR-QUAD-MS (hundreds). This not only increases the amount of information collected but also the complexity of data

handling. The use of both machines and differences in data analysis will be discussed in Chapters 3-7.

2.3.5 Apple texture

Textural characteristics of apple cultivars preferred by consumers have been discussed in Section 2.1. This section discusses the mechanical, acoustic and structural aspects of texture that influences eating quality. Apples are semi-solids where a liquid-phase exists within a cellular structure. During storage or maturation, the cells deteriorate and become pliable affecting texture and eating quality. For example, in highly matured apples, cells tend to separate across the lamella without bursting resulting in a mealy and grainy mouth feel. In comparison, the cells in firm fresh apples rupture releasing their encapsulated juice upon mastication (Lill, 1985; Harker and Hallett, 1992; De Belie and others, 2000; Harker and others, 2002a; Tournier and others, 2009). These textural changes are caused by starch degradation, cell wall dissolution or turgor loss (Johnston and others, 2002).

While numerous researchers have looked at the physiological aspects of apple texture softening, this review will consider a different approach by investigating the mechanical structure of the apple parenchyma. One of the earliest studies on the morphology of apples was carried out by Tukey and Young (1942). Subsequently, interest on different cellular packing and intercellular matrices of the apple parenchyma increased (Reeve, 1953). Later, Vincent (1989) and Khan and Vincent (1990) investigated the anisotropy of apple parenchyma and the relationship between the size of cells, intercellular spaces (IS) and the orientation of the cells (radially or tangentially) that may influence texture. It was shown that the mechanical breakdown of apple parenchyma was related to the microstructural orientation of IS in which less energy was required to crack an apple radially (as opposed to tangentially), due to the presence of radially elongated IS (Khan and Vincent, 1993). The use of Scanning Electron Microscopy (SEM) revealed the differences in cell

morphology of unripe and mature Braeburn apples and a relationship between large IS and poor apple texture was reported (Harker and Hallett, 1992). In other words, an increase in total volume of IS (also referred to as porosity) is positively correlated with apple softness/mealiness.

Acoustic textural properties of apples

Before delving into the properties of apple microstructure, an acoustic textural attribute known as crispness must be addressed (Hampson and others, 2000). In crunchy apples, sound is generated through the rupturing of turgid cells that produces a sound wave during mastication (Duizer, 2001). Conversely, in soft apples, low turgidity cells do not rupture but separate along the cell walls giving off a mealy mouth feel during mastication (De Belie and others, 2002). Although apple firmness was believed to be a solely mechanical attribute, sound has been shown to influence it. When a veridical recording of a crunchy apple was played to panellists while they were consuming apples, crunchiness and hardness were rated higher (Demattè and others, 2014). Therefore, acoustic sounds can influence the eating quality of apples. These findings were in-line with that of Vickers (1987) and Duizer (2001) who stated that to fully understand crunchy texture, sound and force displacement need to be considered. When sound and force-displacement data was combined with sensory data, crunchy texture was better predicted. These studies highlight the importance of incorporating acoustic detail into texture measurements (Corollaro and others, 2014).

Apple microstructure

Apple microstructure has piqued the interest of many research groups due to its influence on the mechanical properties of fresh fruit with respect to texture as well as the presence of intercellular spaces (IS). The volume of air in the IS present at harvest occupies a considerable proportion of the fruit and is predominantly used in gas exchange during respiration (Kuroki and others, 2004; Mebatsion and others, 2008). The total volume of air present in the IS reported as a percentage is commonly referred to as porosity. During maturation, the IS increase in size as the apple cells loose cell to cell adhesion owing to the degradation of the middle lamella, while still maintaining cell wall strength. The loss of cell to cell adhesion causes the IS to

radially elongate. As the degree of cell separation increases, the texture becomes soft and mealy (Reeve, 1953).

Gas exchange in apples

IS greatly affects gas exchange in fruit as it serves as a pathway of the lowest resistance for gas movement (Raven, 1996). Apples with more IS have a higher rate of internal gas diffusion (Rajapakse and others, 1990; Harker and Hallett, 1992; Ho and others, 2009). However, cellular fluids can also be found in the IS due to loss of cell membrane integrity. This can lead to loss of cellular compartmentalization where for example phenolic substrates may become available for enzymatic oxidation through the LOX pathway (Franck and others, 2007) (Section 2.3.3). A mixture of air space and cellular fluid within IS has also been observed in high moisture produce such as cucumbers (Kuroki and others, 2004).

Through the use of cryo-SEM, Varela and others (2007) observed the loss of membrane integrity leading to a porous structure. Membrane weakening was often followed by a decrease in cell-to-cell adhesion as exhibited in a loss of roundness (fullness) indicating cellular collapse (Figure 2.6a_1 and 2.6b_1) The amount of degradation is obvious as the cells became flaccid and less turgid (Figure 2.6 b_2) after 14 days of storage. A large amount of exudate is secreted into the IS (indicated by arrows in Figure 2.6) due to the advancement of membrane disruption. The secretion of exudate from cells and its migration through the IS also contributes to moisture loss and the formation of mealy apples.

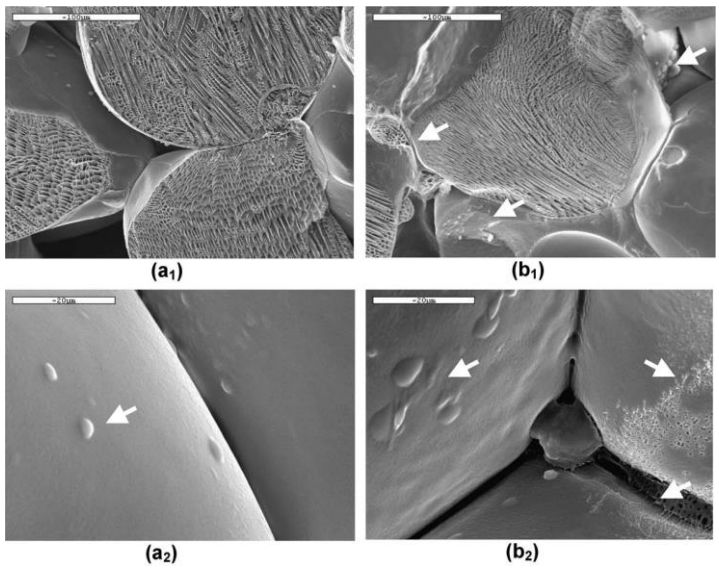

Figure 2.6: A Cryo-SEM micrograph of Golden Delicious apple at (a) day 0 and (b) 14 days after storage at 20°C. Adapted by author and reprinted from Journal of Food Engineering, 78, Varela, P., Salvador, A., and Fiszman, S., Changes in apple tissue with storage time: Rheological, textural and microstructural analyses, 622 – 629, Copyright (2007), with permission from Elsevier.

Visualization of apple microstructure

Based on the examples discussed in the previous sections, the visualization of apple microstructure could be of great value in understanding IS structure and connectivity. This can be used to define apple age, effects of post-harvest regimes on the fruit and/or internal injuries sustained within the fruit such as Braeburn browning disorder (BBD) (Khan and Vincent, 1990; Herremans and others, 2013a). Early studies to visualize the apple cellular matrix concluded that apples are largely anisotropic (Bain and Robertson, 1951; Khan and Vincent, 1990) due to the orientation of parenchyma tissue, IS and radiating vascular veins, a fact which makes apples rather complex to study (Esau, 1977; Vincent, 1989). These factors highlight the need for the systematic selections of apple portions for study away from the peel, vascular strands and core line.

Apple flesh contains an intercellular volume gradient which varies with location. IS immediately under the skin are small and compact, gradually increases in size approach the core line where it dips near the vascular tissue and increases again near the apple core (Figure 2.7 and Figure 2.8). IS on either side of the vascular tissue bundle are small and tend to be elongated radially along the fruit (Bain and

Robertson, 1951; Reeve, 1953; Vincent, 1989; Dražeta and others, 2004) (Figure 2.7 – 2.8). Various traditional microscopy techniques (light, stereoscopic, electron, confocal) have been used to study apple cell size and the distribution of IS (Bain and Robertson, 1951; Reeve, 1953; Khan and Vincent, 1990; Dražeta and others, 2004; Pieczywek and Zdunek, 2012). However, these are limited to the depth associated with the thickness of the specimen used.

An SEM micrograph (Figure 2.9) clearly shows that the outcome of apple image analysis is dependent on the location of sampling for microscopy studies, clearly illustrating the points made by Vincent (1989) and Dražeta and others (2004). Given that apples are highly anisotropic which is defined as the heterogeneous distribution of different sized IS, care must be taken when selecting regions for IS analysis because different areas of an apple will give different results. For example, quantification of IS should be carried out away from the vascular tissue region and hypodermis otherwise IS volume may be underestimated.

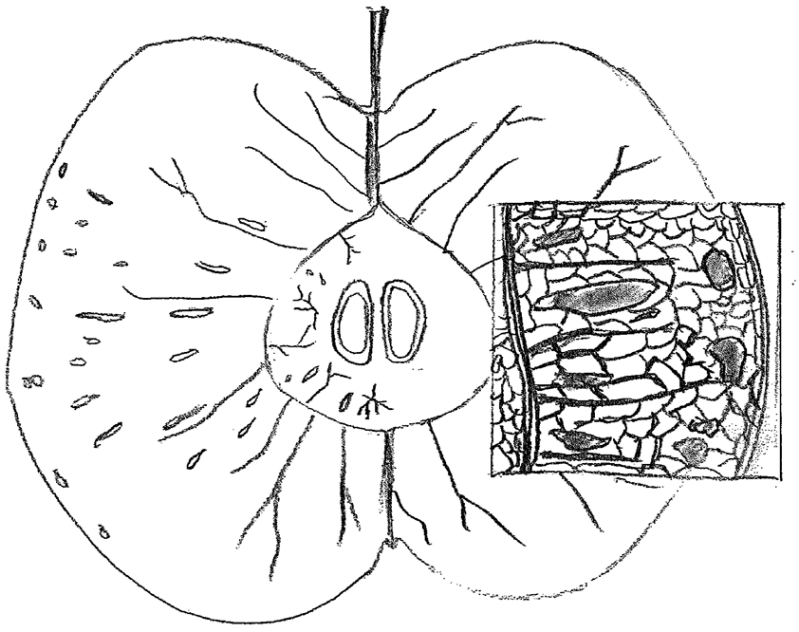

Figure 2.7: Diagram representation (not to scale) of cell, IS and vascular strand orientation in an apple cortex. The diagram illustrates cells and IS near the skin are spherical and less orientated in comparison to the radially elongated cells and IS oriented into the radial columns. Shaded regions represent intercellular spaces and thick bold lines represent vascular strands (Adapted from Khan and Vincent (1993)).

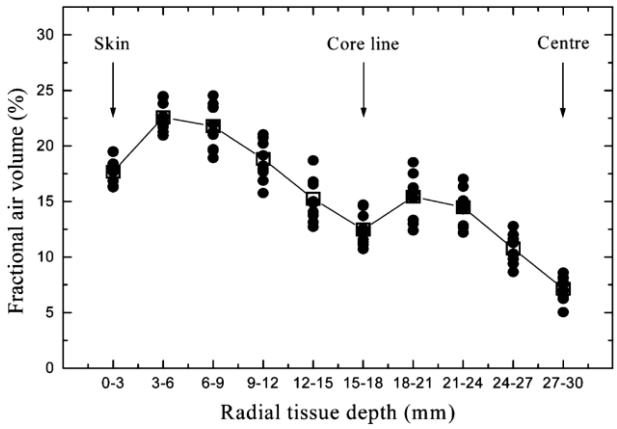

Figure 2.8: The changes in measured fractional air volume (%) using Archimedes' principle for Braeburn apples along a radius based on different radial tissue depths (mm) illustrating air volumes fluctuate when measured at multiple tissue depths and decreases near the centre of the apple. Reprinted from Journal of Experimental Botany, 55, Dražeta, L., Lang, A., Hall, A.J.,Volz, R.K., and Jameson, P.E., Air volume measurement of 'Braeburn' apple fruit, 1061 – 1069, Copyright (2004), with permission from Oxford University Press.

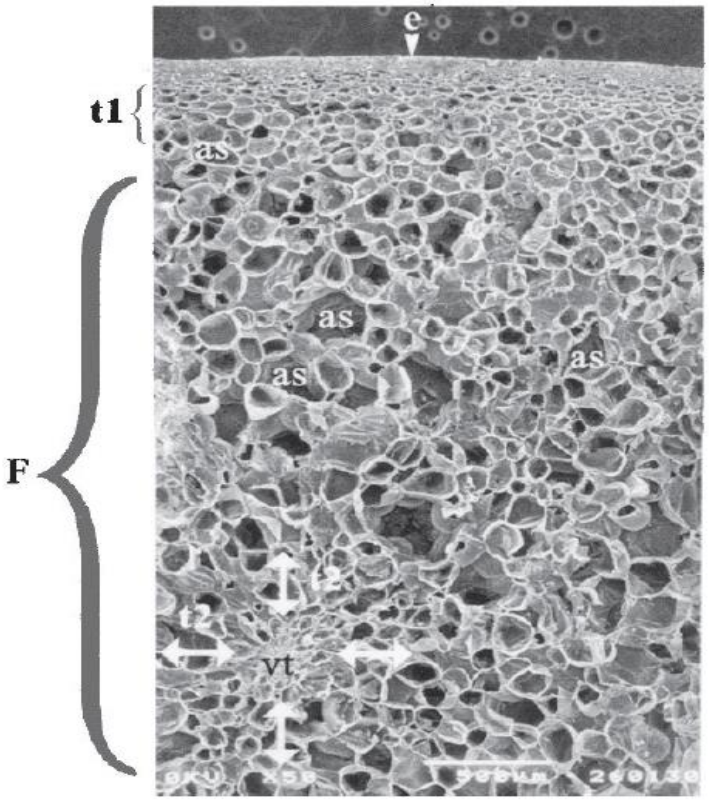

Figure 2.9: Scanning Electron Microscope, SEM micrograph of a 'Golden Russet' apple showing structures important for texture. Abbreviations: e = epidermis; F = flesh; as = air space; t1 = hypodermis, the transition zone between skin and flesh; vt = vascular tissue and t2 = transition zone between vascular tissue and flesh. Scale bar = 500µm. Reprinted from Journal of the American Society for horticultural science, 3, Allan-Wojtas, P., Sanford, K.A., McRae, K.B., and Carbyn, S., An integrated microstructural and sensory approach to describe apple texture, 381 – 390, Copyright (2003), with permission from American society for horticultural science publications.

Conventionally, IS size is measured from acquired 2-D images (Khan and Vincent, 1990) and porosity measured using the Archimedes principle (Bain and Robertson, 1951; Dražeta and others, 2004). With advancing technology, a direct observation method with little sample preparation is available to study the complex and random nature of apple IS orientation and to quantify porosity. X-ray Microcomputer Tomography imaging (µ-CT) is a powerful technique enabling the acquisition of 3D spatial data of IS networks. This technique has been implemented to show high-resolution separation of the IS network in apple samples (Kuroki and others, 2004; Mendoza and others, 2007; Verboven and others, 2008; Mendoza and others, 2010; Herremans and others, 2013b; Warning and others, 2014). It has also been used to study other food products such as cookies (Pareyt and others, 2009), cucumber (Kuroki and others, 2004), bread crumb (Falcone and others, 2005) and deep fried chicken nuggets (Adedeji and Ngadi, 2009). The following section will cover information on X-ray µ-CT scanning which is used in this thesis.

X-ray micro-computer tomographic scanning

In many food industries, visual assessment of quality is performed manually by highly-trained inspectors. However, this method is tedious, labour intensive and unreliable due to its subjective nature. Food imaging has evolved towards maintaining accurate and consistent imaging results eliminating the subjectivity of humans. The increasing demands for objectivity, consistency and efficiency have highlighted the need to use more objective computer based image processing techniques. To fully understand quality such as the occurrence of water-core or internal breakdown of tissues within a plant or food product, it is necessary to apply a non-destructive technique that allows the visualization of the internal features of an object (Du and Sun, 2004).

X-Ray CT, also known as µ-CT, was developed on the same basis as regular X-Ray CT scanners with higher resolution imaging (Sasov and Van Dyck, 1998). During the last decades, numerous types of µ-CT scanners differentiating in resolution ranging from 10 µm/pixel (µ-CT) to 1 µm/pixel (synchrotron radiation x-ray tomography) have been developed (Mendoza and others, 2007; Verboven and others, 2008). For the purpose of this study, only the µ-CT scanner referring to the SkyScan

1172 high-resolution X-ray µ-CT system (Bruker microCT, Kontich, Belgium) will be discussed.

A generic diagram of the important components needed for an X-ray µ-CT scanner is illustrated in Figure 2.10. These include: an X-ray tube acting as a beam source, specimen manipulator, Charge Coupled Device (CCD) camera and a computer equipped with the appropriate software for image processing of 3-D images.

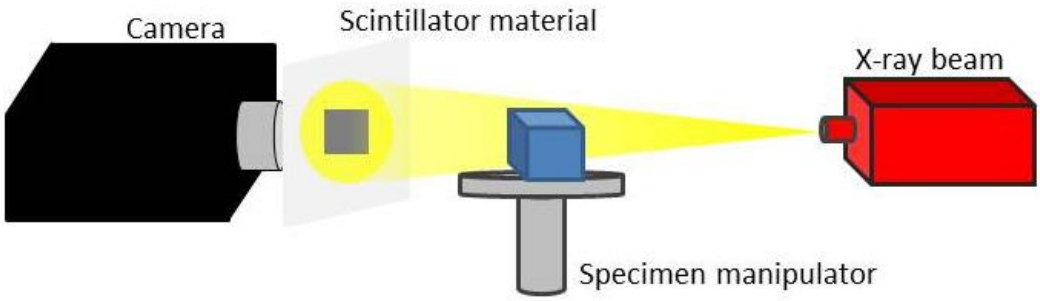

Figure 2.10: Generic set-up of a desktop X-ray µ-CT scanner.

X-ray tube beam

X-rays beams are charged particles (range of 10 - 100keV) that accelerate toward the target of interest. The specific energy range chosen for each experiment depends on the type of materials measured. In the acquired images, intensities and contrasts are based on the differences in the X-ray absorption from the different regions of the sample. Due to the high moisture contents in apples, water dominates the X-ray absorption (Nicolaï and others, 2014). The use of aluminum or copper filters can increase the range of materials to be scanned by concentrating the energy spectrum of the x-ray beam (Stock, 2009).

Specimen manipulator

Desktop µ-CT scanners project a stationary beam onto the sample that is placed on a rotating specimen holder. This can be adjusted for height and degree of rotation which corresponds to resolution and region of interest. The camera will take photos of the object at each degree of rotation until a full 360^{o} rotation is achieved.

CCD camera/detector

The CCD camera captures high resolution images and is equipped with a scintillator material to perform 'scintillation', counting x-ray radiation into visible light to be acquired by the machine. When the distance between the CCD camera and source is decreased or increased, the resolution increases or decreases respectively (Russ, 2005).

Summary of Section 2.3

Section 2.3 has explored the role of ethylene in apple ripening which lead to changes in apple taste, VOCs and texture. Although ripening increases sweet taste and emitted VOCs, it may lead to undesirable textural changes such as excessive softening. Therefore postharvest treatments have been developed to control the effects of ripening due to ethylene production. This will be discussed in the following section.

2.4 Postharvest treatments: Cold storage and 1-MCP

Horticulturists try to extend apple shelf life using various methods. The ripening of apples can be modified at three stages: preharvest; at harvest; or postharvest before the apples reach the consumer. The goal of postharvest treatments is not to improve the initial raw product but rather to prolong the time quality of the stored fruit is comparable to that of freshly harvested produce. This review will focus on the effects of commercially available postharvest treatments on consumer bought apples namely, the use of an ethylene inhibitor, 1-methylcyclopropene (1-MCP) and the use of cold storage. Both of these commercial treatments are commonly used and affect the texture and the production of VOC in apples.

2.4.1 An ethylene inhibitor: 1-MCP

In the late 1990's Sisler and Serek (1997) discovered a compound known as 1-MCP which shares a similar chemical structure to ethylene (Figure 2.11). The initial commercialization of 1-MCP focused on its uses with decorative crops such as flowers by Floralife, Inc (Walterboro, SC, USA). It was later marketed by Agrofresh (2013) (Dow Chemical Company, USA) as SmartFresh™ (containing 0.14% 1-MCP) for use on fresh produce (Blankenship and Dole, 2003; Sisler and Serek, 2003). The proposed mechanism by which 1-MCP extends shelf life involves binding onto ethylene receptors in apples, thereby competing with ethylene (Sisler and Serek, 1997). In the absence of 1-MCP, available ethylene binds onto the ethylene receptor and rapidly diffuses off the receptor. The affinity of 1-MCP to the ethylene binding site is estimated to be ten times greater than that of ethylene itself. As a consequence, the receptor is not induced, nor is it available to ethylene. The action of diffusing off the receptor causes a cascading effect that induces an intracellular response. It also allows for multiple ethylene bindings per receptor. In contrast, upon binding 1-MCP does not dissociate from the receptor readily (often for days). In some products, 1-MCP can also deter ethylene biosynthesis by impeding feedback responses (Blankenship and Dole, 2003).

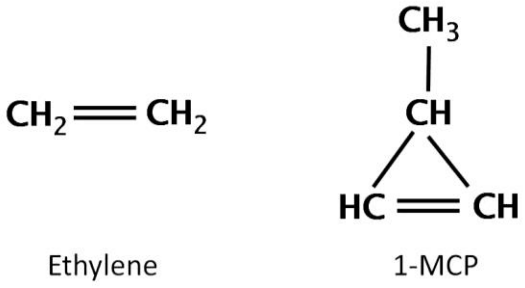

Figure 2.11: Chemical structure of ethylene in comparison to 1-MCP.

Commercially, 1-MCP is applied in an encapsulated powder form, mixed with water and evaporated into an enclosed area. For apples, 1-MCP is normally applied at room temperature (20-25^{o}C) for ~20 hours before they are placed in cold storage (Fan and others, 1999; Mir and others, 2001; DeEll and others, 2002; Blankenship and

Dole, 2003). The effects of cold storage on apples will be discussed later. Although 1-MCP has a high affinity towards ethylene receptors, its efficacy can be influenced by cultivar type, fruit maturity, application temperature, time, concentration and frequency of application (Blankenship and Dole, 2003). Usually these factors are interrelated, where the potency of 1-MCP increases as the frequency of application and temperature is increased (Mir and others, 2001). The time of 1-MCP application is also influenced by temperature and cultivar (DeEll and others, 2002). Cortland cultivars needed at least 9 hours of 1-MCP treatment at 3ºC to retain firmness. However, when Cortland cultivars were treated with 1-MCP at higher temperatures (13 or 23ºC), firmness retention was achieved in less time (6 hours) illustrating the effects of temperature on the efficacy of 1-MCP. In the same study, Empire cultivars retained firmness when treated with 1-MCP for 3 hours regardless of the temperature (3, 13 or 23 ºC) used, suggesting differences in the efficacy of 1-MCP on cultivar type.

As VOC production is also regulated by ethylene, inhibition of ethylene activity by 1-MCP greatly suppresses VOC production. However, this suppression is not permanent (Lurie and others, 2002; Mattheis and others, 2005; Ferenczi and others, 2006). VOCs from 1-MCP treated apples have a higher proportion of alcohols and aldehydes compared to the control apples which showed greater increases in acetate and butyrate esters over time (Lurie and others, 2002). An increase in esters was observed in 1-MCP treated apples that were subsequently stored in air after 20 - 28 weeks of storage and overtime the concentrations of ester VOCs approached similar concentrations to that of untreated apples (Mattheis and others, 2005).

2.4.2 Cold storage

The use of low temperatures during storage increases the shelf life of climacteric produce such as apples as it slows down their respiration rates. Respiration is a plant process that utilizes food reserves within the plant to produce energy to maintain normal physiological functions. Ethylene is produced as a result of normal metabolism, therefore temperature indirectly affects the rate of ethylene production because the enzymes responsible for ethylene production, ACC oxidase and ACC synthase are temperature dependent (Brady and Morris, 2005).

The optimal long term storage conditions for fresh apples in regular atmosphere is in a relative humidity (RH) range of 90 – 95% and a temperature range between -1°C - +4°C (Paull, 1999; Johnston and others, 2002; Watkins and others, 2005). However, at temperatures between -1°C - 0°C some cultivars are susceptible to chilling injuries, which is manifested as symptoms such as soft scald, brown core and internal browning (Brady and Morris, 2005; Watkins and others, 2005). This effect has been reported for McIntosh apples stored below 3°C. Note that although the recommended storage temperature is 0°C, as most apple cultivars are resistant to chilling injuries, it is easier to maintain a RH>90% at temperatures higher than 1°C (Paull, 1999; Watkins and others, 2005).

Maintenance of RH is important since changing its level can alter apple texture and weight depending on the cultivar. A high RH (95%) environment during refrigeration and on the retail shelf is important as it predominantly prevents excessive weight loss. Braeburn and Jonagold cultivars stored at 20°C with a low RH (30% and 65%) showed a faster decrease in weight loss and firmness compared to those stored at the recommended RH (95%) (Tu and others, 2000). Apart from textural, moisture loss in Jonagold cultivars stored at low RH (40%) was also associated reduced levels of specific VOCs (isopentyl, hexyl and butyl acetates) (Wills and McGlasson, 1970). The manipulation of RH and temperature in cold storage may also be combined with CA. When compared to regular atmosphere, CA was better at texture retention and inhibition of VOC production. Studies have also shown that the use of 1-MCP with regular atmosphere could be used as an alternative to CA (Bai and others, 2005; Akbudak and others, 2009).

This section has covered the commercial postharvest treatments used to prolong the initial quality of fresh apple fruits. Cold storage and 1-MCP can maintain the firm texture of fresh apples but, will also defer and alter the production of VOCs. Cultivar differences appear to determine the efficacy of both treatments and will be investigated throughout this thesis.

A significant amount of research on apple flavour and texture has been carried out. However, a full understanding of the flavour perception of fresh apples, and the impact of apple microstructure and cultivar may influence perception is limited. It is clear that texture influences flavour perception but, whether this relationship solely depends on the VOC composition of specific cultivars is unknown. The increase of IS in apple microstructure has been linked to inferior texture. However, information on IS size and distribution in apple flesh is sparse. In addition, although the contribution of IS to gas transport within the fruit has been established, the relationship between apple microstructure and VOC release is not completely understood.

The following working Chapters 3 – 7 have been written in the format of journal articles. The literature used in this chapter will be cited where necessary in the respective chapters.

Chapter 3: *In vitro* and *in vivo* flavour release from intact and fresh-cut apple in relation with genetic, textural and physicochemical properties

Published in Journal of Food Science

Valentina J.L. Ting, Christos Soukoulis, Patrick Silcock, Luca Cappellin, Andrea Romano, Eugenio Aprea, Phil J. Bremer, Tilmann D. Mark, Flavia Gasperi, and Franco Biasioli. 2012. *In vitro* and *in vivo* flavour release from intact and fresh-cut apple in relation with genetic, textural and physicochemical properties. Journal of Food Science, 77 (11), C1226 – C1233.

Names	***Role in Manuscript***
Valentina J.L. Ting	Execution of lab work, design of experiment, data analysis and writing.
Christos Soukoulis	Guidance and help in lab work, design of experiment, guidance, data analysis and writing.
Patrick Silcock	Supervisor.
Luca Cappellin	Proof reading.
Andrea Romano	Proof reading.
Eugenio Aprea	Proof reading.
Phil J. Bremer	Supervisor.
Tilmann D. Mark	Supervisor of student who contributed.
Flavia Gasperi	Proof reading and critical remarks on manuscript.
Franco Biasioli	Supervisor.

3.1 Introduction

The acceptability of apples is related to their texture, flavour, taste and appearance (Dixon, 2000; Johnston and others, 2002). Previous studies have indicated that consumers prefer crisp, crunchy, and juicy apples with characteristic good strong apple flavour (Dixon, 2000; Johnston and others, 2002). Extrinsic parameters such as cultivation and pre-harvesting conditions (sunlight manipulation, coverage during growth), maturity at harvest, post-harvest storage conditions (temperature and duration of cold storage, humidity, application of short term stress treatments, modified atmospheres, use of ethylene inhibitors etc.), and genetic factors can result in significant variations in the structural and flavour traits of commercially harvested apples (Dixon, 2000; Johnston and others, 2002; Marin and others, 2009). A better understanding of the parameters influencing apple flavour will help in the commercial delivery of apples with desirable traits.

Fresh apple aroma is a complex mixture of hundreds of volatile compounds including alkyl esters, alcohols, aldehydes, ketones, sesquiterpenes, terpenoids and carboxylic acids all of which contribute to the overall sensorial perception of flavour-aroma-taste related attributes (Sanz and others, 1997; Dixon, 2000; Harker and others, 2006). Alkyl esters, which generally comprise about 90% of the total volatile organic compounds (VOCs) present have been reported to have the key impact on apple character (Dixon, 2000).

The concentration of VOCs in intact or cut apples has previously been measured under static conditions using chromatographic techniques (Saevels and others, 2004; Komthong and others, 2006). These studies have shown that the flavour of apples is affected by factors such as: their proximate composition (proteins, carbohydrates, lipids), and colloidal structure (microstructural characteristics of peel and flesh), the cultivar type and storage conditions (Sanz and others, 1997; Karlsen and others, 1999; Altisent and others, 2009).

Flavour release under dynamic conditions such as during consumption and structural changes in food matrices is influenced by both the kinetic movement during mastication and thermodynamic parameters of the food itself. Therefore, flavour release during oral processing is not only dependent on VOC partition coefficients that play a significant role but also other aspects related to the mass transfer of volatile compounds from the bulk interior to the product/gas interface (Land, 1996). As a result other phenomena occurring during mastication such as structure deformation, phase transitions, admixture with saliva and hydration coupled with the individual oral physiology variations affects the *in vivo* flavour release (van Ruth and others, 2004). Ultimately, the actual stimulus perceived as apple flavour also relies on the volatile flavour compounds released during consumption reaching the retro-nasal cavity.

Chromatographic techniques that were initially employed for the measurement of *in vivo* flavour release (Linforth and Taylor, 1993) have been surpassed with the use of current direct injection mass spectrometric techniques (Biasioli and others, 2011b) pioneered for *in vivo* applications by Taylor and others (2000). Some examples include those based on proton transfer reaction mass spectrometry, PTR-MS (Biasioli and others, 2011a), atmospheric pressure ionization, API-MS (Taylor and others, 2000) and selected ion flow tube (SIFT-MS) (Ozcan and Barringer, 2011). This is due to their fast data acquisition, little, if any, sample preparation, non-destructive sampling and efficient quantification or semi-quantification of flavour compounds. The coupling of these techniques with nosespace analysis during consumption (Taylor and others, 2000) has enabled, in the case of PTR-MS, analysis of the direct measurement of flavour release foods such as cheese, kidney beans, candies, strawberry flavoured custards and cereal bars (van Ruth and others, 2004; Aprea and others, 2006a; Déléris and others, 2011; Heenan and others, 2012).

The present study aims to study the feasibility of *in vivo* measurements using a PTR-MS on a raw unprocessed food system by using different commercially available apple cultivars. This will be compared to more standard evaluations such as semi-static headspace, texture and physicochemical data.

3.2.1 Chemicals

An antioxidant solution was prepared from 0.2% w/w ascorbic acid (Carlo Erba Reagents, Rodano, Italy), 0.2% w/w calcium chloride (Carlo Erba Reagents, Rodano Italy) and 0.5% w/w citric acid monohydrate (Fluka Analytical, Steinheim, Belgium) balanced with distilled water to stabilize fresh-cut samples.

3.2.2 Fruit samples

Six apple cultivars (Granny Smith, Golden Delicious, Jonagold, Fuji, Morgen Dallago and Red Delicious) were sourced from local supermarkets. Each cultivar was bought and sampled daily to mimic consumer behaviour. For each cultivar, three apples were randomly chosen for headspace analysis. Two of these apples were first used for intact headspace analysis before they were cut using a stainless steel corer into cylinders (18mm diameter) parallel to the core region avoiding the mesocarp. The cylinders were cut into different lengths and dipped in antioxidant solution prior to being assessed for texture (length = 15mm), *in vivo* nosespace and *in vitro* headspace (length = 20mm). Apple cylinders from the texture test were subsequently juiced for pH and titratable acidity (expressed as % malic acid) measurements. The remaining third apple was used only for intact and cut fruit headspace VOC analysis.

3.2.3 Physicochemical properties

Fresh-cut cylinders were juiced to enable pH and titratable acidity measurements (Mehinagic and others, 2004). Moisture content was measured in triplicate using apple discs taken from the upper and lower parts of the fruit by drying in an oven (103°C for 24h) according to the Association of Official Analytical Chemists (AOAC, 2000) official method. Results were expressed as follows:

$$\text{MC (\%)} = \frac{W_0 - W_f}{W_0} \times 100 \qquad \textit{Equation 3.1}$$

Where: W_0, W_f the weights (g) of the apple discs before and after drying.

3.2.4 Textural properties

Penetration tests were carried out using a Texture Analyser (TA-XTi plus, Stable Microsystems Ltd., Godalming, UK) equipped with a 5kg load cell and a cylindrical stainless steel probe (4mm diameter) attachment. Penetration tests were performed with a crosshead speed of 30mm/min, and a trigger force of 5g. The following parameters were calculated from the acquired force-distance curves according to the definitions of Bourne (2002) and Mehinagic and others (2004):

Fracturability (N) = Force at the first significant break during sample penetration.

Firmness (N) = Maximum force recorded over the probe's travel.

*Area (N*mm)* = Area under force curve from start of measurement to maximum force.

*Gradient (N*mm)* = Slope of the force curve from start measurement to max force.

Distance (mm) = Probe displacement at maximum force. Each cultivar was measured in triplicate.

3.2.5 *In vitro* measurements of intact and fresh cut fruits

To measure a whole apple or a cylinder of fresh cut apple, the fruit was placed into either 1L or 250mL glass jar (Bormioli Srl., Bologna, Italy) and incubated at 30^0C for 30min in a water bath. The air inlet was at the top of the jar and the outlet near the base on the opposite side of the jar. VOCs were measured in triplicate for each

cultivar. Headspace analysis was carried out in accordance to Biasioli and others (2006). Air was sampled at a flow rate of 40 standard cm^3 per minute (sccm) over a mass range of m/z 20 to 208 with a dwell time of 0.2s per mass (where an entire mass spectrum cycle was completed in 37.6s), via a heated polyetheretherketone (PEEK) tube (at 80°C) connected to the inlet of a PTR-Quad-MS instrument (Ionicon Analytik GmbH, Innsbruck, Austria). The instrument was operated under drift tube conditions of 138Td (Td=Townsend; 10^{-17} V cm^2mol^{-1}). Each sample was measured for 8 cycles with a mean of cycles 2-8 used for data analysis.

3.2.6 *In vivo* measurements of fresh cut fruits

The importance of serving samples of a controlled and optimal size during nosespace analysis has been previously reported (Yagi and others, 2006; Hutchings and others, 2009). Therefore, preliminary trials were carried out to select a standard apple sample size for *in vivo* nosespace measurements of ∅ 18mm and length 20mm. Five panellists (three male and two female) carried out the *in vivo* nosespace analysis. Instrumental conditions were similar to *in vitro* measurements. Exhaled air from the nose of each panellist was sampled via short PTFE tubes (∅ 6mm, length 50mm) inserted into a heated (95°C) nosepiece (Ionicon Analytik GmbH, Innsbruck, Austria) at a flow rate of 40 sccm connected to the PTR-MS inlet. After normal breathing through the nosepiece for about 30s, each panellist was asked to consume an apple sample. A free mastication profile was chosen to closely simulate consumer's consumption behaviour. Panellists were asked to signal with their hand at the time of sample introduction; at each swallowing event; and when the entire sample was consumed. After the apple was consumed, panellist continued breathing normally for about 30s, before the nosepiece was removed. Panellists were given a 3 minute break to rinse their palate with plain water before proceeding to the next sample. Each cultivar was done in duplicate (two apple cylinders per cultivar). The breathing pattern of each panellist was evaluated by monitoring m/z 59, which is associated with the presence of acetone in the exhaled air. Breath acetone is an endogenous compound which has a concentration much higher than any apple related VOC. Nosespace analysis was measured with the PTR-MS in the selected ions mode. Four

ion peaks of high signal intensities that differentiated apple cultivars were chosen. These were esters (m/z43, 61), acetaldehyde (m/z 45) and ethanol (m/z 47). Data collected was averaged between duplicates and graphs were plotted as a four period moving average trend lines.

3.2.7 Data analyses

VOC analysis

Headspace raw data (counts per second) was converted to concentration according to Lindinger and others (1998). VOCs identified as water clusters or machine derived compounds were removed from the mass spectra (Table 3.1). Some masses such as m/z 45, 55, 57 and 63 were retained as they are isobaric compounds associated to both machine related compounds and VOCs contributing to apple aroma. To clarify, ethylene, as a VOC of interest has a proton affinity lower than H_3O^+ so a H^+ reaction transfer does not occur between H_3O^+ and ethylene. However, ethylene reacts with O_2^+ and its concentration can be calculated by normalizing ppbv28_H_3O^+ to O_2 using equation 3.2.

$$ppbv28\_O_2 = 10 * ppbv28\_H_3O^+ * \frac{cps\_19}{cps\_32}\ ;\ where\ cps\_19 = cps\_21*500 \qquad Equation\ 3.2$$

Table 3.1: List of compounds identified as water clusters or machine derived compounds using the PTR-ToF-MS which were removed from mass spectra. With regards to PTR-QUAD-MS, the nominal mass equivalents were removed.

m/z	Ions
15.9944	O^+
16.0182	H_2N^+
16.0308	CH_4^+
17.0022	OH^+
18.0100	H_2O^+
19.0178	H_3O^+
20.0143	$H_2{}^{[18]}O^+$
21.0221	$H_3{}^{[18]}O^+$
28.0057	N_2^+
29.9975	NO^+
31.9893	O_2^+
32.0018	$N^{[18]}O^+$
33.9936	$O^{[18]}O^+$
37.0283	$(H_2O)_2H^+$
39.0326	$H_2OH_2{}^{[18]}OH^+$
43.9893	CO_2^+
44.9971	CO_2H^+
47.0245	$H_3N_2O^+$
55.0388	$(H_2O)_3H^+$
57.0431	$(H_2O)_2H_2{}^{[18]}OH^+$
63.0082	$CH_3O_3^+$

Statistical analysis

A two-way analysis of variance (ANOVA) was performed on the headspace analysis spectral data in order to evaluate the effects of cultivar and the differences between intact and cut fruit. One way ANOVA was used to investigate the effects of cultivar type on the textural and *in vivo* flavour analysis parameters. Results that were significantly different were separated using a Duncan's post-hoc comparison test. Principal components analysis was carried out to understand the interrelationships between all data sets collected which comprised of standardized headspace spectral data, *in vivo* nosespace data and the physicochemical and textural

properties. All statistical analyses were performed using the STATISTICA release 9 software (StatSoft Inc., Tulsa, OK, USA).

3.3 Results and discussion

3.3.1 Textural and physicochemical properties of fresh cut apple samples

Morgen Dallago and Golden Delicious apples had the lowest values for all texture measurement attributes (Table 3.2) indicating that they had mealy flesh. Granny Smith apples were the hardest and had the highest fracturability (1169g), firmness (1318g) and area value (3826g·s). Jonagold and Fuji apples had the highest gradient values (22g/s and 21 g/s respectively) indicating they were firmer samples and more resistant to rupture than Morgen Dallago or Golden Delicious apples (both 14 g/s). These results are in accordance to previously reported studies (Saftner and others, 2005; Corollaro and others, 2011; Costa and others, 2011).

Physicochemical properties of the cultivars including moisture content, pH and titratable acidity are shown in Table 3.2. Firmer apples such as Red Delicious had a lower moisture content than soft apples like Morgen Dallago. However, instrumental measurements of moisture content and juiciness cannot be used to predict perceived juiciness in apples (Harker and others, 2006) as structural differences between mealy and juicy apples means that juiciness can vary independently of moisture content (Harker and Hallett, 1992; Harker and others, 2006). Measured values of pH and titratable acidity for different apple cultivars were not significantly different with the exception of Granny Smith apples which are well known for their tart flavour (Table 4.1).

Table 3.2: Physicochemical and texture characterization of fresh cut samples taken from different apple cultivars (Morgen Dallago, Golden Delicious, Jonagold, Fuji, Red Delicious, and Granny Smith). Apple cultivars with different superscripts are significantly different ($p < 0.05$).

Apple cultivar	Weight (g)	Moisture content (g/100g)	pH	Titratable acidity (% malic acid)	Fracturability (g)	Firmness (g)	Gradient (g/s)	Area (g*s)	Distance (mm)
Morgen Dallago	223^{c}	76.9^{c}	3.83^{b}	0.17^{a}	420^{a}	646^{a}	14^{a}	1849^{a}	0.98^{a}
Golden Delicious	210^{b}	74.8^{b}	3.64^{b}	0.21^{a}	527^{a}	726^{a}	14^{a}	2115^{a}	1.54^{b}
Jonagold	241^{d}	75.8^{bc}	3.85^{b}	0.29^{ab}	819^{b}	1025^{b}	22^{b}	3143^{b}	1.37^{ab}
Fuji	227^{c}	74.9^{b}	3.84^{b}	0.24^{ab}	975^{bc}	1094^{bc}	21^{b}	3333^{bc}	1.57^{b}
Red Delicious	179^{a}	72.5^{a}	3.81^{b}	0.16^{a}	1008^{bc}	1208^{bc}	17^{ab}	3412^{bc}	1.82^{b}
Granny Smith	229^{c}	75.6^{bc}	3.14^{a}	0.43^{b}	1169^{c}	1318^{c}	20^{b}	3826^{c}	1.57^{b}

3.3.2 In vitro flavour release from intact and fresh cut fruit

Mass ions detected by PTR-MS in the headspace above intact or fresh cut apples included alkyl esters, alcohols, monoterpenes, alpha-farnesene, and carbonyl compounds (Table 3.3a and 3.3b). Note that the mass ions listed in Table 3.3 only include those previously tentatively identified by earlier PTR-MS experiments conducted in conjunction with GC-MS measurements on apple flavour or related compounds (Buhr and others, 2002; Aprea and others, 2006b; Aprea and others, 2007; Cappellin and others, 2011). Isotopes of parent ions were excluded from the results. Overall, mass ions associated with alkyl esters accounted for at least 90% of the total concentration of the volatile flavour compounds present in the headspace.

For intact apples, Granny Smith and Fuji cultivars were characterized by having the lowest concentrations in the headspace of almost all of the mass ions detected. In contrast Red Delicious apples had the highest volatile compound concentrations mainly associated with alkyl esters, carbonyl compounds and ethanol. Golden Delicious and Jonagold apples had a similar total concentration of volatile flavour compounds, but they had a different VOC composition. The concentrations of both monoterpenes, sesquiterpenes (m/z 81, 137, 149 and 205) and carbonyl compounds (m/z 59, 73 and 101) were highest in Golden Delicious apples, while the concentration of alkyl ester (m/z 43) was highest in Jonagold apples. Morgen Dallago apples contained the lowest total concentration of volatile flavour compounds apart from Granny Smith and Fuji cultivars but, had the highest concentrations of alcohol (m/z41) and ester fragments (m/z 75, 89, 103 and 117).

Table 3.3a: Headspace concentration (ppbv) and their standard deviations of different flavour volatile compounds for intact apple samples as they tentatively were identified using PTR-MS. Different superscripts for the same volatile chemical compound are significantly different based on intact fruit and cut fruit respectively (Frag. = fragment, nd= none detected).

m/z	Compounds tentative identification	Intact fruit (FI)						
		Granny Smith	Fuji	Morgen Dallago	Golden Delicious	Jonagold	Red Delicious	p
28	Ethylene	1100 (1200[a])	3348 (1945[a])	70256 (13522[a])	819638 (168088[c])	228704 (72458[b])	5154 (4751[a])	0.000
29	$C_2H_5^+$ (Ethanol frag.)	1.6 (0.11[a])	2.13 (0.34a)	7.19 (1.73b)	9.3 (2.72b[c])	12.83 (4.14[c])	1.11 (0.69[a])	0.000
31	CH_2OH^+	5.35 (3.91[a])	1.59 (0.28[a])	1.82 (0.94[a])	6.89 (0.69[b])	3.11 (0.56[a])	9.87 (2.18[c])	0.000
33	Methanol	28.8 (16.8[a])	27 (8.0[a])	139 (94[b])	235 (49[b])	179 (57[b])	151 (52[b])	0.002
41	$C_3H_5^+$ (alcohol, esters frag.)	33.3 (16.2[b])	75 (20.1[c])	299 (42[d])	4.91 (1.48[a])	276 (167[d])	3.12 (0.49[a])	0.001
42	Acetonitrile	2.19 (1.02[a])	2.89 (0.90[a])	15.1 (2.5[b])	17.4 (2.7[bc])	23.1 (8.8[c])	3.18 (0.25[a])	0.001
43	$C_3H_7^+$ (alcohol, esters, acetates fragment)	38.7 (12.9[a])	117 (44[b])	676 (59[c])	87.8 (10.2[b])	741 (139[c])	466 (14[c])	0.005
45	Acetaldehyde	26 (7.8[ab])	18.8 (2.8[a])	77.1 (88.8[b])	98.3 (55.8[b])	128 (72[b])	197 (4[c])	0.011
47	Ethanol	6.1 (0.97[a])	4.26 (0.98[a])	18.2 (9.2[a])	64.1 (9.5[b])	17.1 (2.3[a])	156 (6[c])	0.000
57	$C_4H_9^+$ (alcohol, esters frag.)	15.3 (2.1°)	19.9 (1.4[a])	176 (19[b])	268 (16[c])	127 (48[b])	323 (4[c])	0.000
59	Acetone	25 (1.6[b])	17.8 (1.0[a])	17.8 (2.1[a])	106 (13[c])	33 (2.0[b])	158 (12[c])	0.000
61	Acetic acid, acetates	7.9 (0.5[a])	43.7 (18.4[a])	349 (9.0[b])	72.4 (15.0[a])	375 (286.1[bc])	618 (32.6[c])	0.000

m/z	Compounds tentative identification	Intact fruit (FI)						
		Granny Smith	Fuji	Morgen Dallago	Golden Delicious	Jonagold	Red Delicious	p
67	$C_5H_7^+$,	0.67 (0.08^a)	1.22 (0.08^a)	1.17 (0.07^a)	1.11 (0.30^a)	2.37 (0.39^b)	1.2 (0.13^a)	0.003
69	Isoprene, furan, aldehyde frag.	7.92 (2.27^b)	2.55 (0.45^a)	51.9 (5.0d)	7.58 (1.32^b)	22.6 (3.5^c)	46.2 (4.3^d)	0.000
71	$C_5H_{11}^+$ (alcohol, ester frag.)	7.43 (0.94^a)	21 (4.7^{ab})	60 (13.2^b)	147 (13^c)	58.1 (21.8^b)	973 (19^d)	0.000
73	2-Butanone/ butanal	2.23 (0.08^a)	1.86 (0.28^a)	2.28 (0.22^a)	421 (11^b)	3.41 (0.49^a)	407 (3^b)	0.000
75	Propanoates/propionic acid	3.82 (0.43^a)	2.32 (0.79^a)	61.7 (14.1^c)	13.4 (1.5^{ab})	21.1 (9.6^{ab})	27.4 (4.4^b)	0.001
81	Monoterpenes, alpha-farnesene, cis-3hexenal, trans-2-hexenal	20 (0.8^a)	35 (4.4^b)	32.7 (5.4^b)	147 (9^d)	88.3 (3.9^c)	32.6 (0.3^b)	0.000
83	Hexanal/ trans-2-hexenol/ cis-3-hexenol	3.46 (0.41^a)	2.13 (0.29^a)	2.62 (0.30^a)	16 (1.6^b)	5.35 (0.29^a)	32.2 (2.5^c)	0.000
85	Pentenal/alcohol/ester frag.	1.19 (0.12^a)	1.89 (0.30^a)	12.8 (2.2^b)	31.9 (1.1^c)	13.7 (5.0^b)	63.7 (2.7^d)	0.000
87	2-pentanone, pentanal	0.96 (0.15^a)	0.57 (0.03^a)	2.1 (0.30^{ab})	3.53 (0.56^b)	2.32 (0.75^{ab})	4.16 (1.54^b)	0.035
89	Butyrates,	2.31 (0.74^a)	5.13 (1.01^a)	36.1 (2.8^c)	6.32 (0.19^a)	15.6 (5.5^b)	20.6 (2.5^b)	0.000
99	Hexanoic acid/ trans-2-hexenal	0.61 (0.01^a)	1.17 (0.23^b)	3.82 (0.66^b)	8.61 (0.99^c)	3.64 (1.22^b)	22.3 (2.9^d)	0.000
101	2-Hexanone/ hexanal	1.06 (0.01^a)	0.82 (0.04^a)	1.53 (0.23^a)	10.4 (0.43^b)	2.67 (0.67^a)	12.4 (1.8^b)	0.000
103	Pentanoates/isoamyl esters	2.59 (0.88^a)	4.89 (0.65^a)	44.3 (9.1^c)	2.67 (0.35^a)	32.8 (11.7^c)	13.6 (0.05^b)	0.001

m/z	Compounds tentative identification	Intact fruit (FI)						
		Granny Smith	Fuji	Morgen Dallago	Golden Delicious	Jonagold	Red Delicious	p
107	Benzaldehyde/ farnesene frag.	0.93 (0.17^{a})	1.84 (0.10^{ab})	2.24 (0.29^{b})	5.99 (0.40d)	3.27 (0.11^{c})	8.51 (0.50e)	0.000
111	1-octen-3-ol/farnesene frag.	0.49 (0.05^{a})	0.41 (0.05^{a})	0.66 (0.06^{a})	7.21 (0.58^{c})	0.61 (0.05^{a})	3.73 (0.01^{b})	0.000
117	Hexanoates/hexanoic acid	0.61 (0.03^{a})	2.85 (0.53^{a})	19.8 (2.8^{b})	2.97 (0.33^{a})	16.5 (6.1^{b})	24.8 (2.4^{b})	0.000
121	Acetophenone/ farnesene frag.	0.84 (0.10^{a})	2.31 (0.40^{b})	2.33 (0.36^{b})	9.54 (0.22d)	6.04 (0.41^{c})	5.22 (0.30^{c})	0.000
131	Heptanoates/heptanoic acid/methylhexyl-esters	0.12 (0.02^{a})	0.5 (0.17^{ab})	2.53 (0.80^{b})	3.13 (0.81^{b})	1.75 (0.94^{b})	40.9 (1.5^{c})	0.000
135	P-cymene/ farnesene frag.	0.97 -0.1	1.74 -0.31	2.13 -0.49	8.85 -0.71	4.37 -0.24	8.17 -0.34	0.000
137	Monoterpenes/alpha-farnesene	1.41 (0.43^{a})	1.61 (0.17^{a})	2.42 (0.30^{a})	12.4 (0.6d)	4.91 (0.29^{b})	6.07 (0.04^{c})	0.000
145	Octanoates/ethylhexyl-esters	0.16 (0.09^{a})	0.33 (0.06^{a})	3.05 (0.70^{b})	1.86 (0.73^{ab})	1.49 (0.60^{ab})	14.7 (1.1^{c})	0.000
149	Alpha-farnesene	0.72 (0.00^{a})	1.58 (0.43^{a})	1.41 (0.05^{a})	10.6 (0.61^{c})	4.06 (0.04^{b})	12.1 (0.13d)	0.000
159	Nonenates	0.03 (0.02^{a})	0.1 (0.05^{a})	0.77 (0.23^{a})	1.32 (0.11^{a})	0.54 (0.17^{a})	6.77 (1.32^{b})	0.000
173	Decanoates	0.09 (0.00^{a})	0.11 (0.05^{a})	0.61 (0.24^{b})	1.43 (0.30^{c})	0.47 (0.29^{ab})	7.94 (0.60d)	0.000
187	Undecanoates	0.04 (0.02^{a})	0.15 (0.04^{a})	0.27 (0.09^{a})	1.64 (0.66^{b})	0.22 (0.07^{a})	6.24 (0.02^{c})	0.000
205	Alpha-farnesene	0.56 (0.17^{a})	1.24 (0.16^{ab})	1.11 (0.12^{a})	13.4 (0.7^{d})	2.97 (0.34^{c})	2.31 (0.02^{bc})	0.000
Volatiles abundance (ppbv)		252	426	2139	2225	2267	3999	

Table 3.3b: Headspace concentration (ppbv) and their standard deviations of different flavour volatile compounds for fresh cut apple samples as they tentatively were identified using PTR-MS. Different superscripts for the same volatile chemical compound are significantly different based on intact fruit and cut fruit respectively. (Frag. = fragment; nd= none detected).

m/z	Compounds tentative identification	Cut fruit (FC)						
		Granny Smith	Fuji	Morgen Dallago	Golden Delicious	Jonagold	Red Delicious	p
28	Ethylene	5508 (5720^{ab})	1567 (1373^{a})	11037 (6691^{b})	7038 (900^{ab})	24969 (1999^{c})	4123 (1544^{ab})	0.000
29	$C_2H_5^+$ (ethanol frag.)	5.92 -0.63	2.13 -0.54	6.68 -3.71	4.14 -0.65	4.39 -1.86	2.11 -0.21	**0.381**
31	CH_2OH^+	4.92 (1.25^{ab})	5.91 (0.62^{ab})	18.21 (1.60^{c})	10.36 (2.93^{b})	19.32 (3.65^{c})	1.98 (0.14^{a})	0.006
33	Methanol	84.3 (47^{a})	973 (254^{c})	4853 (878^{d})	1770 (620^{c})	3390 (632^{d})	209 (25^{b})	0.009
41	$C_3H_5^+$ (alcohol, esters frag.)	63.6 (29.4^{c})	3.94 (0.69^{a})	45.7 (14.1^{c})	51.2 (2.1^{c})	15.3 (5.3^{b})	144 (52^{d})	0.028
42	Acetonitrile	4.92 (1.44^{a})	6.65 (0.70^{a})	45.9 (2.7^{c})	11.3 (1.6^{b})	65.1 (16.1c)	6.69 (2.01^{a})	0.000
43	$C_3H_7^+$ (alcohol, esters, acetates fragment)	156 (28^{a})	409 (45^{b})	5703 (1298d)	425 (68^{b})	1597 (481^{c})	305 (75^{b})	0.004
45	Acetaldehyde	7333 (1449^{a})	35550 (3295^{b})	335110 (22066d)	33223 (1628^{b})	109483 (2696^{c})	5176 (391^{a})	0.005
47	Ethanol	76.4 (10.1^{a})	1080 (238^{b})	11778 (5406^{c})	537 (136^{b})	7239 (2202^{c})	63.7 (9.9^{a})	0.018
57	$C_4H_9^+$ (alcohol, esters frag.)	22.7 (9.2^{a})	645 (44^{b})	9540 (2150d)	37.3 (2.1^{a})	2400 (763^{c})	49.6 (11.9^{a})	0.001
59	Acetone	24.8 (7.7^{a})	119 (16^{b})	679 (239d)	26.3 (5.4^{a})	252 (59^{c})	31.8 (4.4^{a})	0.004
61	Acetic acid, acetates	6.18 (0.53a)	152 (13c)	3510 (1295e)	30.8 (11^{b})	1009 (442d)	88.9 (43c)	0.005

m/z	Compounds tentative identification	Cut fruit (FC)						
		Granny Smith	Fuji	Morgen Dallago	Golden Delicious	Jonagold	Red Delicious	p
67	$C_5H_7^+$,	0.77 (0.08^a)	10.1 (1.8^a)	71.65 (32^b)	1.56 (0.3^a)	43.17 (11.9^{ab})	1.53 (0.3^a)	0.017
69	Isoprene, furan, aldehyde frag.	1.34 (0.3^a)	127 (18.6^{ab})	433 (52^c)	3.43 (0.18^a)	293 (124^{bc})	3.5 (0.45^a)	0.000
71	$C_5H_{11}^+$ (alcohol, ester frag.)	7.84 (2.76^a)	1009 (163^a)	5917 (1794^b)	10.5 (1.24^a)	1201 (416^a)	39 (15.6^a)	0.000
73	2-Butanone/ butanal	3.44 (0.65^a)	1399 (85^c)	1734 (982^d)	9.3 (1.94^a)	1092 (28^b)	4.5 (0.54^a)	0.000
75	Propanoates/propionic acid	8.65 (6.39^a)	20.04 (1.66^a)	272 (115^b)	4.5 (1.56^a)	164 (86^{ab})	3.43 (2.48^a)	0.029
81	Monoterpenes, alpha-farnesene, cis-3hexenal, trans-2-hexenal	1.63 (0.11^a)	233 (36^a)	1459 (412^b)	2.27 (0.55^a)	448 (78^a)	1.37 (0.60°)	0.000
83	Hexanal/ trans-2-hexenol/ cis-3-hexenol	2.2 (0.16^a)	134 (32^a)	1045 (337^b)	5.91 (0.60^a)	646 (152^b)	4.61 (1.32^a)	0.001
85	Pentenal/alcohol/ester frag.	2.76 (0.51^a)	63.64 (7.14^a)	1787 (540^b)	6.52 (1.10^a)	554 (148^a)	4.6 (0.62^a)	0.001
87	2-pentanone, pentanal	1.18 (0.26^a)	12.11 (1.32^a)	61.14 (19.26^b)	1.4 (0.33^a)	21.52 (4.20^a)	1.25 (0.20^a)	0.001
89	Butyrates	7.91 (5.12^a)	114 (19^a)	5922 (3385^b)	10.91 (1.85^a)	891 (358^a)	4.7 (2.12^a)	**0.062**
99	Hexanoic acid/ trans-2-hexenal	0.74 (0.14^a)	12.46 (1.57^{ab})	47.85 (10.23^c)	0.94 (0.02^a)	20.67 (1.53^b)	0.94 (0.09^a)	0.000
101	2-Hexanone/ hexanal	0.78 (0.18^a)	25.54 (3.94^b)	74.45 (16.62^c)	3.12 (1.15^a)	28.37 (2.16^b)	1.52 (0.23^a)	0.000
103	Pentanoates/isoamyl esters	3.64 (2.03^a)	8.42 (0.73^a)	90.41 (44.70^b)	0.17 (0.07^a)	14.78 (5.17^a)	1.18 (0.54^a)	0.031

m/z	Compounds tentative identification	Cut fruit (FC)						
		Granny Smith	Fuji	Morgen Dallago	Golden Delicious	Jonagold	Red Delicious	p
107	Benzaldehyde/ farnesene frag.	0.49 (0.03^{a})	45.04 (24.67^{a})	201 (66^{b})	1.09 (0.33^{a})	52 (20^{a})	0.54 (0.09^{a})	0.003
111	1-octen-3-ol/farnesene frag.	0.13 (0.13^{a})	5.47 (0.76^{a})	53 (22^{b})	0.26 (0.09^{a})	13.99 (5.11^{a})	0.18 (0.07^{a})	0.011
117	Hexanoates/hexanoic acid	1.37 (0.96^{a})	22.07 (5^{a})	501 (216^{b})	0.23 (0.08^{a})	118 (53^{a})	0.81 (0.27^{a})	0.012
121	Acetophenone/ farnesene frag.	0.13 (0.09^{a})	2.49 (0.21^{a})	61.3 (24^{b})	0.29 (0.09^{a})	18.28 (5.09^{a})	0.18 (0.02^{a})	0.005
131	Heptanoates/heptanoic acid/methylhexyl-esters	0.44 (0.21^{a})	21.33 (1.26^{a})	126 (50^{b})	0.1 (0.1^{a})	25.81 (10.26^{a})	0.78 (0.19^{a})	0.008
135	P-cymene/ farnesene frag.	nd	2.65 (0.64^{a})	113 (37^{b})	0.17 (0.03^{a})	25.81 (11.08^{a})	0.03 (0.03^{a})	0.002
137	Monoterpenes/alpha-farnesene	0.24 (0.09^{a})	1.73 (0.24^{a})	6.94 (2.04^{b})	0.38 (0.09^{a})	1.69 (0.24^{a})	0.18 (0.03^{a}a)	0.001
145	Octanoates/ethylhexyl-esters	0.14 (0.10^{a})	2.59 (0.56^{a})	121 (49^{b})	1.12 (0.02^{a})	32.14 (13.36^{a})	0.34 (0.07^{a})	0.008
149	Alpha-farnesene	0.07 (0.07^{a})	5.17 (1.14^{a})	33.78 (11.86^{b})	0.06 (0.03^{a})	7.2 (2.61^{a})	0.27 (0.13^{a})	0.003
159	Nonenates	0.03 (0.03^{a})	0.55 (0.11^{a})	5.58 (2.17^{b})	0.03 (0.03^{a})	1.43 (0.66^{a})	0.03 (0.03^{a})	0.007
173	Decanoates	nd	nd	3.48 (2.06^{b})	nd	0.79 (0.61^{ab})	0.13 (0.07^{a})	**0.092**
187	Undecanoates	nd	nd	2.41 (1.37^{b})	nd	0.47 (0.30°)	nd	**0.065**
205	Alpha-farnesene	nd	nd	0.99 (0.54^{b})	0.04 (0.04^{a})	nd	nd	0.045
Volatiles abundance (ppbv)		7830	43418	396851	36237	134939	6160	

The concentration of m/z 28, tentatively identified as charged ethylene (Zini and others, 2005) was significantly different ($p<0.001$) between cultivars where Granny Smith apples had the lowest (1100 ppbv) and Golden Delicious the highest value which was 819637 ppbv in accordance to previous studies (Song and Bangerth, 1996; Rudell and others, 2000). Apple cultivars with high m/z 28 concentrations also had high concentrations of monoterpene and alpha-farnesene related mass ions as shown for Golden Delicious and Jonagold apples which was also in accordance to the findings of previous studies (Rudell and others, 2000; Golding and others, 2001). These observations suggest that ethylene may be related to the previously mentioned green/herbaceous flavour in Mookherjee and others (1984) study from alpha-farnesene although it is not well understood.

Removal of the peel or cutting the flesh of the apples led to pronounced changes in both the concentration and composition of volatile flavour compounds in the headspace (Table 3.3a and 3.3b). For cut apple sections Morgen Dallago apples had the highest concentrations of the majority of the mass ions detected, followed by Jonagold and Fuji apples. Acetaldehyde (m/z 45) was the most abundant compound present in the headspace above fresh cut samples regardless of cultivar type. The concentration of mass ions related to alkyl esters (m/z 61, 75, 89, 103, 117, 131, and 145) displayed a dependence on cultivar type (Aprea and others, 2011), with the amount of alkyl esters in the headspace above fresh cut compared to intact apples increasing for Morgen Dallago, Jonagold and Fuji apples and decreasing for both Golden and Red Delicious apples. The impact of peel on flavour compound concentration, particularly esters, has been reported in previous studies (Guadagni and others, 1971; Dimick, 1983; Defilippi and others, 2005). These earlier studies stated that the peel was a major source of alkyl esters compounds; however its removal did not limit ester production. Defilippi and others (2005) reported that alkyl ester amounts were higher in peel tissue however, they found that its formation in peel and flesh was regulated by different biosynthetic pathways supporting the increased headspace volatile concentration of cut fruit in which the cutting of the fruit would lead to the formation of secondary VOC formation mainly derived from the lipoxygenase pathway. Monoterpenes and sesquiterpenes production are more

related to peel and therefore decreased in the cut fruit headspace concentration (Yahia, 1994).

3.3.3 *In vivo* flavour release from fresh cut fruit

The concentration of four significant mass ions (m/z 43, 45, 47 and 61 were measured using a free mastication protocol (Figure 3.1). Flavour time intensity curves for in-nose flavour release were found to be affected by apple cultivars, panellist's oral physiologies and their mastication patterns. In these studies, the observed inter-individual variability was high due to personalized chewing and swallowing modalities and the differences in oral cavity physiology as well as the adoption of a free mastication protocol (Buettner and Beauchamp, 2010; Gierczynski and others, 2011). However, the shape of the flavour release curves revealed lesser variation suggesting that the phenomena related with different steps of flavour release (structural decomposition, admixture with saliva, size reduction of food particles and excretion of intercellular fluids) were similar for all assessors (Figure 3.1A and B).

The PTR-MS release profiles were used to calculate: the maximum concentration attained during *in vivo* analysis (I_{max}), the time taken to reach I_{max} (T_{max}), area under the release curve (AUC), the time required for the completion of consumption (T_{con}) and the time needed for the first swallowing event (T_{swal}) (Table 3.4). The T_{swal} was significantly ($p<0.05$) lower in Fuji apples compared to Morgen Dallago, Golden Delicious and Jonagold apples. The T_{swal} is a parameter that reflects the impact of both structural breakdown as well as the volume of fluids excreted from the intracellular matrices. T_{swal} is also related to the time needed for bolus formation. T_{swal} was negatively correlated ($p <0.05$) with firmness, fracturability and area (-0.76, -0.70 and -0.76 respectively) suggesting that softer apple matrices were associated with longer chewing times and consequently an intense structure decomposition and particle size reduction prior to the first swallowing event. These observations infer that an increased amount of liquid matter (juice mixed with saliva) during mastication is a possible driving force for the reduction of T_{swal}. In general,

with the exception of Fuji apples, the higher the apple's matrix fracturability, firmness and flesh rupture work, the longer it took panellists ($p<0.001$) to consume the sample (Pearson's correlation coefficients were 0.97, 0.98 and 0.95 respectively). Our results, therefore, suggest that in addition to T_{swal}, the total consumption time, T_{con} could be used as a measure of *in vivo* sample decomposition where T_{swal} can indicate how juicy the sample is and T_{con} is an indicator of firmness as firmer samples take longer to reduce to a point where they can be swallowed. The T_{max} values were not significantly influenced by apple cultivar with the exception of m/z 43 which had an increasing trend in samples with high firmness and fracturability ($p<0.05$) with softer apples having a lower T_{max} value and *vice versa*.

The AUC values were significantly different amongst all cultivars ($p<0.01$) for all mass ions selected (Table 3.4). A PCA plot of the physicochemical, textural, headspace and nosespace data (Figure 3.2), where 64.1% of the total variation is explained by PC1 and PC2 indicates that Morgen Dallago, Golden Delicious, Red Delicious and Jonagold cultivars were highly associated with I_{max} and both fresh cut (FC) and fresh intact (FI) fruit headspace measurements for m/z45, 47 and 61, on the basis of AUC and T_{max} values. In comparison the firmer Fuji and Granny Smith cultivars were associated with T_{max}, T_{con}, textural and physicochemical properties. This observation implies that although the volatile compound profile is dependent on the VOC concentration within each cultivar, the time needed to reach T_{max} is highly dependent on the breakdown of the structure of the apple. However, T_{max} values in Table 3.4 were not significantly different due to the high inter-individual variation between panellists.

The results on the whole therefore show that *in vivo* release of flavour compounds is a multifaceted process that is affected by both intrinsic (oral physiology, chewing and swallowing pattern heterogeneity of bolus etc.) and extrinsic factors (cultivars, inter-individuals variation) (Kühn and others, 2009; Heenan and others, 2012).

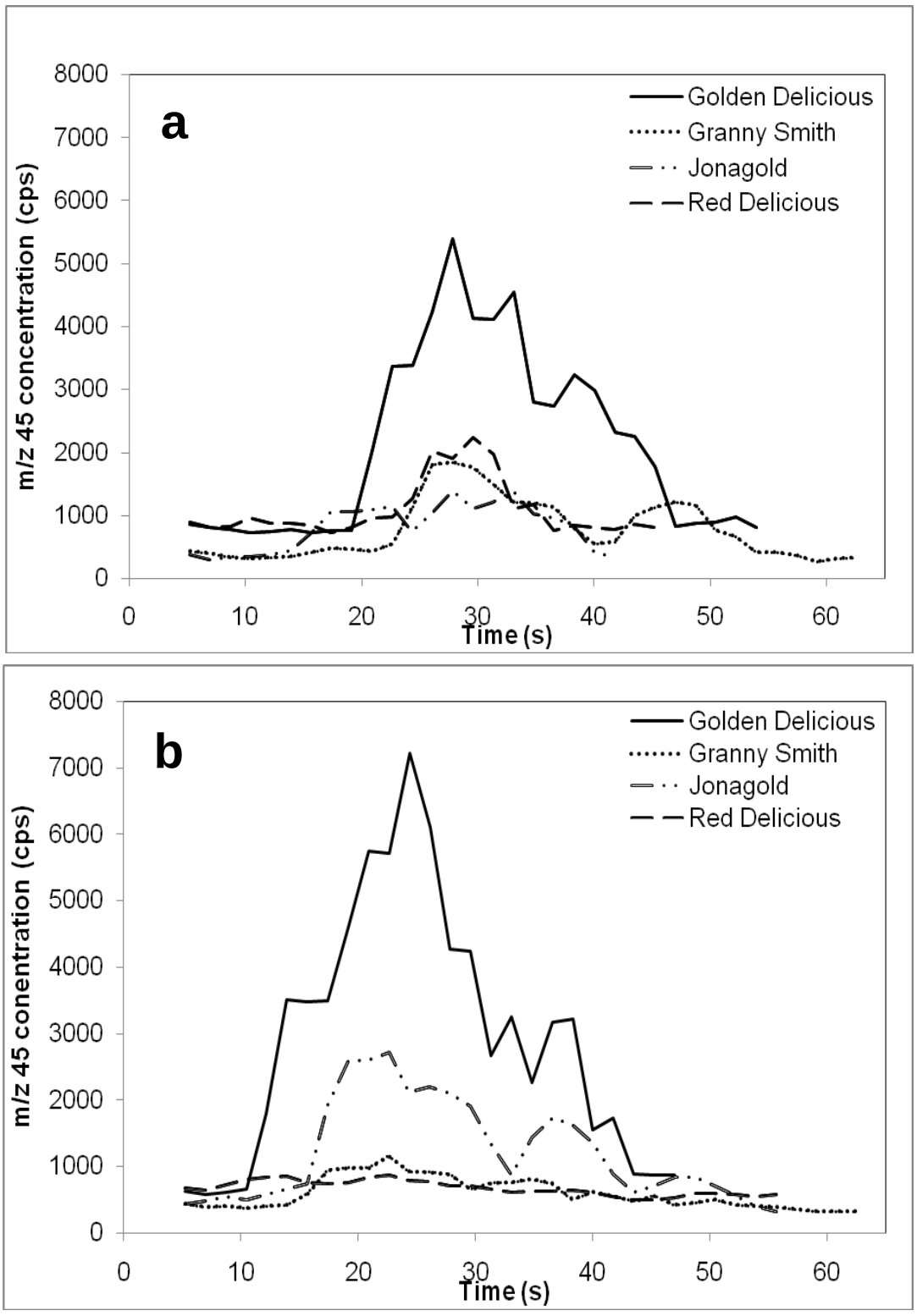

Figure 3.1: Examples of nosespace release profiles of acetaldehyde (m/z = 45) for two assessors, a male (a) and female (b), during the consumption of four different fresh cut apple cultivar samples.

Table 3.4: Mean values (n= 5) of T_{swal} (time required for the first swallowing event), T_{max} (time when I_{max} was attained) in fresh cut apple cultivars, AUC (area under the flavour release curve), I_{max} (maximum intensity of the released flavour compound) and T_{con} (time required for completing the consumption process). Different superscript indicate a significant difference ($p<0.05$) between cultivars.

Cultivar		m/z 43			m/z 45			m/z 47			m/z 61			
	T_{swal}	T_{max}	AUC	I_{max}	T_{max}	AUC	I_{max}	T_{max}	AUC	I_{max}	T_{max}	AUC	I_{max}	T_{con}
Morgen Dallago	14.25^{b}	20.00^{a}	5325^{a}	837^{b}	19.57^{a}	32364^{b}	6931^{b}	21.72^{a}	985^{a}	148^{a}	18.92^{a}	1963^{b}	385^{bc}	30.75^{a}
Golden Delicious	14.25^{b}	20.86^{a}	8095^{c}	612^{ab}	23.44^{a}	74650^{c}	13949^{c}	21.29^{a}	3903^{b}	443^{c}	18.49^{a}	2543^{b}	280^{b}	36.75^{b}
Jonagold	13.25^{b}	20.86^{a}	6132^{bc}	482^{a}	24.51^{a}	30170^{b}	5223^{b}	18.92^{a}	5528^{c}	563^{d}	20.86^{a}	3493^{c}	401^{c}	39.38^{bc}
Fuji	10.38^{a}	26.23^{ab}	3770^{a}	395^{a}	21.29^{a}	10166^{a}	1156^{a}	17.20^{a}	1122^{a}	118^{a}	20.43^{a}	1008^{a}	164^{a}	34.75^{ab}
Red Delicious	12.50^{ab}	22.15^{ab}	12043^{d}	857^{b}	20.64^{a}	25864^{b}	2294^{a}	16.99^{a}	3259^{b}	283^{b}	18.06^{a}	3667^{c}	438^{c}	43.88^{cd}
Granny Smith	12.25^{ab}	29.24^{b}	6815^{bc}	625^{ab}	24.30^{a}	21270^{bc}	2535^{a}	24.51^{a}	1664^{a}	152^{a}	27.74^{a}	2142^{b}	280^{b}	46.38^{d}

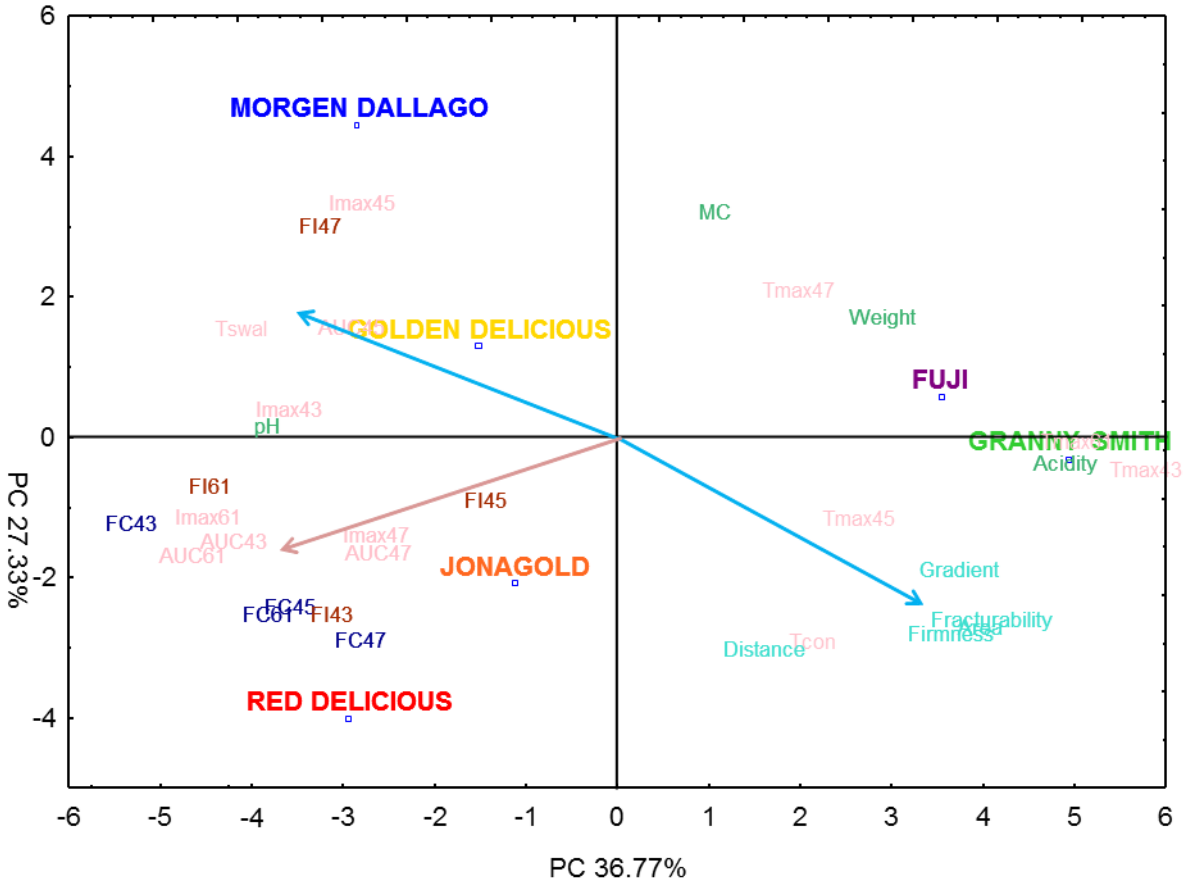

Figure 3.2: Principal component analysis (PCA) on the physicochemical, textural, headspace and nosespace data. The arrows indicate the instrumental drivers of flavour quality for the apple cultivars used in the present study. Headspace, nosespace and texture measurement data was well associated with specific cultivars hence differentiating them. Abbreviations: FI - Fresh Intact; FC - Fresh Cut; MC - Moisture Content.

According to our results, Fuji and Granny Smith apples were generally characterized by the lowest concentrations of volatiles released during the consumption process regardless of the mass ion measured and Golden Delicious, Red Delicious, and Jonagold apples had the highest AUC values though their variability was more dependent on the mass ion. Similar results were also obtained for I_{max} values which were highly correlated with AUC data ($p<0.05$, correlation coefficient ranged from 0.92 to 0.99). I_{max} is an important parameter that enables comparisons to be made between the actual amount of volatile compounds within the sample and amount released through the nasal cavity during consumption. This study highlights the possibility of achieving *in vivo* discrimination of apple cultivars through the use of $I_{max,}$ a parameter that in many other cases has not been achieved

due to the effect of inter-individual variations when a free mastication protocol has been imposed (van Ruth and others, 2004; Kühn and others, 2009). As a whole, the PCA analysis indicates the presence of similarities and differences among the cultivars considered based on texture – flavour release interactions. Observations of trends are as follows: a) firm, crunchy apples characterized by low amounts of flavour compounds released during mastication (Fuji, Granny Smith), b) mealy apples, that texturally deform easily with high amounts of flavour compounds release during consumption (Golden Delicious, Morgen Dallago) and c) firm apples that texturally are resistant to structural breakdown but for which high amounts of flavour compounds are released (Red Delicious, Jonagold).

3.4 Conclusions

The practicality of PTR-MS as a technique for *in vitro* and *in vivo* flavour release studies in complex real food systems such as fruits has been demonstrated. While flavour release under static conditions relies on varietal and post harvesting parameters (cultivar type, peel or flesh flavour composition, maturity at harvest, storage) the dynamic release of volatile compounds during mastication is complex. Thus, parameters like T_{con} and T_{swal} were affected not only by varietal differences among cultivars but also through aspects associated with texture, structural breakdown and temporal particles size reduction. The *in vivo* analysis parameters related with the release of major flavour compounds during sample consumption were influenced by the flavour and textural profile of each cultivar. This in turn characterized Fuji and Granny Smith apples as having low concentrations of flavour volatiles released during mastication and Morgen Dallago and Red Delicious apples with the highest concentration of VOCs.

Further investigations using the recently commercialized PTR-ToF-MS instrument can overcome the limitations of the quadrupole configuration (slower data acquisition, lower temporal resolution and limited mass range). A mechanistic

approach based on the combination of nosespace data with textural, physicochemical, and structural deformation properties for a larger number of cultivar is needed to effectively elucidate the flavour perception during consumption of real food systems. This will be carried out in the next chapter.

Chapter 4: PTR-ToF-MS: A rapid technique to investigating the *in vitro* and *in vivo* flavour release from fresh cut apple fruits

4.1 Introduction

The influence of texture on VOC release during flavour perception explored via *in vivo* nosespace (Chapter 2 and 3) methods has received much interest. Earlier work has generally used model food type systems such as whey protein (Baek and others, 1999; Mestres and others, 2005) and gelatine gels (Boland and others, 2006) of different firmness spiked with known aroma compounds. Both of these studies concluded that a faster and higher release of the targeted VOCs occurred in softer gels compared to the firmer gel matrices. This trend was also observed for the apple cultivars studied in Chapter 3. Softer cultivars such as Golden Delicious and Morgen Dallago were consumed faster and released a higher amount of VOCs in comparison to firmer cultivars such as Fuji and Granny Smith which released a lower VOC concentration and a longer consumption time.

Apart from apples, PTR-QUAD-MS have been used to investigate the flavour release from other natural food products such as fresh banana and canned kidney beans (Mayr and others, 2003; van Ruth and others, 2004). However, the PTR-QUAD-MS has limitations when used to investigate complex food matrices due its acquisition rate and lower time resolution (Poinot and others, 2013). These disadvantages have been overcome by coupling the PTR with a time of flight mass analyser which increases the sensitivity and time resolution compared to its QUAD predecessor. This allows the user to simultaneously monitor VOCs without the need for VOC selection (Chapter 3.2.6). Differences in acquisition rate and data collection between the QUAD and ToF have been elaborated in Chapter 2 (Table 2.1). PTR-ToF-MS have been successfully used in *in vivo* studies focusing on inter-individual variability on brewed coffee (Romano and others, 2013) and flavour release in cereal bars of varying sugar concentration (Heenan and others, 2012).

Preliminary results in Chapter 3 using the PTR-QUAD-MS showed strong relationships between texture and VOC release. To effectively understand the relationship between *in vivo* analyses with regards to textural breakdown, a larger set

of cultivars was needed. Therefore, this chapter aims to expand on the previous chapter using a PTR-ToF-MS to measure the *in vitro* and *in vivo* VOC profiles of 21 apple cultivars. Additionally, an improved simultaneous instrumental measurement of force-displacement curves and acoustic properties was included. Previously combined mechanical and acoustic texture measurements have provided strong correlations to sensory firmness and crunchiness (Costa and others, 2011; Corollaro and others, 2014). This chapter was carried out with the following objectives: A) Characterizing the VOC profiles of fresh cut apple discs based on *in vitro* headspace measurements; B) Measuring the texture and physico-chemical properties of the apples; C) Performing *in vivo* nosespace analysis and lastly; D) Exploring possible relationships between VOC release and texture during *in vivo* measurements.

4.2 Methods and materials

4.2.1 Apple samples

Twenty one apple cultivars (*Malus Domestica* Borkh.) were sourced from an experimental field maintained by the Research and Innovation Centre, Fondazione Edmund Mach, FEM (San Michele a/A, Trento, Italy), where fourteen were commercially available cultivars and seven were from the FEM breeding program (Table 4.1). Apples were harvested upon commercial maturity standards based on soluble solids content, starch degradation index, firmness and fruit colour. A minimum of twenty apples of consistent size and no visible exterior damage were chosen per cultivar. Apples were held at 2ºC for two months (95% RH) and were brought up to room temperature 24 hours before experimentation.

Table 4.1: Apples (n=21) used for this study as indicated by their cultivar, harvest date, type, Soluble Solids Content %, SSC (n=2) and titratable acidity (n=2) reported as mean ± standard deviation, σ.

Cultivar	Harvest Date	Code	Type[a]	SSC% (mean ± σ)	Titratable Acidity[b] (mean ± σ)
FEM 4	8/09/2011	FEM4	Bp	15 ± 0.0	4 ± 0.0
Gloster	8/09/2011	GLO	C	12 ± 0.2	6 ± 0.2
Pilot	8/09/2011	PIL	C	13 ± 0.2	8 ± 0.5
Rubens	8/09/2011	RUB	C	11 ± 1.9	4 ± 0.1
Golden Delicious	12/09/2011	GOL	C	12 ± 1.3	4 ± 0.5
Pinova	13/09/2011	PNV	C	13 ± 0.5	6 ± 0.2
Renetta Bianca	13/09/2011	RNB	C	14 ± 0.1	13 ± 0.2
Renetta Grigia	13/09/2011	RNG	C	14 ± 0.5	15 ± 0.2
FEM 11	15/09/2011	FEM11	Bp	14 ± 0.5	3 ± 0.3
Pinova Evelina	15/09/2011	PNEV	C	13 ± 0.0	8 ± 0.6
Topaz	15/09/2011	TOP	C	12 ± 0.3	11 ± 0.54
Kanzi	16/09/2011	KAN	C	12 ± 0.1	5 ± 0.2
Braeburn	27/09/2011	BRN	C	12 ± 0.2	7 ± 0.2
FEM 3	29/09/2011	FEM3	Bp	14 ± 0.2	10 ± 0.4
Fuji Kiku 8	6/10/2011	FUJ	C	14 ± 0.6	3 ± 0.4
FEM 10	10/10/2011	FEM10	Bp	14 ± 0.6	9 ± 0.3
Dalinette	18/10/2011	DAL	C	15 ± 0.2	7 ± 0.2
FEM 5	18/10/2011	FEM5	Bp	14 ± 0.9	4 ± 0.5
FEM 2	20/10/2011	FEM2	Bp	18 ± 0.6	11 ± 0.5
FEM 7	20/10/2011	FEM7	Bp	15 ± 0.7	9 ± 0.0
Gold Rush	24/10/2011	GDR	C	15 ± 2.0	9 ± 1.0

[a] Abbreviations: Breeding Program (Bp) and Commercial (C)

[b] Malic acid/100g juice

4.2.2 Sample Preparation

For each cultivar, apples of a consistent size with no visible exterior damage were selected and cut to yield 15-18 discs (18 mm Ø 12 mm height) from the flesh region parallel to the core (the skin and mesocarp removed) using a stainless steel corer. The apple discs were treated with an antioxidant dip (0.2% w/w calcium chloride, 0.2% w/w ascorbic acid, 0.5% w/w citric acid monohydrate) to prevent oxidative browning. The apple discs were used in the following analyses.

4.2.3 PTR-ToF-MS measurements

In-vitro headspace analysis

The headspace of fresh cut apple discs was measured by placing one apple disc in a 250mL lidded glass jar (Bormioli Srl. Bologna, Italy) which had a silicon septa on the top and the opposite base side of the jar. The apple disc was incubated prior to measurement in a water bath (30 °C) for 30 minutes. The headspace VOCs were then measured through a direct injection into the PTR-ToF-MS 8000 (Ionicon Analytik GmbH, Innsbruck, Austria) using a heated PEEK tube (110 ºC, 0.055" Ø) at a flow rate of 50 sccm.

Standard machine operating conditions were: drift voltage 600V; drift pressure, 2.25mbar; and E/N, 140 Td (Td: Townsend; 10^{-17}/Vcm^{2}s) were maintained throughout the experiment. A 0.1ns per channel sampling time in the ToF analyser amounting to 350 000 channels resulted in a mass spectrum range of m/z 15 – 400. Each sample was measured for 50 cycles resulting in an analysis time of 50s/sample. A total of six replicates were measured for each cultivar and sample order was randomized to decrease the occurrence of systematic memory effects.

In-vivo nosespace analysis

In vivo nosespace analysis was carried out using the same settings for the *in vitro* measurements except the flow rate was increased to 300 sccm. Four panellists (2 males; 2 female) performed the analysis where their exhaled air was sampled via short teflon tubes (ø 6 mm, length 50 mm) which was inserted in their noses and into a heated (95ºC) Nosespace Air Sampling Extension (NASE) (Ionicon Analytik GmbH, Innsbruck, Austria) apparatus that was directly attached to the PTR-ToF-MS. A slide show was used to guide panellists through the *in vivo* test. The breathing pattern of each panellist was monitored by tracking their breath acetone (m/z 59.049) which is an endogenous compound present in exhaled air. Only when the breathing profile was stable did the *in vivo* nosespace measurement and slideshow began. Each panellist was to breath normally (30 sec) into the nose piece until prompted by the slideshow to consume the sample. Panellists were allocated 45 seconds to consume the entire sample using a free mastication protocol. A hand signal for every consequent swallow was shown until the entire sample was consumed. Upon

finishing, panellists were told to remain seated and to breathe normally for another 30 sec before the nose piece was removed. Panellists were given a one minute break between samples to rinse their palate with plain water and unsalted crackers before proceeding to the next sample. All cultivars were measured in duplicate.

4.2.4 Texture analysis

Texture was measured using a Texture analyser (TA-XTi plus) equipped with an Acoustic Envelop Detector (AED) device (Stable Microsystems Ltd, Goldalming, UK) to extract force-displacement and acoustic curves acquiring twelve mechanical and four acoustic properties simultaneously. Texture measurements were performed on 15 apples per cultivar and a single disc per apple. A penetration test was performed using the texture analyzer loaded with a 5 kg cell and a cylindrical 4mm diameter flat head probe attachment. The probe penetrated the sample at a test speed of 300 mm/min and an auto-trigger of 5 g to a 90% deformation. Mechanical parameters were calculated from the acquired force (N) – distance (mm) curves. Acoustic properties of the sample were measured simultaneously, with the AED device placed approximately 5 cm from the sample. The device was synchronized to the texture analyser and the acoustic data acquisition started when the auto-trigger was activated by the probe. Parameters calculated from these curves can be found in Table 4.2.

Table 4.2: The definitions of mechanical (n = 12) and acoustic (n = 4) parameters from acquired force-displacement and acoustic curves using a texture analyser.

Mechanical Properties	**Abbreviation**	**Definition**
Yield Force	Yield_F (N)	Force indicating sample transition from elastic to plastic phase
Max Force	Max_F (N)	Maximum force
Final Force	Final_F (N)	Force after 90% compression
Mean Force	Mean_F (N)	Average force over curve
Force Peak	Force_P (-)	Number of force peaks
Work	Work (N.mm)	Area under the force curve
Force Linear Distance	F_Linear_Dist (-)	Force curve length
Young's Module	Youngs_M (Nmm^{-2})	Slope between stress and strain signifying elasticity
Peaks/Distance	Peaks.mm (-)	Number of peaks based on physical distance travelled by probe
Linear Distance/Distance	Linear_Dist.mm (-)	Average length of linear distance
Difference in Force	Diff_F (N)	Difference between yield and final force indicating force curve direction
Force Ratio	F_ratio (-)	Ratio between yield and final force indicating magnitude of change
Acoustic Properties		
Acoustic Peak	A_Peak (-)	Number of acoustic peaks higher than 10dB
Maximum Acoustic Pressure	Max_A_Press, (dB)	Maximum acoustic peak
Mean Acoustic Pressure	Mean_A_Press, (dB)	Mean of sound pressure measured over acoustic wave
Acoustic Linear Distance	A_Linear_Dist (-)	Length of acoustic profile

4.2.5 Physico-chemical properties

Using a garlic crusher, four apple discs from each cultivar were crushed to express juice and reported as expressed juice per fresh weight (%). The soluble Solids Content (SSC) (DBR35 refractometer, XS Instruments, Poncarale, Brescia, Italy) and titratable acidity (TA) (Compact Titrator, Crison Instruments S.A., Alella, Barcelona, Spain) of the juice was measured. The residual apple palette obtained after juice expression was used to measure dry matter content (DMC %) by oven drying (103ºC for 24 hours). Results were calculated based on weight loss percentage before and after drying (Chapter 3) (Equation 3.1). These measurements were performed in duplicate.

4.2.6 Data analysis

PTR-ToF-MS spectrum analysis

In-vitro headspace analysis

The 50 cycles for each sample measured were averaged and pre-processed by baseline removal to reduce the noise in the ToF spectra. The resulting raw data was expressed as counts per second (cps). This was converted into concentration (ppbv) using the rate coefficient ($k=2*10^{-9}cm^3/s$) in the formula derived by Lindinger and others (1998). Dead time correction, peak extraction and internal calibration of the mass spectral data was carried out using specialized scripts (Cappellin and others, 2011) performed in MATLAB R2012a (The Mathworks Inc, Natic, MA). Subsequently, 429 peaks were detected over the resulting m/z15 - 250 mass range, and these peaks were pre-processed to remove water clusters, machine derived compounds (for list of compounds refer to Table 3.1), compound isotopologues and peaks with averages across all samples lower than 1ppbv. This decreased the dataset for statistical analysis to 183 abundant peaks.

In-vivo nosespace analysis

A total of 120 cycles (1 second/cycle) was measured for each *in vivo* nosespace measurement. The ToF spectra were processed similarly to *in vitro* headspace spectra except, the 120 cycles were not averaged but extracted individually in order to study the breath curve of each m/z. A custom script was used to select masses containing

peak-like curves (Romano and others, 2013). Important nosespace parameters extracted from selected m/z were: Area under the Curve, AUC; maximum intensity, I_{max}; and time to maximum intensity, T_{max} were extracted. Other consumption parameters measured: time of consumption, T_{con}; time to first swallow, T_{swal} and number of swallows, N_{swals}.

4.2.7 Statistical analysis

To investigate cultivar effects on *in vitro* headspace, *in vivo* nosespace, texture and physico-chemical datasets, a one-way Analysis of Variance (ANOVA) was performed. M/z and texture attributes that significantly differentiated the 21 cultivars were $\log_{10}$ transformed before being subjected to a heat map (R package 'gplots') to visualize differences between cultivars (Warnes and others, 2014). Hierarchical cluster analysis was performed using the Ward's agglomeration method to cluster based on rows (cultivars) and columns (measured headspace masses, texture or nosespace attributes). Lastly, all datasets were collated, standardized ($1/\sigma$) and subjected to a Multi Factor Analysis, MFA (Lê and Husson, 2008). Statistical analyses and graphics were performed using the statistical package R (R Core, 2014).

4.3 Results and Discussion

The heat maps (Figure 4.1 - 3) contain a colour key that ranges from dark to light blue thru white to light to dark red. The density plot indicates the data distribution based on the calculated variables (per column). On the main heat map, each rectangle contains a the vertical dotted line representing the mean from 21 cultivars of a single variable (column) whereas the solid line indicates if a specific cultivar is higher (shares of red) or lower (shades of blue) in intensity for each variable compared to the sample set mean.

4.3.1 *In-vitro* headspace analysis.

Mass peaks (n=51) that significantly differentiated ($p<0.05$) the cultivars were used as variables to cluster cultivars and mass ions based on their similarity. However, VOCs were generally grouped together based on their log_{10} transformed concentrations rather than their chemical fragments despite strong correlations ($p<0.05$; $r<0.90$) (Figure 4.1) (Farneti and others, 2014). The m/z listed in Table 4.3 were tentatively identified based on previously published literature (Cappellin and others, 2012b; Soukoulis and others, 2013; Farneti and others, 2014). Lower molecular weight (MW) m/z are coloured red which graduates to green for higher MW masses (Figure 4.1). For ease of reading, the m/z are reported as nominal masses in the text unless, two compounds share the same nominal mass, they are reported to three decimal places.

FEM4, FEM11 and Pinova were cultivars that emitted numerous VOCs (> 2σ) at higher concentrations compared to other cultivars (Figure 4.1). The VOCs that differentiate these cultivars include esters such as ethyl acetate/butyrate (m/z 89), butanoate/acetate esters (m/z71.050), hexyl acetate/hexanal (m/z 99.080), hexyl acetate (m/z 145), and aldehydes or ester related compounds measured by the PTR-ToF-MS as low MW fragments (m/z 41, 43.018, 43.054, 55, 57.034, 57.070, 61). FEM4, FEM11 and Pinova also emitted higher amounts of estragole (m/z 149.096). In contrast, these cultivars were low emitters of terpene and α-farnesene related compounds.

Table 4.3: The list of tentatively identified VOCs from the headspace (HS) and nosespace (NS) of fresh cut apples

True mass	Chemical formula	HS m/z	NS m/z	Tentatively identified VOCs
28.031	$C_2H_4^+$	28.029	n.a	Ethylene
31.018	CH_3O^+	31.018	31.018	Formaldehyde fragment
33.034	CH_5O^+	33.034	33.034	Methanol
41.039	$C_3H_5^+$	41.038	41.039	Fragment of diverse origin
43.018	$C_2H_3O^+$	43.017	43.018	Ester fragment
43.054	$C_3H_7^+$	43.050	43.054	Alcohols fragment
45.033	$C_2H_5O^+$	45.030	45.033	Acetaldehyde
47.013	$CH_3O_2^+$	47.013	47.016	Formic acid
47.045	$C_2H_7O^+$	47.050	47.045	Ethanol
53.039	$C_3H_5^+$	53.039	53.039	Ester fragment
55.054	$C_4H_7^+$	55.055	55.056	Aldehyde fragment
57.034	$C_3H_5O^+$	57.034	57.034	Aldehyde/ester fragment
57.07	$C_4H_9^+$	57.070	57.070	Alcohol fragment
59.049	$C_3H_7O^+$	59.050	59.048	Acetone
61.028	$C_2H_5O_2^+$	61.028	61.030	Acetate esters, acetic acid
67.054	$C_5H_7^+$	67.055	67.055	Terpene fragment
69.07	$C_5H_9^+$	69.070	69.071	Isoprene
71.05	$C_4H_7O^+$	71.050	71.050	Esters (ethyl butanoate, butyl butanoate, ethyl hexanoate, 2-methylbutyl acetate, isoamyl acetate)
71.086	$C_5H_{11}^+$	71.086	71.087	Alcohol fragment
73.065	$C_4H_9O^+$	73.065	73.066	Butanal
75.044	$C_3H_7O_2^+$	75.044	75.045	Methyl acetate
77.039	C_6H_5	77.040	77.035	Benzene related phenyl group
79.054	$C_6H_7^+$	79.054	79.056	Benzene
81.07	$C_6H_9^+$	81.070	81.071	Terpene fragment
83.086	$C_6H_{11}^+$	83.086	83.087	Hexanal
85.101	$C_6H_{13}^+$	85.102	85.103	Hexane

True mass	Chemical formula	HS m/z	NS m/z	Tentatively identified VOCs
87.044	$C_4H_7O^+$	87.045	87.046	Diacetyl
87.08	$C_5H_{11}O^+$	87.082	87.082	Methyl butanal
89.06	$C_4H_9O_2^+$	89.060	89.060	Ethyl acetate/ butyrates
91.054	$C_7H_7^+$	91.057	91.056	Benzyl Radical
93.07	$C_7H_9^+$	93.071	93.072	Terpene fragment
95.049	$C_6H_6O^+$	95.050	95.051	Phenol
95.086	$C_7H_{10}^+$	95.086	95.088	Terpene
97.065	$C_6H_9O^+$	97.065	97.066	2,5 Dimethylfuran
99.081	$C_6H_{11}O^+$	99.081	99.081	Esters fragment (hexyl acetate), aldehydes (hexanal)
101.09	$C_6H_{13}O^+$	101.090	101.061	Hexanal
103.075	$C_5H_{11}O_2^+$	103.075	103.076	Methyl butanoate
105.071	$C_8H_9^+$	105.072	105.073	Styrene
107.086	$C_8H_{11}^+$	107.086	107.088	Ethyl benzene
109.101	$C_8H_{13}^+$	109.102	109.104	α -farnesene fragment
117.091	$C_6H_{13}O_2^+$	117.091	117.091	Ethyl butanoate
121.065	$C_8H_9O^+$	121.065	121.067	Acetophenone
121.101	$C_9H_{13}^+$	121.102	121.105	α -farnesene fragment
123.117	$C_9H_{15}^+$	123.117	123.119	α -farnesene fragment
131.107	$C_7H_{15}O_2^+$	131.107	131.095	2-methyl butyl acetate
135.117	$C_{10}H_{15}^+$	135.117	135.119	α -farnesene fragment
137.132	$C_{10}H_7$+	137.132	137.137	Terpene/ α -farnesene fragment
145.122	$C_8H_{17}O_2^+$	145.122	145.124	Hexyl acetate
149.096	$C_{10}H_{13}O$+	149.096	149.103	Estragole
149.133	$C_{11}H_{17}^+$	149.133	149.139	α -farnesene fragment
205.195	$C_{15}H_{25}^+$	205.195	205.202	α -farnesene

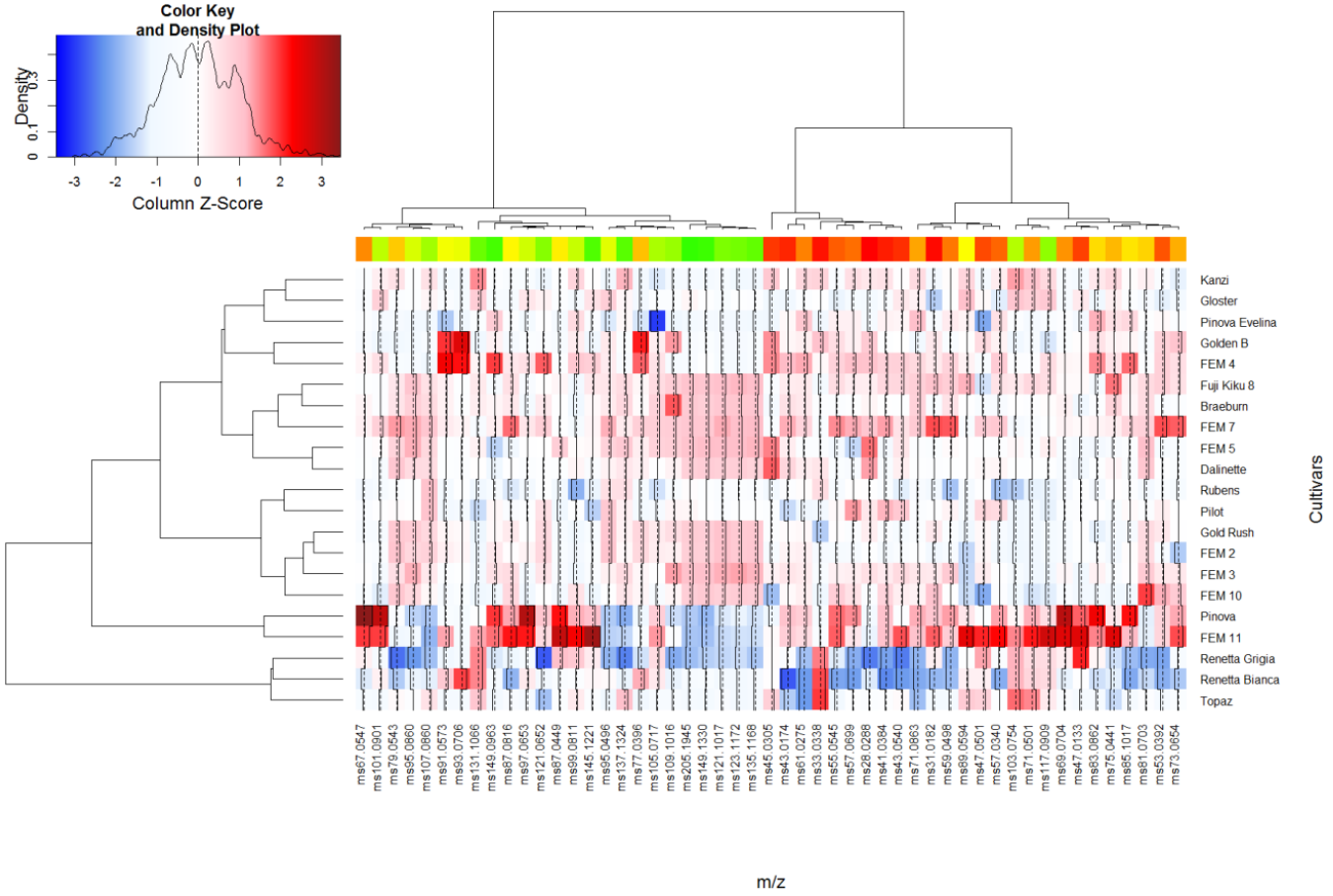

Figure 4.1: Heat map of the significantly different mass ions from the in vitro headspace analysis of fresh cut apple discs from 21 different cultivars. Ward's method of cluster analysis was performed on log_{10} transformed after pre-processing the data where apples were clustered by rows and VOCs, by column.

Nine cultivars (Fuji Kiku 8, Braeburn, FEM7, FEM5, Dalinette, Gold Rush, FEM2, FEM3 and FEM10) were categorized as high emitters of terpenes (m/z 81.070), α-farnesene (m/z 205) and its related fragments (m/z 95.086, 109.101, 121.101, 123.117, 135.117, 149.133). Ethylene (m/z 28) was positively correlated ($p < 0.05$; $r = 0.67$) to α-farnesene (m/z 205) as reported for previous studies (Chapter 3) (Ju and Curry, 2000; Golding and others, 2001). Other tentatively identified VOCs emitted by these cultivars were aromatic hydrocarbons such as phenol (m/z 95.049), acetophenone (m/z 121.065), benzene (m/z 79.054), styrene (m/z 105.071) and ethyl benzene (m/z 107.086). However, within this subset, Braeburn, Fuji Kiku 8, FEM7 and FEM3 also emitted ester related fragments, which were detected as lower MW mass ions (m/z 41, 43.018, 57.034, 61.

In comparison to the other cultivars, Renetta Grigia, Renetta Bianca and Topaz were classified as cultivars with the lowest VOC concentrations, despite Renetta Bianca, Renetta Grigia and Topaz being the highest emitters of methanol (m/z 33.034), ethanol (m/z 47.045), butanoate/acetate (m/z 71.050), methyl butanoate (m/z 103.075), ethyl butanoate (m/z 117.091) and 2-methyl butyl acetate (m/z 131.107).

4.3.2 Texture and physico-chemical properties

Physico-chemical properties, texture and acoustic parameters significantly differentiated (ANOVA, $p < 0.05$) the different apple cultivars (Figure 4.2). In this figure, the colour band represents the different attributes with the red colours being acoustic properties, orange, the physico-chemical properties and yellow-green colours mechanical force-displacement related parameters. Visually, cultivars were well separated based on their acoustic properties.

Based on texture the 21 cultivars were separated into four main groups/ branches by the cluster analysis (Figure 4.2). Based on the order the cultivars have been clustered, the first group were FEM3, FEM2, Pinova Evelina, FEM7 and Dalinette. These were firm cultivars of high DMC and SSC with intermediate acoustic properties. The second group were cultivars high in both mechanical and

acoustic texture properties, suggesting that cultivars such as Gold Rush, FEM4, FEM10, Pilot and Braeburn are firm and crunchy cultivars (Costa and others, 2011; Corollaro and others, 2013). Other cultivars were high in acoustic properties but were not as firm in texture as those in the second group. These characteristics were represented by the third group of cultivars such as Fuji Kiku 8, FEM5, Pinova and FEM11. The forth group of seven cultivars (Topaz, Kanzi, Gloster, Rubens, Golden Delicious, Renetta Grigia and Renetta Bianca) were the lowest in both mechanical and acoustic texture properties representing soft cultivars.

Apart from the conventional mechanical and acoustic parameters used to differentiate soft and firm cultivars, two other parameters were of interest. Difference in force (Diff_Force) and force ratio are two parameters that can be used to indicate the direction and magnitude of force. In this instance, they can be interpreted as the behaviour of the flesh fracture after compression. A positive value suggests less resistance from the flesh as it fractures which is indicative of soft flesh, while a negative value indicates a higher resistance as seen with firm apple flesh (Costa and others, 2012). These two parameters can be used to predict flesh break-down behaviour in the mouth during first bite and mastication, where a positive value is indicative of soft mealy cultivars that have a floury mouthfeel during consumption; and a negative value suggests more force is needed during mastication to break down the apple flesh indicating firm flesh.

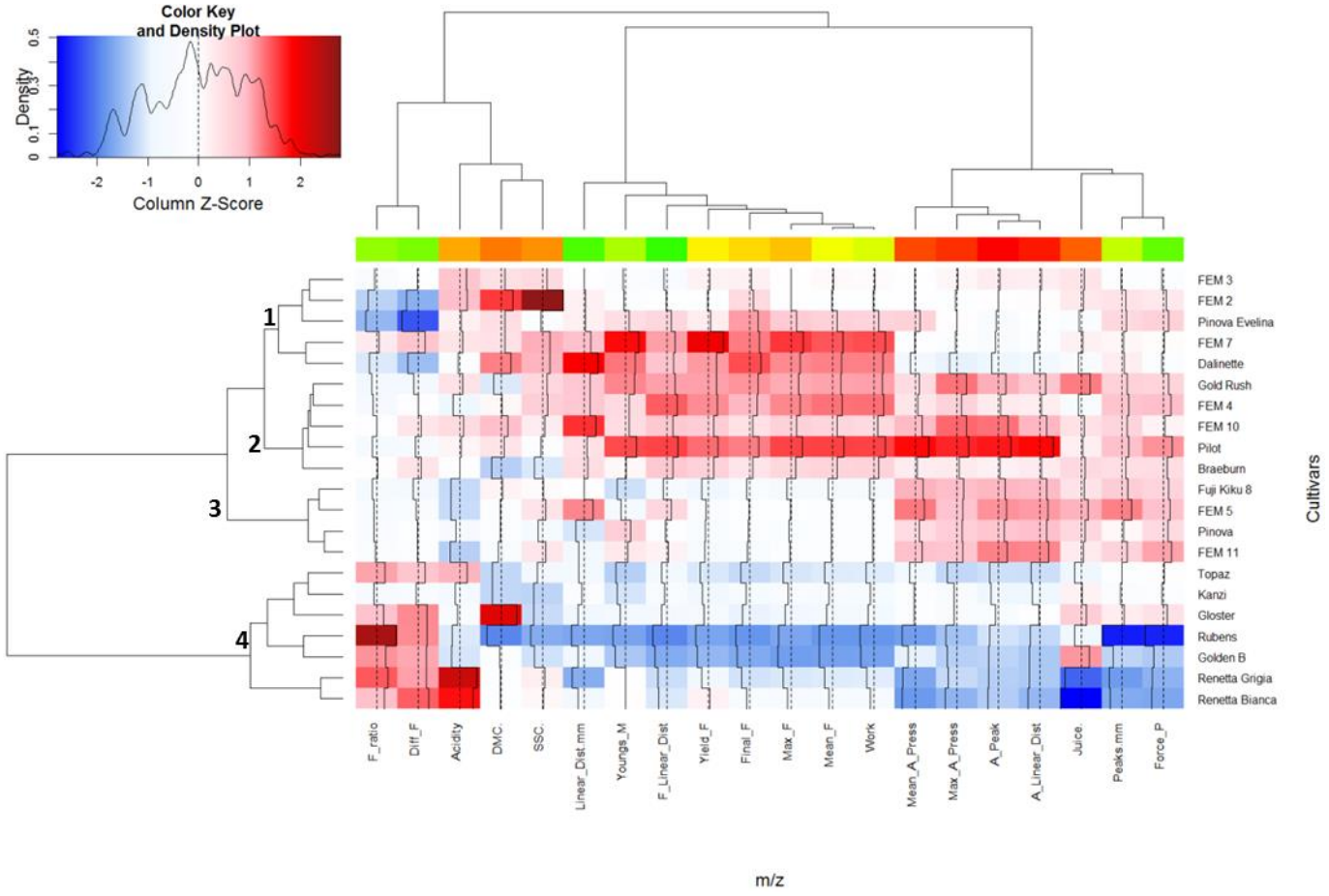

Figure 4.2: Heat map of the log_{10} transformed physico-chemical properties, mechanical and acoustic texture parameters that significantly differentiated (P<0.05) the 21 apple cultivars.

Focusing on the maximum acoustic pressure, ten cultivars were found to have intensities higher than the log_{10} transformed sample set mean. Although high values of acoustic parameters are indicative of sensory crunchiness (Costa and others, 2011), four cultivars (Pinova, Fuji Kiku 8, FEM5 and FEM11) were mechanically softer than the other crunchy cultivars. This was shown by their lower intensities in mechanical texture properties such as yield, final, max, mean and work force values. In contrast, Pinova Evelina, Dalinette and FEM7 were firm cultivars, especially the latter two, which were higher in yield, mean and max force compared to other crunchy cultivars but the lower values for acoustic properties indicate these cultivars were less crunchy. This suggests differences in apple texture profiles are not limited to just soft-not crunchy and firm-crunchy but could also include firm-less crunchy and less firm-crunchy (Costa and others, 2011). Nevertheless, for acoustic properties to be present in apple flesh the underlying structural mechanical properties must be present.

Expressed juice was positively correlated ($p < 0.05$, mean $r = 0.51$) to all acoustic properties, with cultivars that were higher in acoustic parameters contained more expressed juice (Figure 4.2). This correlation could be interpreted as the emission of sound in apples when the turgid cell walls are ruptured due to an application of external pressure. As a result, the cells rupture producing a sound whilst releasing its juices (Duizer, 2001). Titratable acidity was the highest in Renetta Bianca and Renetta Grigia and the lowest in FEM5 and FEM11. DMC% was high in cultivars that were very firm such as Dalinette, Pinova Evelina and FEM7 and intermediate for crunchiness

4.3.3 *In vivo* nosespace analysis

Compared to the 51 mass ions used to differentiate the cultivars in Figure 4.1, only 15 mass ions (Figure 4.3) were compiled using the *in vivo* nosespace data selection criteria (Section 4.2.6). However, this was still four times more than the number obtained using the PTR-QUAD-MS (Chapter 3). The selected m/z included ester related fragments (m/z 43.018, 43.054, 57.034, 57.070), acetic acid/acetate esters (m/z 61), butanoate/acetate esters (m/z 71.05), methyl acetate (m/z 75), methyl

butanal (m/z 87.082), ethyl acetate/ butyrates (m/z 89.060), hexyl acetate/ hexanal (m/z 99), hexanal (m/z 101), methyl butanoate (m/z 103), ethyl butanoate (m/z 117), acetophenone (m/z 121.065) and phenyl/ estragole (m/z 149.096). No terpene or sesquiterpene related VOCs were detected in the *in vivo* nosespace data, owing to the low concentrations present within the apple discs as these compounds are mainly associated with the peel (Guadagni and others, 1971). Preliminary results from the Chapter 3 implied that I_{max} could be used as a variable to discriminate between samples. As the values of I_{max} in Chapter 3 were strongly correlated ($p < 0.05$; $r \sim 0.99$) to their AUC values for the same m/z only I_{max} will be used in Figures 4.3. Swallowing parameters reported as number of swallows, N_{swals}, time to first swallow, T_{swal} and time for entire consumption, T_{con} were also included.

As reported in Figure 4.1, clustering of the *in vivo* variables in Figure 4.3 was influenced by the measured abundance. However, the *in vivo* nosespace profile of the cultivars did not fully reflect the *in vitro* VOC profiles in all cultivars. For example, although *in vitro* measurements for FEM11, FEM4 and Pinova cultivars were high in estragole and numerous ester related compounds (Section 4.3.1), these trends were similar only for Pinova and FEM4. Both cultivars were high in AUC and I_{max} for all ester related fragments, acetic acid/acetates and methyl acetate. In comparison, FEM11 contained high concentrations for estragole (m/z 149.096) and hexanal (m/z 101) and had a longer durations for T_{max} for the majority of the measured VOCs (Figure 4.3).

Six cultivars which had a longer T_{con} compared to the other cultivars (Pinova Evelina, Braeburn, Pinova, FEM3, FEM7 and FEM4) had the highest intensities for a majority of the I_{max} variables compared to the others cultivars. These were all ester related fragments (m/z 43.018, 43.054, 57.034, 57.070), acetate esters (m/z 61), hexyl acetate (m/z 99), hexanal (m/z 101), acetophenone (m/z 121.065) and estragole (m/z 149.096). The accompanying T_{max} values of the I_{max} variables listed were intermediate suggesting the times needed for the mass ions to reach maximum intensity were similar. Other cultivars such as Pilot, Dalinette and Kanzi had lower I_{max} intensities and longer T_{max} values compared to the other cultivars owing to the fact that these cultivars, although firm, did not contain high *in vitro* concentrations of the selected 15

nosespace mass ions (Section 4.3.1). Therefore, a lower I_{max} and a longer T_{max} was measured for these cultivars. Renetta Grigia, Renetta Bianca and Topaz were soft cultivars that were characterised with high I_{max} values for butanoate/acetate esters (m/z 71.05), ethyl acetates/butyrates (m/z 89), methyl butanoate (m/z 103) and ethyl butanoate (m/z 117). These cultivars were also characterised similarly in the i*n vitro* headspace measurements (Section 4.3.1).

Although not significantly different, Time to first swallow, T_{swal} was the longest for Pilot, Topaz and FEM11 and the shortest for Fuji Kiku 8, FEM5 and FEM3. Previously, T_{swal} showed potential to be used to explain sample breakdown within the mouth. A shorter T_{swal} indicated a juicy apple and the need to swallow even before bolus formation. On the contrary, a longer T_{swal} indicates a dry, mealy apple with less juice and therefore first swallow is made after bolus formation (Chapter 3). Chapter 3 showed similar findings of short T_{swal} times for firm Fuji Kiku 8, FEM5 and FEM3 cultivars. However, T_{swal} was the longest for both a soft (Topaz) and a firm cultivar (FEM11). The longer T_{swal} could be due to FEM11 being a firm and crunchy cultivar but not perceived as juicy by the panellists (in Chapter 5 more details on perceived and instrumental measured juiciness are discussed).

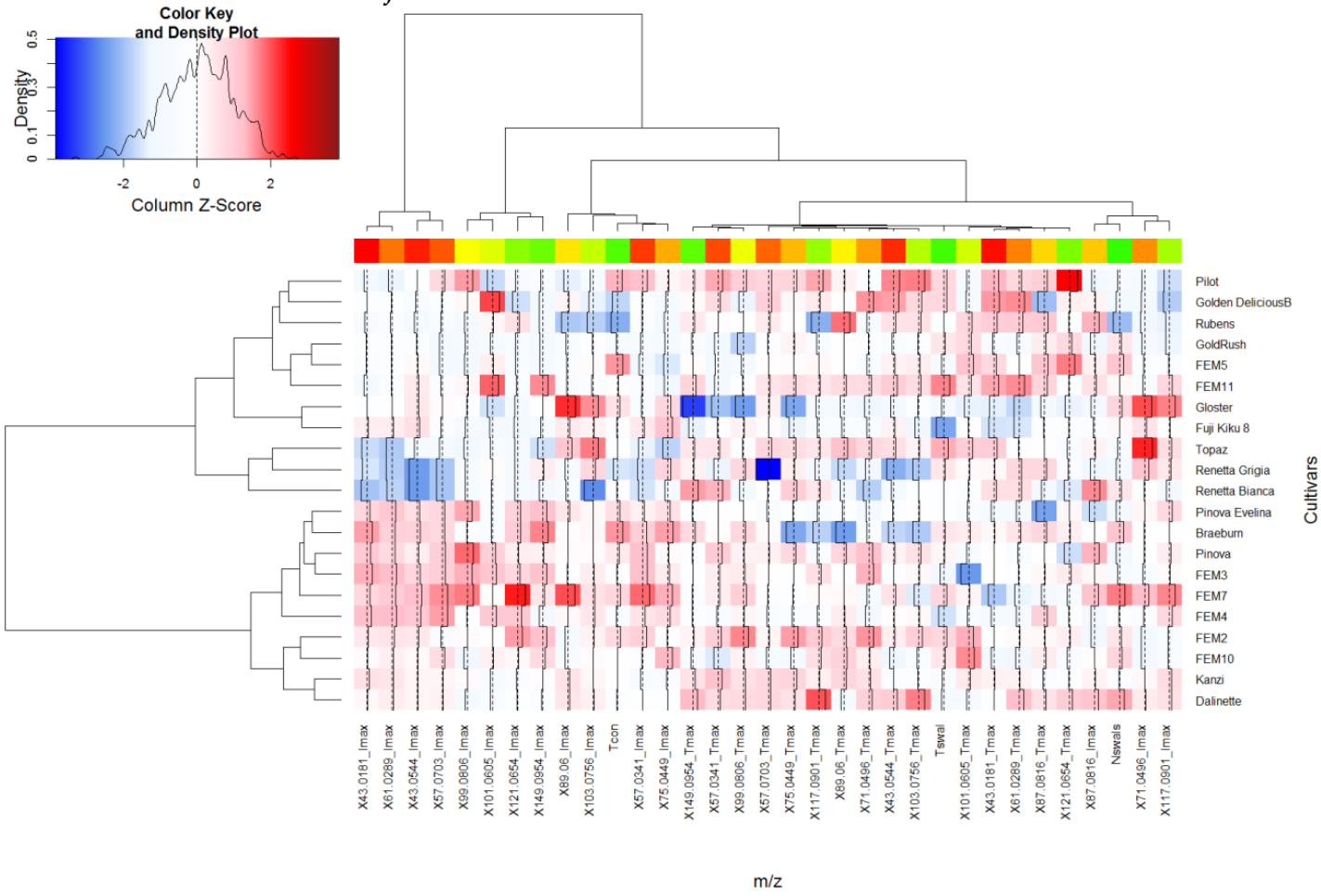

Figure 4.3: Heat map of log_{10} transformed nosespace parameters from mass ions that significantly differentiated ($p<0.05$) the cultivars. For ease of reading, I_{max} was not plotted as it was strongly correlated to AUC ($P<0.05$; $r=0.99$).

4.3.4 Multi Factor Analysis (MFA)

Using the *in vivo* nosespace parameters, *in vitro* headspace VOCs, texture, physico-chemical and swallowing parameters an MFA was plotted to identify interrelationships between these variables (Figure 4.4). The first three dimensions of the MFA explained 51.7% of the total variance. There was a separation on Dim1 based on *in vivo* nosespace parameters, textural and physico-chemical properties. A general observation showed *in vitro* headspace parameters were orthogonal to both texture and nosespace parameters which were correlated to each other. The main variables that separated cultivars on positive Dim1 were physico-chemical, texture parameters and a majority of the I_{max} and AUC variables associated with ester related compounds. These variables were associated with firm cultivars such as Braeburn, FEM7 and FEM4 that contained high amounts of VOCs; or cultivars that were firm with low amounts of ester related VOCs (Dalinette, Pilot, Gold Rush). Negative Dim1 was associated with force ratio, difference in force, acidity, most T_{max} variables and hexanal (m/z 101), butanoate/acetate esters (m/z 71.05), methyl butanal (m/z 87.082) and methyl butanoate (m/z 103) I_{max} and AUC variables. These variables were strongly associated with soft cultivars such as Renetta Grigia, Renetta Bianca and Topaz. Dim 2, separated cultivars based on their VOC composition and abundance. Positive Dim 2 was explained by a majority of the *in vitro* VOCs whereas negative Dim 2 was only associated with aldehydes, ethylene and terpene/sesquiterpene related compounds.

Time of consumption, T_{con} was longer for firmer cultivars indicating panellists took a longer time to finish an entire sample when given a firm sample to consume (Chapter 3). This was based on the association of T_{con} with textural properties (Figure 4.4). T_{con} was positively correlated to force peaks ($r = 0.627$) in which a higher number of force peaks indicated firmer and crunchier cultivars (Section 4.3.2). N_{swals} was found in the same quadrant and was positively related to T_{con} ($r = 0.538$). This could be interpreted as panellists taking longer and having more swallows when consuming firmer samples.

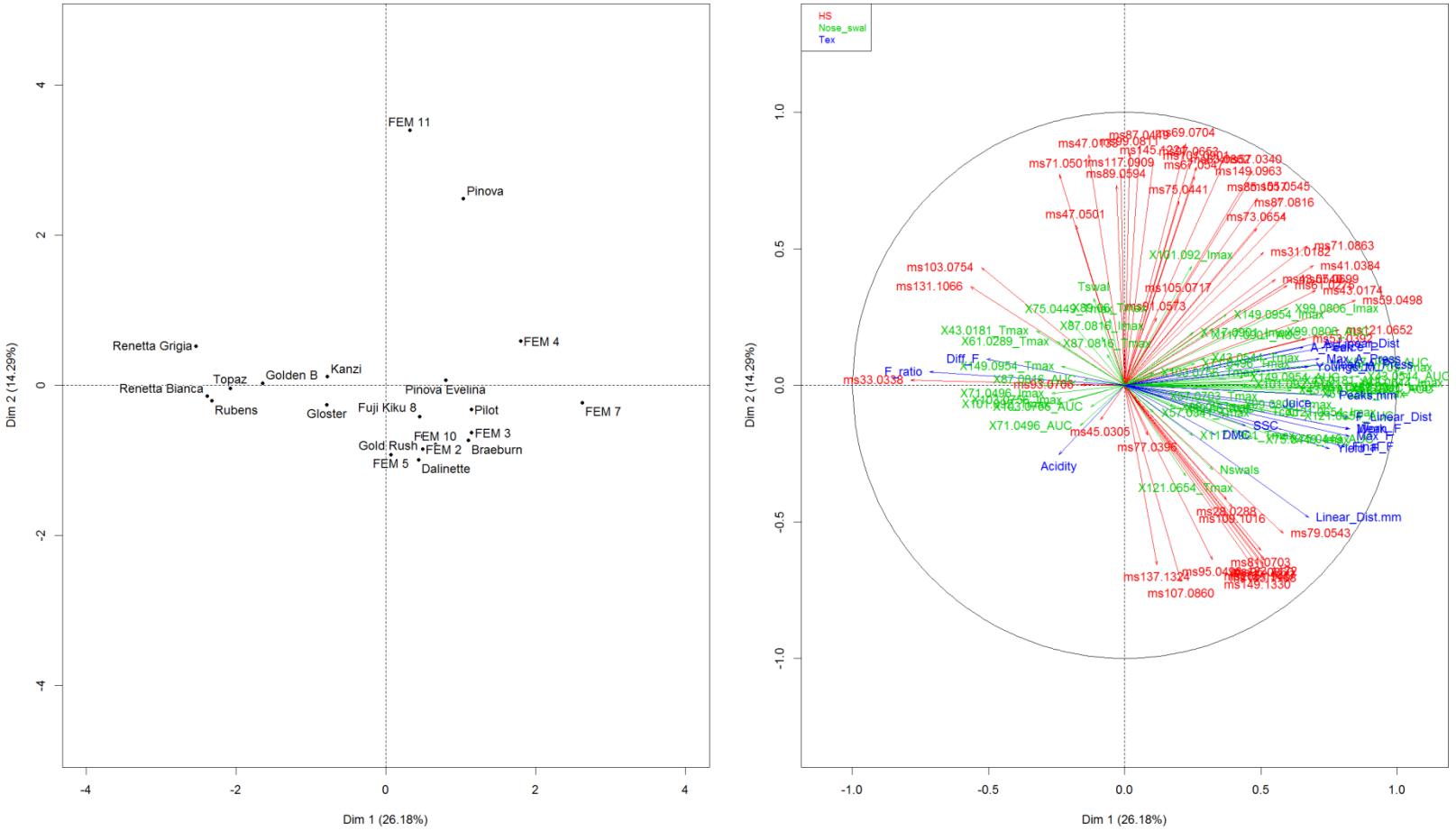

Figure 4.4: MFA of Dim 1-2 (A) using in vitro headspace VOCs, in vivo nosespace and swallow parameters, texture and physico-chemical properties. Abbreviations on figure legend include: HS - Headspace; Nose_swal - Nosespace and swallows; and Tex - Texture.

The relative abundance of VOCs in the *in vivo* nosespace profiles of each cultivar is dependent on the available *in vitro* VOC concentration. However, the release of flavour depicted by T_{max} is observed to be dependent on the behaviour of texture breakdown. These two concepts coincide with the findings from Chapter 3. In the current study apple cultivars on Dim 2 were separated by their differences in VOC abundance and composition whereas Dim 3 separated cultivars based on texture and nosespace parameters (Figure 4.5). Firm cultivars (Braeburn, FEM7 and FEM4) that emitted high intensities of ester related VOCs for both *in vitro* and *in vivo* measurements were associated with T_{con}, N_{swals} and the accompanying I_{max} and AUC variables on negative Dim 3. A negative association to T_{max} indicated that a shorter time was needed to reach I_{max} despite the cultivars firm texture. This was contradictory to previous research which reported a shorter T_{max} was associated to soft gel matrices due to the ease of gel breakdown during mastication causing a faster increase in surface area (Baek and others, 1999; Mestres and others, 2005; Boland and others, 2006).

In the current study, the shorter T_{max} could be driven by a shorter T_{swal} for Braeburn, FEM7 and FEM4 cultivars. The short T_{swal} times for these cultivars could be due to juice expressed during mastication causing a greater need to swallow as previously discussed. This resulted in faster swallows and expiration of retronasal air which other studies did not involve due to their gel system. Therefore, apart from texture and VOC composition, other factors such as juiciness may influence T_{swal}, T_{max} and I_{max} (Frank and others, 2012). Additionally, T_{swal} could also be related to ease of swallow which has been observed to be dependent on sample juiciness or the time needed during mastication to reduce the sample size to form a bolus.

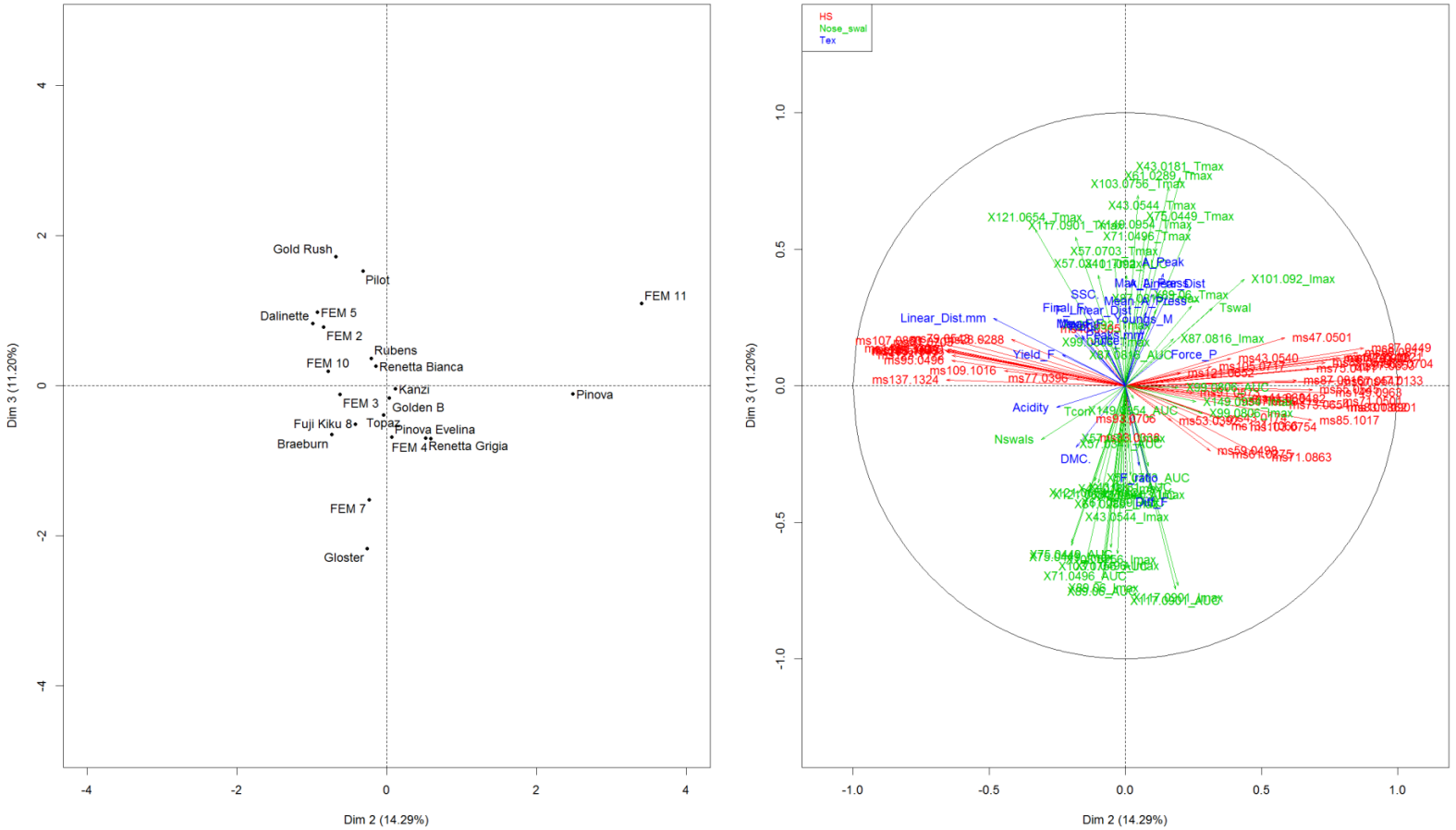

Figure 4.5: MFA of Dim 2-3 using in vitro headspace VOCs, in vivo nosespace and swallow parameters, texture and physico-chemical properties. Abbreviations on figure legend include: HS - Headspace; Nose_swal - Nosespace and swallows; and Tex - Texture.

Using a larger collection of apple cultivars to measure VOCs provided additional information towards understanding the *in vitro* and *in vivo* flavour release for fresh apples. *In vitro* analysis separated cultivars based on their differences in VOC composition and abundance into cultivars characterised as containing: A) high amounts of ester related VOCs, B) predominantly terpenes, sesquiterpenes and aromatic hydrocarbons, C) sesquiterpenes, terpenes and ester VOCs and D) alcohols and butanoate related esters. In the *in vivo* study, only fifteen m/z were detected from the *in vivo* nosespace measurements. These were mainly ester related compounds, acetophenone, estragole and hexanal. Cultivars containing high amounts of these VOCs in the *in vitro* measurements showed a similar separation in the *in vivo* results as indicated by the nosespace parameters of AUC and I_{max}. Swallowing parameters such as T_{swal} and N_{swals} could be used to identify differences in cultivar texture. Additionally, to better understand the influence of texture on flavour release during consumption the use of T_{max} is proposed. Conventionally, only soft cultivars containing high VOC concentrations were proposed to have short T_{max} values. This was due to a more efficient breakdown of the flesh during mastication increasing the surface area, hence the emission of VOCs. However, this study illustrates firm cultivars containing high amounts of ester related VOCs could also achieve a shorter T_{max} due to a faster T_{swal}. Faster swallows induced by the release of juices during mastication increases the rate of VOCs passing through the nasal cavity which may heighten flavour perception during consumption.

Based on these findings, *in vivo* methods showed that flavour release is not just dependent on the VOC composition and texture but also on the available juice that induces swallowing during consumption. Importantly, it was also found that the amount of expressed juice instrumentally measured may not reflect the perceived sensory juiciness. This is because instrumental juiciness does not consider the mastication effects on juice release nor the breakdown behaviour and mouth feel of apple flesh that could induce other tactile sensations which may decrease perceived

juiciness. Therefore, it would be beneficial to understand the differences or similarities between cultivars analysed using analytic and sensory measurements. This will be explored in the next chapter.

Chapter 5: Apple flavour: Linking sensory perception to volatile release and textural properties

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Names	***Role in Manuscript***
Valentina J.L. Ting	Execution of instrumental lab work, design of experiment, data analysis and writing.
Andrea Romano	Guidance in data analysis and instrumental lab work
Patrick Silcock	Supervisor
Phil J. Bremer	Supervisor
Maria Laura Corollaro	Execution of instrumental lab work and descriptive sensory analysis
Christos Soukoulis	Guidance in instrumental lab work and data analysis
Luca Cappellin	Proof reading and guidance in data analysis
Flavia Gasperi	Supervisor of student who contributed
Franco Biasioli	Supervisor

5.1 Introduction

In apples, flavour and texture traits are complementary eating qualities which change at varying rates over time depending on cultivar and storage conditions (Dixon, 2000). Consumers generally place a greater importance on a firm and juicy texture than on flavour meaning that technologies to enhance such texture over time such as ultra-low, controlled and modified atmosphere storage or the application of 1-MCP have been developed (Daillant-Spinnler and others, 1996; Sisler and Serek, 1997; Harker and others, 2003). However, these techniques are known to alter the VOCs produced by apples during storage and may also alter their sensory properties. Apples exposed to such postharvest treatments have delayed or disrupted ripening patterns which results in them having a good texture but little or unpleasant flavour as elaborated in Chapter 2.4.

Previous studies profiling the sensory properties of apple cultivars have provided a lexicon describing important attributes within the modalities of odour, texture and flavour (incorporating retronasal taste and odour) (Corollaro and others, 2013; Seppä and others, 2013). Although these studies provide an extensive list of texture attributes, the number of flavour attributes has normally been limited and has often been termed as a generic 'apple flavour/overall odour' attribute based on intensity. In some cases, a few attributes associated with flavour, including green/red apple, grassy, pear-like and fruity have been listed (Daillant-Spinnler and others, 1996; Karlsen and others, 1999). The lack of a full descriptive attribute list that differentiates taste and flavour modalities illustrates the complexity of apple flavour and the challenges associated with using consumers or trained panellists to describe it. In part this is due to the fact that flavour is a multi-sensorial experience derived from perceptions of taste, ortho/retronasal olfaction and texture. It has been postulated that by looking at components of flavour independently using instrumental methods a more comprehensive understanding of flavour could be obtained (Cook and others, 2005).

Reviews carried out by Dixon (2000) and Yahia (1994) report that there are more than 300 VOCs associated with apple flavour/odour, further illustrating the challenge consumers and trained panellists face when asked to describe apple flavour. It has however also been reported that perceived sensory odour assessed by trained panellists could be predicted from a selection of VOCs at different ratios detected by GC-MS analyses (Aprea and others (2012). While this analysis approach is promising, GC-MS techniques require longer analysis times than direct injection methods, such as proton transfer reaction time of flight mass spectrometry (PTR-ToF-MS). PTR-ToF-MS is a very fast technique producing a rapid VOC fingerprint within seconds and it can therefore dramatically increase sample throughput. The sensitivity of the high resolution data obtained has previously been used to distinguish apple cultivars and their clones based solely on their VOC fingerprints (Cappellin and others, 2012c; Soukoulis and others, 2013).

Instrumental measurements of firmness have been correlated to sensory crispness in numerous studies (Harker and others, 2002a; Brookfield and others, 2011; Corollaro and others, 2014). The mechanical aspects of a crisp or crunchy texture occur due to a sharp decrease in force followed by a rapid fracture of the apple flesh. Concurrently, during mastication, a series of sounds are produced as the turgid cells rupture (Duizer, 2001). It has been reported that a combined force-deformation and acoustic technique was more effective in predicting crisp and crunchy attributes than either technique alone due to the simultaneous occurrence of crisp sound upon mechanical rupture (Vickers, 1987). Recently, improved combined acoustic and mechanical techniques have been used to characterize apple cultivars (Zdunek and others, 2010; Costa and others, 2011), with sensory crispness being better predicted when both mechanical and acoustic parameters were used (Corollaro and others, 2014).

A relationship between texture and perceived flavour has been reported for apples (Harker and others, 2006) and other food products (Cook and others, 2005; Aprea and others, 2006a). However, studies utilizing both analytical VOC and texture measurements correlated to sensory perception are rare (Karlsen and others, 1999). The use of multivariate analyses can increase the understanding of

relationships between analytical measurements and sensory analysis for different apple cultivars. Such information is important because while sensory analysis is the gold standard in providing sample descriptors based on human perception, descriptive panels are costly and rely on strict selection and specific training before product evaluation (Murray and others, 2001). The development of analytical techniques that could predict sensory perception is therefore beneficial.

Previously Corollaro and others (2014) have used instrumental texture, chemical (% soluble solid composition, titratable acidity, % juice, % dry matter) and sensory data (texture, taste modalities) obtained from a large dataset of 27 apple cultivars to construct a partial least square (PLS) model for sweet taste. The current study uses six of the cultivars used in the Corollaro and others (2014) study plus an additional three experimental cultivars, and focuses on comparing cultivar VOC composition, acoustic and instrumental texture data with sensory data (odour, flavour, taste and texture).

5.2 Methods and materials

5.2.1 Apple sample preparation

Nine apple cultivars (*Malus Domestica* Borkh.) (Dalinette, F1, F2, F3, Golden Delicious, Kanzi, Pinova Evelina, Renetta Bianca, and Topaz) were sourced in 2011 from an experimental field maintained by the Research and Innovation Centre, Fondazione Edmund Mach, FEM (San Michele a/A, Trento, Italy). Six of these cultivars were commercial varieties from this region and F1, F2 and F3 were crossed cultivars from the FEM breeding program. Harvested apples conformed to commercial maturity standards based on starch degradation index, fruit colour, firmness and soluble solids content. A minimum of 20 apples with no visible exterior damage and consistent size were selected for each cultivar. Apples were held at 2ºC for two months (95% RH) before use. Samples were brought to room temperature 24 hours before the experimentation.

Three cultivars were chosen for each of the three experimental sessions. For each cultivar, fifteen apples were selected and cut into discs (18mm Ø 12mm height) using a stainless steel corer parallel to the core where each apple yielded 15-18 discs. Care was taken to avoid sampling the mesocarp region or the skin. The apple discs were dipped in an antioxidant solution (0.2% w/w ascorbic acid, 0.2% w/w calcium chloride, 0.5% w/w citric acid monohydrate) to prevent oxidative browning. For the first eight apples, one disc from each apple was set aside for physico-chemical measurements and another disc was used for PTR-ToF-MS measurement. A total of 8 discs for each of these measurements were used. One disc from each apple was set aside for texture analysis giving a total of 10 discs for each variety. The remaining discs were used for sensory analysis.

5.2.2 PTR-ToF-MS measurement

For PTR-ToF-MS analysis one fresh cut apple disc was placed in a 250mL glass jar fitted with a lid (Bormioli Srl. Bologna, Italy) that contained a silicon septa on the top and opposite base side of the jar. The sample was incubated in a water bath (30°C) for 30 minutes before measurement. Sample headspace was measured through direct injection into the PTR-ToF-MS 8000 (Ionicon Analytik GmbH, Innsbruck, Austria) via a heated PEEK tube (110°C, 0.055" Ø) at a flow rate of 50sccm. Identical instrumental settings for the PTR-ToF-MS were used and have been elaborated in Chapter 4.2.3. Three cultivars were measured per session where a total of six replicates were measured for each cultivar. Sample order was randomized in order to decrease the occurrence of systematic memory effects.

5.2.3 Sensory analysis

Prepared apple discs (15-18 discs per apple) were served in covered plastic pottles (8 discs/ pottle) coded with a 3-Digit random number and presented in a balanced randomized order to each panellist. A total of six apple samples per session were evaluated corresponding to three cultivars in two replicates.

Apple sensory profiling was carried out by a trained panel (6 males, 7 females) according to the quantitative descriptive method reported in Corollaro and others (2013). A lexicon describing the apple samples was developed through a consensus method. Panellists were trained on their agreed list of 33 attributes (2 appearance, 11 odour, 11 flavour/retro-nasal odour, 2 taste, and 7 texture) and the use of scales using a set of references. During sample evaluation, the intensity of each attribute was rated on a 100mm linear line scale anchored at 0 (absence), 50 and 100 (maximum intensity). Unsalted bread and water were provided as palate cleansers to be taken between samples. For odour and flavour attributes, panellists were told to rate only attributes they could perceive in the samples. All sensory tests were carried out using the FIZZ 2.46A software (Biosystemes, Couternon, France). Mean scores from each panellist (average of 2 replicates) were used for further analysis. Only attributes that significantly differentiated ($p<0.05$) the cultivars were discussed in this study (Table 5.1).

Table 5.1: Sensory lexicon of attributes that significantly differentiated ($p<0.05$) apple cultivars.

Modality	*Attribute*	*Definition*	*References (low: 0 to high: 100)*
Appearance	Green Flesh	Green colour of apple flesh	0: Print of white colour (RGB model: red 255, green 255, blue 255); 100: Print of green colour (RGB model: red 207, green 253,blue 203)
	Yellow Flesh	Yellow colour of apple flesh	0: Print of white colour (RGB model: red 255, green 255, blue 255); 100: Print of yellow colour (RGB model: red 252, green 237,blue 150)
Odour	Overall odour	Overall odour sensation	0: Diluted apple juice: water (1:2)[a]; 100: Apple juice (100%)[a]
	Pear	Perceived pear odour	n-hexyl acetate (5.0 mg/kg)
	Banana	Perceived banana odour	Isoamylacetate (5.0 mg/kg)
	Lemon	Perceived lemon odour	d-limonene (50 mg/kg)
	Vanilla	Perceived vanilla odour	Vanillin (10 mg/kg)
	Honey	Perceived honey odour	Methyl-anthranilate (0.5 mg/kg)
Flavour/ retro-nasal odour	Banana	Perceived banana flavour	Isoamylacetate (5.0 mg/kg)
	Lemon	Perceived lemon flavour	d-limonene (50 mg/kg)
	Vanilla	Perceived vanilla flavour	Vanillin (10 mg/kg)
Flavour/Taste	Sweet	Perception of sweet taste	0: Fructose water solution (20 g/kg); 100:Fructose water solution (80 g/kg)
	Sour	Perception of sour taste	0: Citric acid water solution (0.6 g/kg); 100: Citric acid water solution (2.0 g/kg)
Texture	Hardness	Resistance of sample during first chews using molars	0: Carrot boiled for 12 min; 100: Carrot boiled for 4 min
	Flouriness	Degree of breakdown into small and dry fragments during chewing	0: Potato boiled for 4 min; 100: Potato boiled for 12 min

Modality	*Attribute*	*Definition*	*References (low: 0 to high: 100)*
Texture	Juiciness	Amount of juice released during first 3 chews	0: Unripe melon; 100: Ripe melon
	Crunchiness	Sound produced by sample during first 5 chews	0: Soft breakfast cereals [b]; 100: Fresh and dry breakfast cereals
	Graininess	Size of fragments produced during chewing	0: Carrot boiled for 4 min; 100: Short bread biscuit
	Fibrousness	Degree of flesh breakdown into thick and fibrous fragments during chewing until bolus is ready to be swallowed	0: Carrot boiled for 12 min; 100: Fresh raw celery
	Astringency	A dry sensation in mouth	0: Tannic acid water solution (0.1 g/kg); 100: Tannic acid water solution (0.5 g/kg)

[a] Cloudy apple juice produced by Pfanner Getränke GmbH, Lauterach, Austria.
[b] 50g extruded cereal balls (Miel Pops Kellogg's) kept at 23°C for 24 hours in a sealed container containing a 30ml cup of water.

5.2.4 Texture analysis

A comprehensive analysis combining the acquisition of 12 mechanical and 4 acoustic properties of apple texture was performed using a Texture Analyzer (TA-XT plus) equipped with an Acoustic Envelop Detector (AED) device (Stable Microsystems Ltd, Goldalming, UK) in accordance to Costa and others (2011).

A penetration test was carried out using the texture analyser loaded with a 5kg cell and a cylindrical 4mm diameter flat head probe attachment. Texture analysis was carried out on 10 apples per cultivar using a single cylinder per apple. The test was performed by penetrating the sample at a test speed of 300mm/min and an auto-trigger of 5g to a 90% deformation. Mechanical parameters were calculated from the acquired force (N) – distance (mm) curves. Acoustic properties of the sample were measured simultaneously with the penetration test where the AED device was placed approximately 5cm from the sample. The device was synchronized to the texture analyzer and started acquiring acoustic data when the probe sensed the auto-trigger. The following parameters were calculated based on these curves defined by Costa and others (2011) and can be found in Chapter 4, Table 4.2.

5.2.5 Physico-chemical properties

Physico-chemical properties were measured in accordance to Corollaro and others (2014). Briefly, two replicates for each measurement were carried out. For each replicate, the juice from four discs from each cultivar was obtained by mechanical compression using a garlic crusher and reported as the percentage of expressed juice per fresh weight (%). Soluble Solids Content (%), SSC was measured using a DBR35 refractometer (XS Instruments, Poncarale, Brescia, Italy). Titratable acidity, TA (Compact Titrator, Crison Instruments S.A., Alella, Barcelona, Spain) of the juice were measured by titrating 5g of juice up to pH8.16 with NaOH (0.1M) and calculated as malic acid equivalents in 100g juice. The remaining apple palette obtained after the juice has been expressed was used to measure dry matter concentration, (DMC %) by drying in an oven at 103°C for 24 h in accordance to the Association of Official Analytical Chemists method. Results were calculated as a

percentage of weight loss before juice expression and after drying (Chapter 3) (Equation 3.1).

5.2.6 Data analysis

PTR-ToF-MS spectrum analysis

Data pre-processing was carried out after first removing the baseline and reducing the noise in the ToF spectra by averaging over 50 consecutive cycles measured for each sample. Headspace raw data, acquired as counts per second (cps) was converted to concentration (ppbv) based on the formula described by Lindinger and others (1998) using a constant rate coefficient ($k = 2*10^{-9}cm^3/s$). Internal calibration of the mass spectral data, peak extraction and dead time correction was performed offline in accordance to Cappellin and others (2011) using a customized script developed in MATLAB R2012a (The Mathworks Inc, Natic, MA). From this, a mass range of m/z 15 – 250 was selected in which a total of 489 peaks were extracted. Due to the very large number of peaks involved, a pre-processing step was carried out, which included the exclusion of water clusters, machine associated compounds (in accordance to Table 3.1) and compound isotopologues; and peaks having overall average concentration lower than 0.5 ppbv.

Statistical analysis

A one-way Analysis of Variance (ANOVA) was used to investigate cultivar effects on PTR-ToF-MS, sensory, texture and physico-chemical data sets. Results that were significantly different ($p < 0.05$) were subjected to a Tukey's Honest Significant Difference, HSD post-hoc test. A radial plot was drawn for the PTR-ToF-MS data in order to visualize the significant differences in VOC groups emitted by each cultivar. Due to the large variation in intensities (Table 5.3), each m/z was scaled independently across all cultivars to allow the comparison of relative differences of each m/z based on the cultivars. A Multi Factor Analysis (MFA) was carried out using the FactoMineR package (Lê and Husson, 2008) on all significantly different variables from the four variable groups. All data used for the radial plot and MFA

were scaled before plotting (1/Standard Deviation). All statistical analyses and graphs were carried out using the Statistical Package R.

5.3 Results and discussion

5.3.1 Sensory analysis

Descriptive sensory analysis identified 20 attributes which significantly differentiated between the nine apple cultivars (Table 5.2). Pinova Evelina and F2 were not significantly different from each other in overall odour but were significantly higher than all other cultivars. In general, F2 was rated the highest in all sensory odour and flavour attributes with the exception of lemon flavour (Fl) and lemon odour (Od), for which it had the lowest rating (Table 5.2). It was evaluated as having a hard texture, with a juicy and fibrous mouth feel and a sweet taste. Topaz and Renetta Bianca were rated the highest in Lemon.Fl and Lemon.Od and were the lowest in Banana.Od, Banana.Fl and Pear.Od. Both Topaz and Renetta Bianca were rated the highest in graininess, flouriness, astringent mouth feel, not juicy and most sour.

Cultivars of intermediate odour and flavour, i.e. Golden Delicious, Pinova Evelina and Kanzi, were characterized with high Pear.Od, Banana.Od, Vanilla.Od and Vanilla.Fl. Despite their similarities, these three cultivars differed in texture and mouth feel attributes. Of these three, Golden Delicious was rated the least hard, least crunchy and less fibrous but highest in juiciness, flouriness and graininess. Pinova Evelina was a harder apple than Golden Delicious and higher in all texture and mouth feel attributes with the exception of graininess and flouriness. Kanzi scored a high in intensity of juiciness, compared to F2 and F3 and was significantly lower in hardness, crunchiness and higher in grainy mouth feel similar to Renetta Bianca and Golden Delicious. This indicates that, although these apples were comparable in flavour and odour attributes, their difference in texture may influence consumer preference (Harker and others, 2008).

F1 and F3 cultivars were rated higher than the other cultivars in texture and mouth feel attributes such as juiciness, hardness, crunchiness and fibrousness. Dalinette, F1 and F3 were not significantly different in odour or flavour attributes. Although Dalinette was rated a hard apple, it was also at least twice as high in flouriness and graininess but not juiciness than F1 and F3. F1 was rated higher in sour taste in comparison to Dalinette and F3. These apples could be characterized as being firm with low intensities in all odour and flavour attributes. In the current study it was observed that sour taste was positively correlated to Lemon.Fl (r=0.98), Lemon.Od (r=0.80), green flesh (r=0.3) and floury (r=0.58), astringent (r=0.88) and grainy (r=0.48) mouth feel. Previous studies investigating flavour perception and cross modal interactions reported an increase in sour taste when an odour well associated with "sourness" was used (Prescott and others, 2004). Although sour apples are typically hard and juicy, this was not the case with Renetta Bianca. Other studies conducted on the green flesh Renetta Bianca also characterized this cultivar as being mealy and not juicy (Paoletti and others, 1993; Corollaro and others, 2013).

Table 5.2: List of the mean values (n=13) and standard deviations (σ) of all significantly different (p<0.05) sensory attributes tested. Superscripted alphabets indicate there is a significant difference between those cultivars for a specific sensory attribute (Abbreviations: Odour: Od.; Flavour: Fl.).

Sensory	*Dalinette*	*F1*	*F2*	*F3*	*Golden Delicious*	*Kanzi*	*Pinova Evelina*	*Renetta Bianca*	*Topaz*
Odour	52	46	71	49	47	53	56	52	53
	(22) ^{a}	(21) ^{a}	(17) ^{b}	(21) ^{a}	(21) ^{a}	(25) ^{a}	(25) ^{ab}	(14) ^{a}	(17) ^{a}
Od.Pear	17	16	30	15	27	18	18	8.9	12
	(21) ^{abc}	(15) ^{abc}	(19) ^{c}	(17) ^{abc}	(27) ^{bc}	(18) ^{abc}	(15) ^{abc}	(15) ^{a}	(18) ^{ab}
Od.Banana	14	13	37	10	20	15	27	2.1	5.8
	(26) ^{ab}	(21) ^{ab}	(33) ^{c}	(20) ^{ab}	(30) ^{abc}	(28) ^{ab}	(30) ^{bc}	(7.6) ^{a}	(7.7) ^{a}
Od.Lemon	5.4	5.4	3.8	9.5	8.0	6.2	4.9	22	19
	(10) ^{ab}	(15.8) ^{ab}	(10) ^{a}	(15) ^{abc}	(12) ^{ab}	(7.5) ^{ab}	(8.3) ^{a}	(25) ^{c}	(26) ^{bc}
Od.Vanilla	3.4	3.0	14	3.3	6.1	6.8	8.0	3.4	3.8
	(6.3) ^{a}	(5.6) ^{a}	(15) ^{b}	(6.6) ^{a}	(7.5) ^{ab}	(18) ^{ab}	(12) ^{ab}	(8.2) ^{a}	(6.7) ^{a}
Od.Honey	3.5	2.0	12	2.8	7.9	2.9	3.9	3.4	8.1
	(6.6) ^{ab}	(4.3) ^{a}	(19) ^{b}	(6.4) ^{ab}	(13) ^{ab}	(5.8) ^{ab}	(7.3) ^{ab}	(8.8) ^{ab}	(20) ^{ab}
Fl.Banana	11	4.2	33	18	20.9	11.6	17	1.7	6.2
	(14) ^{ab}	(7.9) ^{ab}	(30) ^{c}	(26) ^{abc}	(29) ^{bc}	(23) ^{ab}	(25) ^{abc}	(3.4) ^{a}	(11) ^{ab}
Fl.Lemon	7.1	18	3.3	4.3	5.3	9.5	13	39	30
	(10.2) ^{a}	(15) ^{ab}	(8.8) ^{a}	(8.2) ^{a}	(12) ^{a}	(13) ^{a}	(16) ^{a}	(27) ^{c}	(26) ^{bc}
Fl.Vanilla	6.7	1.7	17	8.6	13.9	9.6	7.2	4.5	1.9
	(14.1) ^{ab}	(4.1) ^{a}	(16) ^{b}	(13) ^{ab}	(26) ^{ab}	(18) ^{ab}	(16) ^{ab}	(13) ^{ab}	(5.0) ^{a}
Green flesh	7.5	2.0	3.9	9.7	6.4	14	6.4	23	1.7
	(13.8) ^{ab}	(4.9) ^{a}	(7.3) ^{ab}	(12) ^{ab}	(11) ^{ab}	(17) ^{bc}	(9.5) ^{ab}	(21) ^{c}	(3.9) ^{a}

Sensory	*Dalinette*	*F1*	*F2*	*F3*	*Golden Delicious*	*Kanzi*	*Pinova Evelina*	*Renetta Bianca*	*Topaz*
Yellow flesh	69	73	63	28	61	40	55	9.3	81
	(23) ^{de}	(21) ^{de}	(22) ^{de}	(19) ^{ab}	(26) ^{de}	(27) ^{bc}	(24) ^{cd}	(16) ^{a}	(26) ^{e}
Juiciness	38	56	63	68	51	66	54	24	37
	(22) ^{ab}	(19) ^{c}	(21) ^{c}	(18) ^{c}	(23) ^{bc}	(20) ^{c}	(24) ^{bc}	(20) ^{a}	(21) ^{ab}
Sweet Taste	57	45	71	55	60	43	43	21	34
	(21) ^{cde}	(24) ^{bc}	(18) ^{e}	(23) ^{cde}	(15) ^{de}	(19) ^{de}	(19) ^{bc}	(16) ^{a}	(18) ^{ab}
Sour taste	23	44	8.5	9.7	10	16	31	73	62
	(24) ^{ab}	(22) ^{c}	(10) ^{a}	(16) ^{a}	(15) ^{a}	(18) ^{ab}	(24) ^{bc}	(19) ^{d}	(23) ^{d}
Hardness	55	68	57	61	10	34	51	29	20
	(24) ^{d}	(25) ^{d}	(24) ^{d}	(20) ^{d}	(11) ^{a}	(22) ^{bc}	(23) ^{cd}	(25) ^{ab}	(16) ^{ab}
Flouriness	26	3.5	4.5	5.1	47	18	8.4	55	56
	(24) ^{b}	(4.6) ^{a}	(6.3) ^{a}	(8.2) ^{a}	(26) ^{c}	(17) ^{ab}	(13) ^{a}	(27) ^{c}	(28) ^{c}
Astringency	23	31	18	18	7.6	11	26	35	32
	(29) ^{ab}	(34) ^{ab}	(30) ^{ab}	(27) ^{ab}	(13) ^{a}	(15) ^{ab}	(30) ^{ab}	(37) ^{b}	(34) ^{ab}
Crunchiness	51	72	67	69	12	45	61	28	27
	(26) ^{cd}	(23) ^{e}	(22) ^{de}	(27) ^{de}	(11) ^{a}	(24) ^{bc}	(23) ^{cde}	(19) ^{ab}	(20) ^{ab}
Graininess	37	14	16	13	48	36	17	48	60
	(28) ^{b}	(13) ^{a}	(13) ^{a}	(11) ^{a}	(28) ^{bc}	(24) ^{b}	(13) ^{a}	(26) ^{bc}	(25) ^{c}
Fibrousness	31	50	47	52	6.0	23	56	6.9	2.8
	(31) ^{bc}	(30) ^{cd}	(31) ^{cd}	(32) ^{cd}	(11) ^{a}	(30) ^{ab}	(32) ^{d}	(11) ^{a}	(3.4) ^{a}

5.3.2 PTR-ToF-MS headspace measurement

The 49 mass ions that were significantly different between the nine apple cultivars are shown in Table 5.3. These mass ions represented compounds such as esters, alcohols, aldehydes, hydrocarbons, acids, monoterpenes and sesquiterpenes, which have all previously been reported to occur in apples (Yahia, 1994). These compounds were tentatively identified based on previous PTR-MS and GC-MS studies on apple flavour, their fragmentation patterns and related compounds (Buhr and others, 2002; Aprea and others, 2007; Aprea and others, 2012; Soukoulis and others, 2013). To increase the legibility of the manuscript, all m/z will be recorded as their nominal mass number, except for compounds sharing the same nominal mass which will be specified to three decimal places.

Mass ions reported in Table 5.3 were grouped based on their tentatively identified compound groups and illustrated as radial plots (Figure 5.1). The compounds were labelled in an anti-clockwise order based on the list provided which starts from the aromatic hydrocarbon compounds and ends with α-farnesene compounds. Visually inspecting Figure 5.1, F1, Dalinette and F3 cultivars were the only cultivars that produced large amounts of α-farnesene and terpene related compounds. Renetta Bianca and Topaz were differentiated by other cultivars through their higher emission of only alcohols and butanoate esters. Golden Delicious, Kanzi and Pinova Evelina were similar compared to other cultivars. These cultivars emitted more hydrocarbons, high molecular weight esters (typically detected as the fragments m/z 43.018, 43.054, 57, 61 by the PTR-ToF-MS) fragments and very little α-farnesene and terpene related compounds. Also, F2 was the only cultivar high in all other compound classes except for α-farnesene and terpene related compounds. F2 and Dalinette cultivars contained the highest abundant VOCs emitted whereas Renetta Bianca and F1 were the lowest (Table 5.3). All five remaining cultivars were intermediate in the intensity of the VOC released, but differed in VOCs composition and could hence be characterized differently.

Table 5.3: Headspace concentration (ppbv) and standard deviations (in brackets) of all significantly different VOCs ($p<0.05$) from cut apple discs with their corresponding tentatively identified compounds. A list of expected masses and sum formulas are included. Superscript alphabets indicate the significant difference between cultivars for a specific VOC.

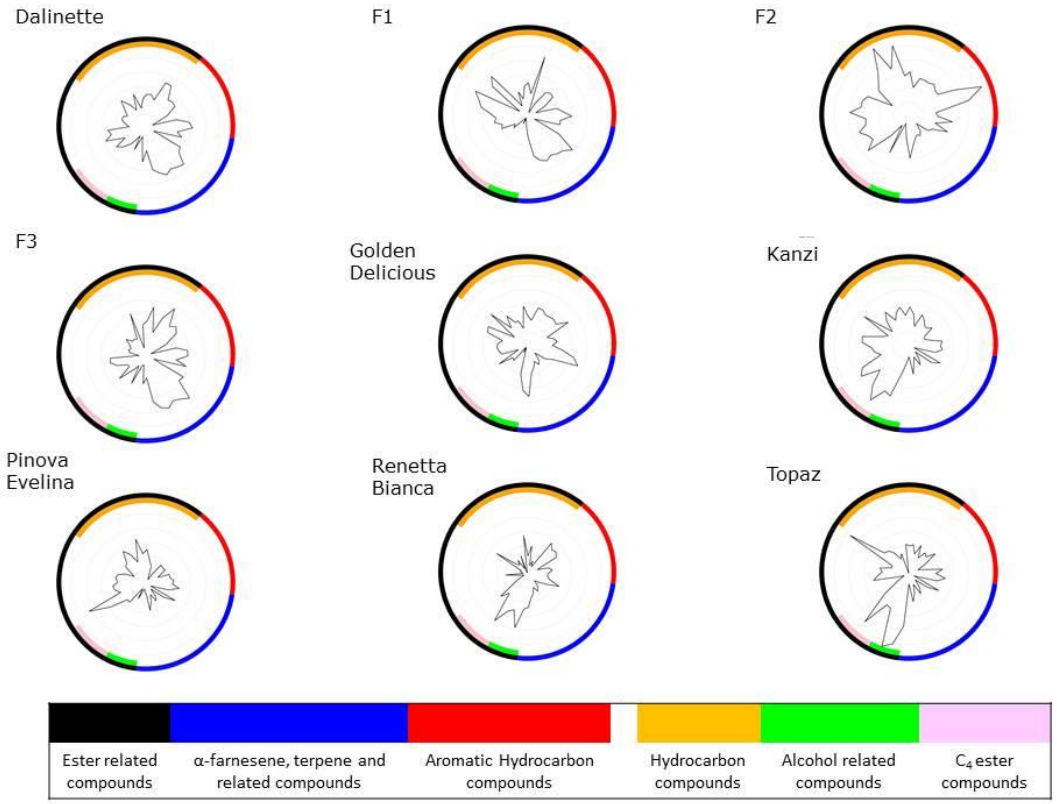
m/z	*Expected*	*Sum formula*	*Dalinette*	*F1*	*F2*	*F3*	*Golden Delicious*	*Kanzi*	*Pinova Evelina*	*Renetta Bianca*	*Topaz*
mz28.027	28.0313	$C_2H_4^+$	1033	320	819	1167	752	629	226	169	390
Ethylene			(260) ^{de}	(41.6) ^{a}	(154) ^{cd}	(270) ^{e}	(193) ^{cd}	(163) ^{bc}	(41.7) ^{a}	(38.0) ^{a}	(117) ^{ab}
mz31.018	31.018	CH_3O^+	69	146	93.0	58.5	93.2	47.6	44.1	20.6	48.4
Methanal			(18) ^{bc}	(37.1) ^{d}	(11.86) ^{c}	(10.7) ^{b}	(15.5) ^{c}	(11.4) ^{ab}	(6.7) ^{ab}	(2.64) ^{a}	(6.66) ^{ab}
mz33.034	33.0337	CH_5O^+	283	82.9	235	167	533	493	94.9	1007	1313
Methanol			(33) ^{b}	(29.0) ^{a}	(87.1) ^{ab}	(27.9) ^{ab}	(76.9) ^{c}	(187) ^{c}	(17.2) ^{a}	(113) ^{d}	(186) ^{e}
mz41.039	41.039	$C_3H_5^+$	383	867	885	288	465	866	565	98.0	535
Ester Fragment			(91) ^{ab}	(162) ^{c}	(306) ^{c}	(45.0) ^{ab}	(115) ^{b}	(109) ^{c}	(88.3) ^{b}	(18.8) ^{a}	(101) ^{b}
mz43.018	43.018	$C_2H_3O^+$	1575	983	1948	1128	1014	1091	991	154	368
Ester fragment			(682) ^{b}	(102) ^{ab}	(1540) ^{b}	(79.2) ^{ab}	(170) ^{ab}	(165) ^{ab}	(132) ^{ab}	(29.5) ^{a}	(197) ^{a}
mz43.054	45.054	$C_3H_7^+$	463	440	573	477	379	456	414	127	471
Ester fragment			(37) ^{b}	(48.2) ^{b}	(277) ^{b}	(19.6) ^{b}	(41.9) ^{b}	(68.8) ^{b}	(55.1) ^{b}	(22.07) ^{a}	(59.1) ^{b}
mz45.030	45.0334	$C_2H_5O^+$	15238	1103	12126	12733	11910	10320	1490	4212	9415
Acetaldehyde			(6450) ^{c}	(498) ^{a}	(2598) ^{c}	(4575) ^{c}	(4094) ^{c}	(4665) ^{bc}	(478) ^{a}	(1266) ^{ab}	(3132) ^{bc}
mz47.013	47.013	$CH_3O_2^+$	8.57	8.70	11.6	9.06	11.3	8.7	8.8	11.0	8.42
Formic acid			(0.78) ^{a}	(0.4) ^{a}	(0.74) ^{b}	(1.17) ^{a}	(0.82) ^{b}	(0.97) ^{a}	(0.84) ^{a}	(1.39) ^{b}	(0.79) ^{a}
mz47.050	47.096	$C_2H_7O^+$	43.8	11.4	29.4	35.9	58.0	21.4	11.3	45.9	68.0
Ethanol			(5.31) ^{bcd}	(3.1) ^{a}	(11.8) ^{abc}	(13.3) ^{abc}	(27.3) ^{cd}	(11.2) ^{ab}	(3.07) ^{a}	(15.2)^{bcd}	(38.6) ^{d}
mz53.039	53.039	$C_3H_5^+$	12.6	28.6	22.4	8.20	21.5	11.3	11.5	3.34	8.92

m/z	*Expected*	*Sum formula*	*Dalinette*	*F1*	*F2*	*F3*	*Golden Delicious*	*Kanzi*	*Pinova Evelina*	*Renetta Bianca*	*Topaz*
Ester Fragment			(3) ^{b}	(7.04) ^{d}	(2.09) ^{cd}	(2.53) ^{ab}	(3.64) ^{c}	(4.72) ^{b}	(1.92) ^{b}	(1.02) ^{a}	(1.43) ^{ab}
mz55.054	55.054	$C_4H_7^+$	123	247	237	88.8	205	135	144	36.7	88.7
Fragment			(24.2) ^{b}	(55.1) ^{c}	(24.0) ^{c}	(26.2) ^{ab}	(35.8) ^{c}	(43.7) ^{b}	(23.5) ^{b}	(10.2) ^{a}	(12.8) ^{ab}
mz57.070	57.070	$C_4H_9^+$	176	383	614	74.0	308	486	295	42.6	279
Ester Fragment			(84.5) ^{ab}	(78.5) ^{cd}	(226) ^{e}	(20.4) ^{a}	(81.9) ^{bcd}	(63.2) ^{de}	(82.2) ^{bcd}	(7.89) ^{a}	(65.6) ^{bc}
mz59.049	59.049	$C_3H_7O^+$	75.0	75.3	99.9	78.2	65.6	86.6	77.9	40.1	62.3
Acetone			(18.1) ^{bc}	(16.4) ^{bc}	(22.2) ^{c}	(15.0) ^{bc}	(6.81) ^{ab}	(23.8) ^{bc}	(8.97) ^{bc}	(5.62) ^{a}	(8.16) ^{ab}
mz61.028	61.028	$C_2H_5O_2^+$	1133	995	4069	447	999	2207	2587	27.7	23.7
Acetic acid/acetates			(575) ^{b}	(225) ^{b}	(437) ^{d}	(223) ^{ab}	(234) ^{b}	(573) ^{c}	(611) ^{c}	(14.3) ^{a}	(3.7) ^{a}
mz67.054	67.054	$C_5H_7^+$	3.15	2.56	3.5	2.9	2.5	3.04	2.7	1.76	2.51
Ester fragment			(0.35) ^{cd}	(0.2) ^{bc}	(0.31) ^{d}	(0.14) ^{bc}	(0.28) ^{b}	(0.25) ^{bcd}	(0.29) ^{bc}	(0.37) ^{a}	(0.23) ^{b}
mz69.070	69.070	$C_5H_9^+$	5.48	5.78	7.62	5.29	6.07	5.82	5.77	3.52	6.37
Isoprene			(0.88) ^{b}	(0.42) ^{b}	(0.69) ^{c}	(0.65) ^{b}	(0.6) ^{b}	(0.83) ^{b}	(0.76) ^{b}	(0.34) ^{a}	(0.69) ^{bc}
mz71.049	71.049	$C_4H_7O^+$	9.73	4.03	8.53	8.37	6.72	23.2	6.13	16.2	30.7
2-Butenal			(2.97) ^{a}	(0.62) ^{a}	(1.3) ^{a}	(1.26) ^{a}	(1.09) ^{a}	(6.11) ^{c}	(0.93) ^{a}	(3.14) ^{b}	(4.11) ^{d}
mz71.086	71.086	$C_5H_{11}^+$	41.3	51.5	80.7	36.3	17.9	92.3	113	6.26	8.9
Ester fragment			(10.8) ^{bc}	(9.48) ^{c}	(11.9) ^{d}	(5.33) ^{bc}	(3.56) ^{ab}	(22.9) ^{de}	(20.5) ^{e}	(0.78) ^{a}	(1.14) ^{a}
mz73.065	73.065	$C_4H_9O^+$	13.2	21.9	18.8	12.2	22.9	10.3	12.6	7.34	15.1
Butanal			(2.39) ^{bc}	(3.89) ^{d}	(2.22) ^{cd}	(3.87) ^{ab}	(5.01) ^{d}	(2.48) ^{ab}	(3.5) ^{ab}	(1.99) ^{a}	(2.68) ^{bc}
mz75.044	75.044	$C_3H_7O_2^+$	21.1	8.78	25.7	11.9	20.4	34.2	25.5	7.1	6.92
Methyl acetate			(5.02) ^{cd}	(2.28) ^{ab}	(7.95) ^{de}	(3.43) ^{abc}	(4.46) ^{bcd}	(11.0) ^{e}	(9.01) ^{de}	(0.92) ^{a}	(1.2) ^{a}
mz77.039	77.06	$C_3H_9O_2^+$	3.09	2.76	5.69	2.97	6.93	1.54	1.31	4.58	1.41

m/z	*Expected*	*Sum formula*	*Dalinette*	*F1*	*F2*	*F3*	*Golden Delicious*	*Kanzi*	*Pinova Evelina*	*Renetta Bianca*	*Topaz*
Propylene glycol			(0.18) ^{ab}	(0.12) ^{ab}	(4.48) ^{ab}	(0.28) ^{ab}	(5.22) ^{b}	(0.18) ^{a}	(0.09) ^{a}	(2.62) ^{ab}	(0.21) ^{a}
mz79.054	79.054	$C_6H_7^+$	7.01	6.7	4.34	6.26	3.77	4.77	3.95	3.1	4.08
Benzene			(0.58) ^{d}	(0.57) ^{d}	(0.57) ^{bc}	(0.57) ^{d}	(0.61) ^{ab}	(0.65) ^{c}	(0.14) ^{b}	(0.67) ^{a}	(0.56) ^{bc}
mz81.070	81.070	$C_6H_9^+$	34.0	46.8	20.0	34.2	18.4	23.5	18.2	12.0	18.5
Monoterpene fragment			(9.29) ^{c}	(10.7) ^{d}	(5.83) ^{ab}	(10.3) ^{c}	(5.05) ^{ab}	(9.69) ^{b}	(2.39) ^{ab}	(2.4) ^{a}	(3.58) ^{ab}
mz83.086	83.086	$C_6H_{11}^+$	11.2	8.3	21.4	8.15	14.3	16.23	15.9	6.38	10.1
Hexanal			(4.4) ^{abc}	(1.36) ^{a}	(3.78) ^{d}	(3.49) ^{a}	(3.54) ^{bc}	(5.04) ^{cd}	(2.9) ^{c}	(1.18) ^{a}	(1.74) ^{ab}
mz85.065	85.065	$C_5H_9O^+$	2.89	2.09	4.07	3.63	2.12	2.86	1.9	1.53	1.7
Methyl butenal			(0.71) ^{bc}	(0.53) ^{ab}	(1.17) ^{c}	(0.75) ^{c}	(0.41) ^{ab}	(0.57) ^{bc}	(0.28) ^{ab}	(0.58) ^{a}	(0.22) ^{ab}
mz85.101	85.101	$C_6H_{13}^+$	15.6	8.79	59.2	14.6	20.1	22.0	26.0	4.06	12.8
Hexane			(8.93) ^{abcd}	(2.52) ^{ab}	(10.3) ^{e}	(7.09) ^{abcd}	(3.29) ^{bcd}	(5.95) ^{cd}	(8.83) ^{d}	(0.47) ^{a}	(2.46) ^{abc}
mz87.045	87.044	$C_4H_7O_2^+$	1.99	1.35	1.71	2.91	1.51	1.87	1.42	1.64	1.73
Diacetyl			(0.44)	(0.22)	(0.29)	(2.25)	(0.34)	(0.48)	(0.14)	(0.31)	(0.29)
mz87.081	87.080	$C_5H_{11}O^+$	1.47	2.15	1.8	0.79	0.95	1.31	1.15	0.46	1.22
Methyl butanal			(0.33) ^{de}	(0.24) ^{f}	(0.33) ^{ef}	(0.1) ^{ab}	(0.17) ^{bc}	(0.27) ^{cd}	(0.2) ^{bcd}	(0.07) ^{a}	(0.12) ^{bcd}
mz89.060	89.060	$C_4H_9O_2^+$	49.1	10.5	59.7	44.3	46.9	123	56.4	61.3	149
Ethyl acetate			(27.2) ^{ab}	(2.83) ^{a}	(10.8) ^{b}	(12.1) ^{ab}	(10.5) ^{ab}	(42.2) ^{c}	(19.1) ^{ab}	(18.7) ^{b}	(39.3) ^{c}
mz91.056	91.054	$C_7H_7^+$	4.09	3.36	9.11	3.9	7.97	3.09	2.34	5.65	3.2
Benzyl acetate			(0.38) ^{ab}	(0.29) ^{a}	(4.2) ^{c}	(0.19) ^{ab}	(4.47) ^{bc}	(0.33) ^{a}	(0.13) ^{a}	(2.18) ^{abc}	(0.35) ^{a}
mz93.070	93.070	$C_7H_9^+$	4.12	3.86	20.4	4.17	26.6	2.84	2.44	16.8	2.84
Monoterpene fragment			(0.5) ^{a}	(0.48) ^{a}	(18.9) ^{ab}	(0.41) ^{a}	(22.3) ^{b}	(0.32) ^{a}	(0.09) ^{a}	(11.5) ^{ab}	(0.68) ^{a}
mz95.049	95.049	$C_6H_7O^+$	1.8	1.6	1.79	2.04	1.82	1.22	1.13	1.66	1.17

m/z	*Expected*	*Sum formula*	*Dalinette*	*F1*	*F2*	*F3*	*Golden Delicious*	*Kanzi*	*Pinova Evelina*	*Renetta Bianca*	*Topaz*
Phenol			(0.16) ^{bc}	(0.15) ^{abc}	(0.36) ^{bc}	(0.66) ^{c}	(0.32) ^{bc}	(0.16) ^{ab}	(0.07) ^{a}	(0.25) ^{abc}	(0.12) ^{a}
mz95.086	95.086	$C_7H_{11}^+$	4.75	4.51	3.24	5.21	3.03	3.2	2.99	2.74	3.14
Monoterpene fragment			(0.88) ^{b}	(0.53) ^{b}	(0.25) ^{a}	(1.02) ^{b}	(0.24) ^{a}	(0.4) ^{a}	(0.15) ^{a}	(0.32) ^{a}	(0.35) ^{a}
mz103.075	103.075	$C_5H_{11}O_2^+$	14.4	4.79	11.6	16.7	4.32	81.1	10.1	57.9	116
Methyl butanoate			(6.02) ^{a}	(1.35) ^{a}	(2.38) ^{a}	(6.18) ^{a}	(1.78) ^{a}	(29.3) ^{b}	(3.47) ^{a}	(13.5) ^{b}	(16.9) ^{c}
mz105.071	105.071	$C_8H_9^+$	2.34	2.06	2.36	2.45	2.3	1.89	1.54	2.33	2.13
Styrene			(0.27) ^{cd}	(0.24) ^{bc}	(0.12) ^{cd}	(0.14) ^{d}	(0.12) ^{cd}	(0.2) ^{b}	(0.07) ^{a}	(0.12) ^{cd}	(0.11) ^{bc}
mz107.086	107.086	$C_8H_{11}^+$	4.4	3.66	2.78	4.25	3.08	3.42	2.86	2.57	3.45
Ethyl benzene			(0.39) ^{d}	(0.34) ^{c}	(0.29) ^{a}	(0.33) ^{d}	(0.2) ^{ab}	(0.36) ^{bc}	(0.09) ^{a}	(0.31) ^{a}	(0.59) ^{bc}
mz109.101	109.101	$C_8H_{13}^+$	2.83	2.59	2.1	3.18	3.69	2.11	1.77	1.75	2.23
Farnesene fragment			(0.51) ^{bc}	(0.33) ^{bc}	(0.12) ^{ab}	(0.98) ^{cd}	(0.33) ^{d}	(0.22) ^{ab}	(0.09) ^{a}	(0.18) ^{a}	(0.15) ^{ab}
mz117.091	117.091	$C_6H_{13}O_2^+$	10.1	3.59	17.0	12.8	3.36	21.1	6.85	17.6	16.1
Hexanoic acid			(4.22) ^{abc}	(0.87) ^{a}	(4.46) ^{cd}	(2.09) ^{bcd}	(0.66) ^{a}	(7.66) ^{d}	(2.27) ^{ab}	(5.14) ^{cd}	(7.87) ^{cd}
mz119.088	119.086	$C_9H_{11}^+$	0.84	0.72	0.96	0.89	0.86	0.60	0.57	0.92	0.62
Propenylbenzene			(0.16) ^{bc}	(0.11) ^{ab}	(0.08) ^{c}	(0.09) ^{bc}	(0.08) ^{bc}	(0.09) ^{a}	(0.05) ^{a}	(0.07) ^{c}	(0.04) ^{a}
mz121.065	121.065	$C_8H_9O^+$	1.55	1.48	3	1.52	1.31	1.02	1.21	1.08	0.77
Acetophenone			(0.22) ^{a}	(0.29) ^{a}	(1.27) ^{b}	(0.19) ^{a}	(0.19) ^{a}	(0.23) ^{a}	(0.11) ^{a}	(0.23) ^{a}	(0.04) ^{a}
mz121.101	121.101	$C_9H_{13}^+$	2.44	2.31	1.15	2.65	1.13	1.15	1	1.03	1.19
Farnesene fragment			(0.54) ^{b}	(0.21) ^{b}	(0.09) ^{a}	(0.61) ^{b}	(0.08) ^{a}	(0.12) ^{a}	(0.06) ^{a}	(0.16) ^{a}	(0.37) ^{a}
mz123.117	123.117	$C_9H_{15}^+$	2.44	2.34	0.9	2.69	0.93	0.79	0.76	0.83	0.84
Farnesene fragment			(0.7) ^{b}	(0.47) ^{b}	(0.11) ^{a}	(0.74) ^{b}	(0.15) ^{a}	(0.12) ^{a}	(0.09) ^{a}	(0.16) ^{a}	(0.15) ^{a}
mz131.106	131.106	$C_7H_{15}O_2^+$	1.83	0.39	2.05	2.22	0.39	12.24	0.98	10.54	6.97

m/z	*Expected*	*Sum formula*	*Dalinette*	*F1*	*F2*	*F3*	*Golden Delicious*	*Kanzi*	*Pinova Evelina*	*Renetta Bianca*	*Topaz*
Methyl butanoate			(0.99) ^{a}	(0.05) ^{a}	(0.71) ^{a}	(0.67) ^{a}	(0.04) ^{a}	(5.75) ^{c}	(0.23) ^{a}	(3.27) ^{bc}	(2.05) ^{b}
mz135.117	135.117	$C_{10}H_{15}^{+}$	2.11	2.07	1.03	2.43	1.03	0.83	0.82	0.94	0.92
Farnesene fragment			(0.52) ^{b}	(0.37) ^{b}	(0.12) ^{a}	(0.59) ^{b}	(0.14) ^{a}	(0.06) ^{a}	(0.04) ^{a}	(0.16) ^{a}	(0.11) ^{a}
mz137.133	137.132	$C_{10}H_{17}^{+}$	2.39	2.23	1.63	2.62	1.73	2.2	2.04	1.32	2.1
Farnesene fragment			(0.5) ^{c}	(0.31) ^{bc}	(0.3) ^{ab}	(0.39) ^{c}	(0.15) ^{ab}	(0.42) ^{bc}	(0.15) ^{bc}	(0.1) ^{a}	(0.54) ^{bc}
mz145.122	145.122	$C_{8}H_{17}O_{2}^{+}$	1.16	0.7	2.07	1.36	0.73	1.47	0.94	1.37	1.23
Hexyl Acetate			(0.31) ^{ab}	(0.15)^{a}	(0.46) ^{c}	(0.23) ^{ab}	(0.14) ^{a}	(0.54) ^{bc}	(0.22) ^{ab}	(0.34) ^{ab}	(0.46) ^{ab}
mz149.094	149.096	$C_{10}H_{13}O^{+}$	0.28	0.36	3.07	0.21	0.83	0.65	0.99	0.42	0.39
Estragole			(0.14) ^{a}	(0.31) ^{a}	(1.76) ^{b}	(0.24) ^{a}	(0.14) ^{a}	(0.26) ^{a}	(0.17) ^{a}	(0.11) ^{a}	(0.08) ^{a}
mz149.131	149.131	$C_{11}H_{17}^{+}$	2.94	2.79	1.17	3.26	1	0.72	0.65	0.87	0.68
Farnesene fragment			(0.87) ^{b}	(0.55) ^{b}	(0.25) ^{a}	(0.81) ^{b}	(0.21) ^{a}	(0.18) ^{a}	(0.06) ^{a}	(0.15) ^{a}	(0.15) ^{a}
mz205.195	205.195	$C_{15}H_{25}^{+}$	6.5	6.15	1.34	7.53	1.33	1.13	0.87	1.26	1.12
a-farnesene			(2.18) ^{b}	(1.48) ^{b}	(0.4) ^{a}	(2.39) ^{b}	(0.41) ^{a}	(0.45) ^{a}	(0.27) ^{a}	(0.36) ^{a}	(0.48) ^{a}
Volatile abundance (ppbv)			***20 909***	***5 927***	***22 176***	***17 038***	***17 074***	***17 368***	***7 296***	***6 261***	***13 518***



Groups	Mass ion	Compounds
Aromatic Hydrocarbons	ms79.054	Benzene
	ms107.086	EthylBenzene
	ms121.065	Acetophenone
	ms149.094	Estragole
	ms91.056	Benzyl Acetate
	ms105.071	Styrene
	ms95.049	Benzenol
	ms119.088	Propenylbenzene
Hydrocarbons	ms28.027	Ethylene
	ms45.030	Acetaldehyde
	ms31.018	Methanal
	ms87.045	Diacetyl
	ms59.049	Acetone
	ms47.013	Formic Acid
	ms61.028	Acetic Acid
	ms85.065	Methyl Butenal
	ms85.101	Hexane
	ms83.086	Hexanal
	ms87.081	Methyl Butanal
	ms73.065	Butanal
	ms71.049	2-Butenal

	Mass ion	Compounds
Ester and Ester related compounds	ms53.039	Ester Frag.
	ms55.054	Ester Frag.
	ms41.039	Ester Frag.
	ms57.070	Ester Frag.
	ms67.054	Ester Frag.
	ms43.054	Ester Frag.
	ms43.018	Ester Frag.
	ms71.086	Ester Frag.
	ms75.044	Methyl Acetate
	ms145.122	Hexyl Acetate
	ms89.060	Ethyl Acetate
	ms131.106	Methyl Butanoate
	ms117.091	Ethyl Butanoate
	ms103.075	Methyl Butanoate

	Mass ion	Compounds
Alcohol	ms33.034	Methanol
	ms47.049	Ethanol
	ms77.039	Propylene Glycol
α-Farnesene and Terpene related compounds	ms95.086	Monoterpene Frag
	ms135.117	Farnesene Frag.
	ms149.131	Farnesene Frag.
	ms121.101	Farnesene Frag.
	ms123.117	Farnesene Frag.
	ms205.195	α-farnesene
	ms81.070	Monoterpene Frag
	ms137.133	Farnesene Frag.
	ms93.070	Monoterpene Frag
	ms109.101	Farnesene Frag.
	ms69.070	Isoprene

Figure 5.1: Radial plots of the 49 significantly different VOCs present in the cut discs of all cultivars. The colours on the other circle depict the main VOC groups whereas; the inner circle indicates the different ester derivatives present. Note that each VOC has been scaled relative to itself across all cultivars.

*Compound list below depicts order of compounds listed in the radial plots starting from Aromatic hydrocarbons, hydrocarbons etc. in an anti-clockwise order.

F2 in general emitted the highest volatile concentration (22 176 ppbv) and this was well associated with hydrocarbons, numerous esters and their associated fragments as illustrated in Figure 5.1. F2 was also the only cultivar that emitted a significantly higher amount of estragole (m/z 149.094) than the other cultivars. In sensory data, F2 was previously rated as containing the highest overall odour intensity and banana and pear attributes (Table 5.2). F2 had the highest concentration of ester fragments (m/z 41, 43.018, 43.054, 55, 57, 85.065), acetic acid/acetates (m/z 61), acetate esters (m/z 75, 145) and butanoate ester (m/z 117). Previous studies have postulated that cultivars described as fruity contain a higher amount of acetate esters (Karlsen and others, 1999; Aprea and others, 2012). Hexyl acetate (m/z 145) has been previously described as fruity and suggestive of pear (Karlsen and others, 1999). In the current study, hexyl acetate was also positively correlated ($p<0.05$) to the sensory attribute pear odour ($r=0.76$) and overall odour ($r = 0.82$). Panellists were also trained on anise flavour (estragole m/z 149.094). However, only one panellist was able to detect anise flavour (estragole) in high amounts and then for only one cultivar (F2) meaning that overall no significant difference between cultivars were observed.

Dalinette also emitted a high amount of total VOC but this value was almost solely related to its large emission of acetaldehyde (15 238 ppbv). It also contained numerous terpene, and aromatic hydrocarbon compounds: aromatic fragment (m/z 79), styrene (m/z 105), ethyl benzene (m/z 107), acetophenone (m/z 121.065) and lower molecular weight hydrocarbon compounds. F1 which had the lowest VOC intensity of the nine cultivars overall emitted some specific VOCs at a significantly higher concentration than the other cultivars including ester related fragments (m/z 53, 55), methanal (m/z 31), terpene fragment (m/z 81) and methyl butanal (m/z 87.080). F3 emitted a high amount of acetaldehyde (m/z 45) and was the highest emitter of diacetyl (m/z 87.045), but was a low emitter of all ester related compounds. Further Dalinette, F1 and F3 cultivars were low in sensory flavour and odour and were generally characterized by their high emission of terpene and aromatic hydrocarbon compounds in PTR-ToF-MS measurements. These three cultivars also emitted the highest amount of α-farnesene which in one study was described as green or herbaceous and being more related to vegetable descriptors (Aprea and others, 2012). However, the trained panel did not significantly

differentiate the cultivars based on vegetable descriptors during their vocabulary development.

F3, Golden Delicious and Kanzi were similar in their VOC intensity but differed in VOC composition (Table 5.3). F3 had a similar VOC composition to F1 and Dalinette. Kanzi was similar to Pinova Evelina and Golden Delicious with the exception of a higher butanoate ester (m/z 103, 117) and 2-butenal (m/z 71.049) emission. These three cultivars were also high in methyl acetate (m/z 75), hexanal (m/z 83) and hexane (m/z 85.101). Golden Delicious had a similar VOC composition containing high intensities of hexanal and acetate esters (Chapter 3) (Karlsen and others, 1999; Aprea and others, 2012).

Renetta Bianca which also contained a low intensity of VOCs was not associated with acetate ester compounds. It mainly emitted methanol, methyl butanoate, ethyl butanoate and ethyl-2-methyl butanoate (m/z 33, 103, 117 and 131 respectively). These findings are comparable to data by Thedy and others (2003) who characterized Renetta apples as containing butanoate esters. Topaz was similar to Renetta Bianca in its emission of alcohol and butanoate esters; but differed in that it had a higher concentration of 2-butenal (m/z 71.049) and some ester fragments (Figure 5.1). The sensory analysis data from these cultivars were also comparable and were rated higher in lemon odour and flavour. Here, the butanoate esters (m/z 103, 117, 131) were positively correlated ($p<0.05$) with both lemon flavour (mean $r = 0.46$) and odour (mean $r = 0.54$). These were similar findings to that of Aprea and others (2012) in which Renetta Bianca and Topaz were associated with sensory lemon attributes and butanoate esters.

5.3.3 Texture and physico-chemical analysis

All the mechanical and acoustic data that were measured significantly differentiated the cultivars (Table 5.4). Dalinette, F1, F2, F3 and Pinova Evelina were similar in all mechanical and acoustic textural properties indicating these cultivars were firmer and crunchier than the other four remaining cultivars. Overall, Dalinette, F1, F2, F3 and Pinova Evelina cultivars were higher in most mechanical texture

properties such as the yield force, max force, final force, work, mean force and linear distance/distance. However, between these five cultivars only F1 and F3 were significantly higher in acoustic textural properties such as acoustic peak and linear acoustic distance. This suggests that although some cultivars were firm, this was not necessarily an indication of crispness indicating the need to assess mechanical and acoustic data simultaneously to detect crispness (Chapter 4).

Focusing on two examples, Dalinette and F2 cultivars were very similar in all mechanical data with the exception of force peak, difference in force and peak/distance. However, F2 was significantly higher in acoustic peak (80.80) compared to Dalinette (21). This indicates that although both cultivars were hard, they were not necessarily crunchy. The sensory data showed a similar profile where sensory hardness of Dalinette and F2 was closely matched but F2 was rated higher in crunchiness thereby supporting the observation that crisp apples derive from hard apples, but all hard apples are not necessarily crisp (Costa and others, 2011). This result also strengthens the findings of Duizer (2001) who suggests that the use of only mechanical or acoustic data is insufficient for reliable analysis of crisp or crunchy texture.

Four mechanical parameters were significantly correlated ($p<0.05$) to acoustic data. These were force peak (mean r = 0.79), peaks/distance (mean r = 0.84), force linear distance (mean r = 0.74) and linear distance/distance (mean r = 0.71) all of which are based on the number of peaks or distance during the entire force curve acquisition. During sample measurement, force peaks are acquired as the probe penetrates the sample for a defined distance and crushing the apple cells. A crisp apple contains more force peaks due to the rupture of turgid cells. This in turn generates sound and increases the number of acoustic peaks measured suggesting that specific mechanical parameters can be used to describe acoustic profiles (Costa and others, 2011). Compared to sensory data, these four mechanical parameters were positively correlated to crunchiness (mean r = 0.86); and negatively correlated to flouriness (mean r = -0.80) and graininess (mean r = -0.75). These parameters have previously been used to successfully predict sensory attributes using PLS regression analyses (Corollaro and others, 2014).

Crisp or crunchy texture is defined by the amount of sound generated upon the first bite or mastication with the molar teeth (Harker and others, 2002a). Soft cultivars such as Golden Delicious, Renetta Bianca and Topaz were very low in all mechanical and acoustic textural properties. Compared to the sensory results, these three cultivars were lowest in hardness and crunchiness but highest in graininess and flouriness. From a structural point of view, 'crispness' occurs during the rupturing of apple parenchyma that has a high turgidity and compact cell to cell adhesion. However, upon storage cell to cell adhesion decreases due to the dissolution of cell walls. This causes a secretion of intercellular fluids which leads to the enlargement of intercellular spaces (Varela and others, 2007). The difference in the parenchyma structures of firm and soft cultivars was studied in Chapter 6 and 7. It was concluded that softer cultivars such as Golden Delicious were highly porous and contained larger intercellular spaces compared to firmer cultivars (Jazz). With regards to eating quality, the parenchyma of softer apples do not rupture but, breakdown into clumps causing a grainy or floury mouth feel (Harker and Hallett, 1992) as observed in the current study in the sensory analysis.

Table 5.4: The mean values of the measured texture (n=15) and physico-chemical (n=8) properties measured. Different superscript alphabets indicate a significant difference between cultivars for that parameter.

Texture and physico-chemical properties	*Dalinette*	*F1*	*F2*	*F3*	*Golden Delicious*	*Kanzi*	*Pinova Evelina*	*Renetta Bianca*	*Topaz*
Yield Force	11.6 (2.89) ^{e}	10.8 (1.41) ^{de}	11.8 (3.91) ^{e}	8.75 (2) ^{bcd}	6.14 (0.97) ^{a}	7.57 (2.1) ^{abc}	9.84 (2.4) ^{cde}	9.72 (1.58) ^{cde}	7.09 (1.68) ^{ab}
Max Force	14.6 (3.7) ^{e}	13.2 (1.32) ^{de}	14.6 (3.79) ^{e}	11.0 (1.79) ^{cd}	6.42 (0.83) ^{a}	10.2 (2.46) ^{bc}	13.4 (2.99) ^{de}	9.94 (1.39) ^{bc}	8.26 (1.48) ^{ab}
Final Force	12.7 (3.82) ^{e}	9.98 (2.3) ^{cd}	11.3 (3.13) ^{de}	8.67 (1.96) ^{c}	4.62 (0.91) ^{a}	7.52 (2.63) ^{bc}	11.7 (2.52) ^{de}	7.82 (1.84) ^{bc}	5.77 (1.48) ^{ab}
Force Peak	20.1 (3.31) ^{b}	21.5 (2.83) ^{bc}	24.1 (4.45) ^{c}	24.0 (2.37) ^{c}	16.4 (2.24) ^{a}	20.1 (4.36) ^{b}	23.2 (4.35) ^{bc}	15.23 (4.09) ^{a}	21.0 (3.29) ^{bc}
Area/Work	1030 (270) ^{d}	897 (81.7) ^{cd}	1046 (271) ^{d}	751 (112) ^{bc}	452 (60.2) ^{a}	694 (188) ^{b}	911 (180) ^{cd}	694 (96.6) ^{b}	590 (98.6) ^{ab}
Force Linear Distance	105.63 (4.91) ^{d}	105 (4.1) ^{cd}	108 (6.66) ^{d}	105 (3.32) ^{cd}	95.2 (1.26) ^{a}	101 (6.14) ^{bc}	104 (4.91) ^{cd}	97.6 (2.23) ^{ab}	99.1 (1.79) ^{ab}
Young's Module	1.55 (0.6) ^{c}	1.33 (0.36) ^{bc}	1.35 (0.51) ^{bc}	1.05 (0.31) ^{ab}	0.86 (0.3) ^{a}	0.93 (0.29) ^{ab}	1.33 (0.29) ^{bc}	1.21 (0.37) ^{abc}	0.88 (0.35) ^{a}
Mean Force	12.05 (3.23) ^{d}	10.6 (0.96) ^{cd}	12.2 (3.29) ^{d}	8.77 (1.39) ^{bc}	5.21 (0.7) ^{a}	8.13 (2.22) ^{b}	10.7 (2.21) ^{cd}	8.10 (1.12) ^{b}	6.82 (1.15) ^{ab}
Difference in force	-1.05 (2.64) ^{ab}	0.81 (2.91) ^{bc}	0.42 (2.44) ^{abc}	0.08 (2.8) ^{abc}	1.52 (0.82) ^{bc}	0.08 (2.37) ^{abc}	-1.87 (2.89) ^{a}	1.88 (1.59) ^{c}	1.31 (1.75) ^{bc}
Force Ratio	0.95 (0.2) ^{ab}	1.14 (0.31) ^{abc}	1.03 (0.23) ^{abc}	1.07 (0.37) ^{abc}	1.36 (0.25) ^{c}	1.06 (0.26) ^{abc}	0.86 (0.2) ^{a}	1.30 (0.4) ^{bc}	1.34 (0.6) ^{c}
Peaks/Distance	2.06 (0.32) ^{cd}	2.17 (0.26) ^{cde}	2.26 (0.43) ^{de}	2.39 (0.2) ^{e}	1.57 (0.21) ^{ab}	1.89 (0.38) ^{bc}	2.16 (0.37) ^{cde}	1.42 (0.4) ^{a}	1.95 (0.3) ^{cd}

Texture and physico-chemical properties	*Dalinette*	*F1*	*F2*	*F3*	*Golden Delicious*	*Kanzi*	*Pinova Evelina*	*Renetta Bianca*	*Topaz*
Linear Distance/Distance	10.8	10.7	10.2	10.4	9.12	9.49	9.78	9.18	9.19
	(0.76) ^{d}	(0.57) ^{d}	(0.89) ^{bcd}	(0.55) ^{cd}	(0.43) ^{a}	(0.66) ^{ab}	(0.79) ^{abc}	(0.36) ^{a}	(0.37) ^{a}
Acoustic Peak	21.0	80.8	53.1	76.6	10.4	19.7	36.4	8.46	13.6
	(15.7) ^{ab}	(22.8) ^{d}	(21.9) ^{c}	(21.0) ^{d}	(9.95) ^{a}	(21.1) ^{ab}	(23.3) ^{bc}	(5.61) ^{a}	(10.2) ^{a}
Max Acoustic Peak	58.6	66.0	63.8	64.5	57.6	60.6	61.8	56.8	57.9
	(4.22) ^{ab}	(3.83) ^{d}	(3.56) ^{cd}	(2.66) ^{cd}	(4.06) ^{a}	(5.28) ^{abc}	(3.78) ^{bc}	(3.62) ^{a}	(3.35) ^{ab}
Mean Acoustic Pressure	43.7	47.9	46.5	48.8	43.0	45.1	47.2	40.9	43.5
	(2.76) ^{bc}	(1.09) ^{e}	(1.31) ^{de}	(1.44) ^{e}	(0.71) ^{b}	(1.67) ^{cd}	(1.53) ^{e}	(0.53) ^{a}	(0.98) ^{b}
Acoustic Linear Distance	3607	6512	5346	6745	2664	3399	4780	2393	3020
	(1194) ^{b}	(895) ^{d}	(985) ^{c}	(984) ^{d}	(578) ^{ab}	(1502) ^{ab}	(1284) ^{c}	(388) ^{a}	(741) ^{ab}
Soluble Solids Content (%)	15.2	14.0	14.7	14.2	11.8	11.6	13.3	13.8	12.4
	(0.21) ^{c}	(0.57) ^{bc}	(0) ^{c}	(0.99) ^{bc}	(1.34) ^{a}	(0.07) ^{a}	(0) ^{abc}	(0.07) ^{abc}	(0.28) ^{ab}
Titratable Acidity (malic acid equivalent of 100g juice)	6.68	9.14	4.47	3.59	3.88	5.40	8.18	13.4	10.7
	(0.24) ^{c}	(0.28) ^{d}	(0.05) ^{ab}	(0.54) ^{a}	(0.5) ^{a}	(0.2) ^{bc}	(0.58) ^{d}	(0.21) ^{f}	(0.47) ^{e}
Juiciness (%)	38.4	45.6	38.2	50.5	51.8	43.3	39.1	16.7	33.7
	(0.81) ^{ab}	(11.6) ^{b}	(2.2) ^{ab}	(3.36) ^{b}	(8.98) ^{b}	(7.25) ^{b}	(3.97) ^{ab}	(0.2) ^{a}	(2.03) ^{ab}
Dry Matter Concentration (%)	18.4	17.9	16.5	15.9	15.9	14.9	17.4	16.4	15.1
	(1.06) ^{d}	(1.17) ^{cd}	(1.02) ^{abc}	(0.8) ^{ab}	(0.84) ^{ab}	(1.83) ^{a}	(1.49) ^{bcd}	(1.31) ^{abc}	(2.11) ^{a}

Differences in the force and the force ratio describe how apple flesh behaves during penetration. A large positive value calculated from the difference of force between the yield and final force shows a decreased apple flesh resistance and implies a soft texture that is easy to penetrate (Golden Delicious, Renetta Bianca, Topaz). Whereas a negative value indicates the yield force is smaller than the final force due to a resistance present in the apple tissue as it is compressed. Apples which show this behaviour are firm cultivars (Dalinette, Pinova Evelina). A difference of force near zero indicates there is not much difference in the yield and final force (Costa and others, 2011). Young's module, also known as the elasticity module, is a parameter linked to the 'stiffness' of a sample. As suggested by Duizer (2001), it can be positively correlated to sensory crispness. However, in this study, Young's module was positively correlated ($p < 0.05$) to sensory hardness ($r = 0.73$) but not crunchiness.

Renetta Bianca and Topaz had the highest measured titratable acidity, corresponding to their perceived sensory sour taste (Table 5.2) and lemon odour and flavour. In contrast, F2, F3 and Golden Delicious had the lowest titratable acidity and the lowest perceived sour taste for sensory. Titratable acidity was strongly correlated ($p < 0.05$) to sensory sour taste ($r = 0.98$) and astringency ($r = 0.89$) and negatively correlated to sweet taste ($r = -0.88$). These results are suggestive of an intra-modal sensory interaction with sourness potentially having a large role in determining perceived sweetness. F3 and Golden Delicious contained the highest amount of juice whereas Renetta Bianca was the lowest. However, Golden Delicious were not perceived as juicy by the panellists in the current study suggesting the influence of its high rating in floury and grainy mouth feel on juiciness. This interaction was also reported by Harker and others (2006) who found that a softer cultivar (Royal Gala) contained a high apparent juice content but was not perceived as juicy in sensory studies. This result also suggests that the measured instrumental technique did not accurately represent juice expressed during mastication.

F1, Dalinette and Pinova Evelina contained a high DMC (%) whereas Kanzi and Topaz had the lowest DMC (%). Dalinette, F2 and F3 contained the highest amount of SSC whereas Kanzi had the lowest. DMC was positively correlated ($p < 0.05$) to

SSC, Yield force, Max force, work and Young's module (0.75, 0.93, 0.73, 0.98 and 0.90 respectively). This indicates a relationship between DMC and SSC with apple flesh firmness as previously reported (Palmer and others, 2010; Saei and others, 2011). It is interesting to note, although titratable acidy was positively correlated to sour taste, sweet taste was not correlated to SSC. Harker and others (2002b) also found difficulty in predicting sweet taste using instrumental methods and later hypothesized that the stimulation of a sensory sweet taste was sufficient with a small amount of juice (Harker and others, 2006). In the current study, there was no trend between SSC and sweet taste, although sweet taste was positively correlated (mean r = 0.57) to fruity ester related compounds (m/z 43, 61, 145). For example, although F2 and F3 were similar in SSC (14.7% and 14.2% respectively) and SSC/ Titratable acid ratio (3.29 and 3.96), F2 contained a higher VOC intensity of fruity ester related compounds and was perceived to be a lot sweeter in sensory compared to F3. Nonetheless the correlation between SSC and VOC production could also arise from biological differences in substrate availability or differences in ripening rates of the cultivars moderated by ethylene (Yahia, 1994).

5.3.4 Multivariate analysis: A comparison between analytical and sensory techniques

A total variance of 60.47% was explained by the first two dimensions of the MFA (Figure 5.2). This MFA illustrates the explained variance between the combined analytical (PTR-ToF-MS headspace measurements, physico-chemical and texture properties) and sensory data. Overall, odour and flavour sensory attributes were associated with PTR-ToF-MS headspace measurements; and sensory texture attributes were well associated with instrumental texture measurements. Cultivars were separated based on their textural attributes on Dim 1 whereas Dim 2 separated the cultivars based on their volatile, flavour and odour associated sensory attributes (Figure 5.2).

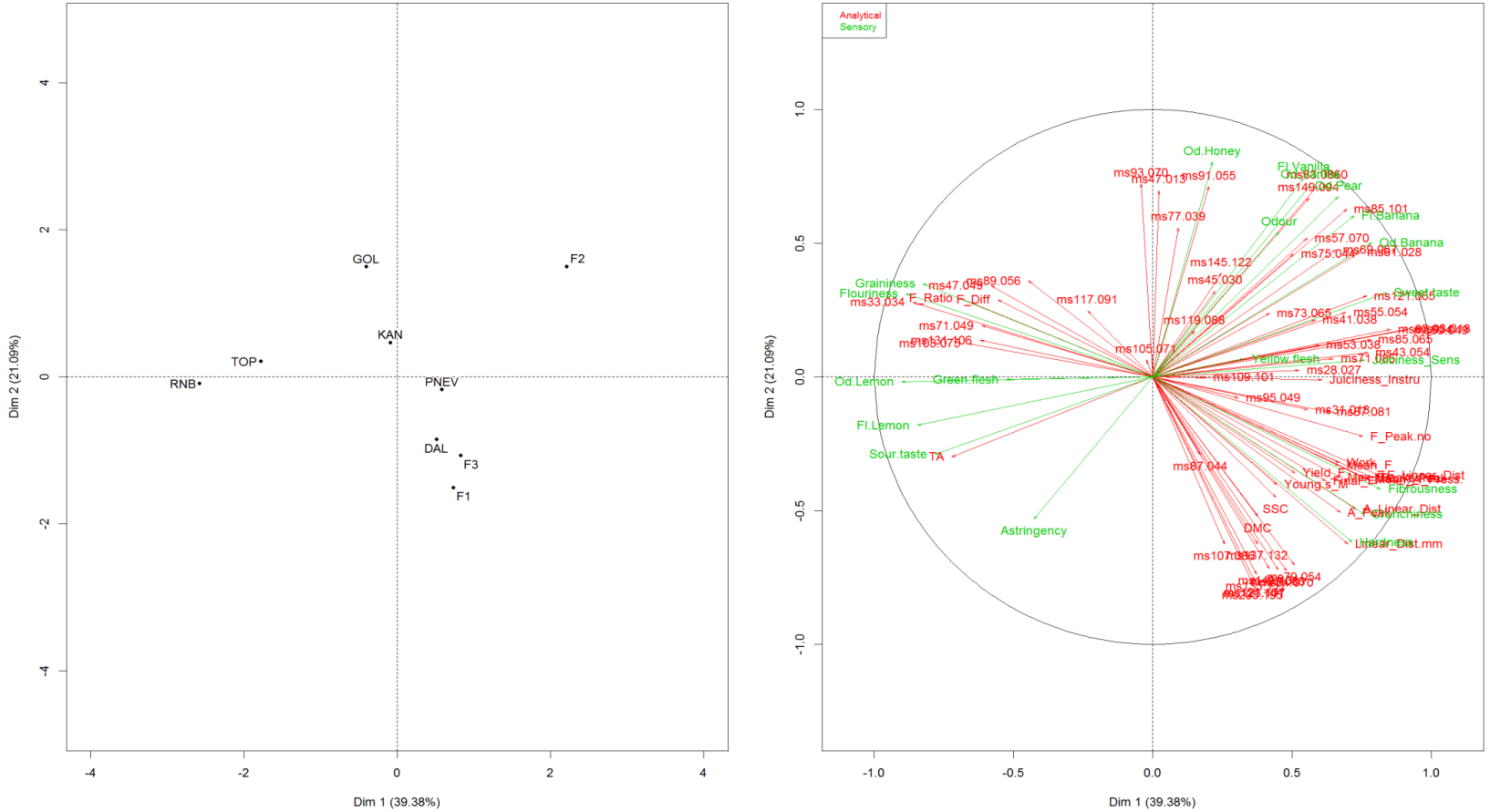


Figure 5.2: MFA of PTR-ToF-MS, sensory, texture and physico-chemical properties of 9 apple cultivars (Abbreviations: GOL: Golden Delicious; KAN: Kanzi; PNEV: Pinova Evelina; TOP: Topaz; RNB: Renetta Bianca; DAL: Dalinette; Abbreviations of variables can be found in the methods and materials section).

As an overview, the MFA score plot showed similar groupings as those discussed in earlier sections. Dalinette, F1 and F3 were well associated with both sensory texture and instrumental texture properties. These three cultivars were not highly associated with sensory odours/ flavours but well associated with the PTR-ToF-MS terpene related compounds. On the positive quadrant of Dim 1 and 2, F2 was well differentiated from all other cultivars. F2 can be characterized as a sweet, juicy, and flavourful cultivar. It was highly associated with sensory flavour and odour attributes, ester related VOCs and sweet taste.

On negative Dim 2, Topaz and Renetta Bianca were separated from the other cultivars. Texturally, these cultivars were associated with sensory and analytical attributes describing soft cultivars (i.e. graininess, flouriness, force difference and force ratio). Based on their VOC compositions, both cultivars were associated with methanol, ethanol and butanoate esters in which the latter was positively correlated to sensory lemon odour and flavour. These cultivars were rated the most sour in taste and were well correlated with titratable acidity which shows potential to be used as an instrumental technique to depict sour taste (Harker and others, 2002b; Corollaro and others, 2014)

Instrumental and sensory juiciness were within the same MFA quadrant, but were not significantly correlated ($p > 0.05$). As mentioned previously, instrumental juiciness alone could not be used as an alternative for perceived juiciness (Harker and others, 2006). This is because the technique used to measure instrumental juiciness (single press using a garlic press) did not reflect the breakdown of the apple disc sample in the mouth (multiple crushing using molar teeth on a smaller contact area). Differences in microstructural breakdown were more apparent during consumption. Although Dalinette and F2 were comparable in mechanical texture and instrumental juiciness, Dalinette was rated significantly lower in juiciness attribute and acoustic properties. Based on texture attributes, Dalinette was perceived more floury and grainy compared to F2. This could be due to the mechanical breakdown of the apple tissue that influences the degree of salivation needed for bolus formation upon swallowing. A mealy dry apple requires a longer time for first swallow as opposed to a juicy crunchy apple leading to a stronger perception of floury and

grainy attributes (Chapter 3 and 4). To help explain sensory perception *in vivo* nosespace measurements could be conducted with PTR-ToF-MS in conjunction with the measurement of oral processing parameters to investigate flavour release. This would help to understand the mechanical breakdown of apple flesh associated with swallowing parameters, juiciness and its influence on flavour release (Sprunt and others, 2002).

Apart from the scores and loadings plot (Figure 5.3), an Individual Factor Map (IFM) (Lê and Husson, 2008) can add further information. MFA creates an easy way to observe inter-relationships between the different group variables on a single space. IFM allows the position of the different scores to be viewed as if placed in their individual PCA plots. A superimposed plot is presented where the MFA scores are subsequently placed on the barycentre of the grouped variables, as shown by the arrow in Figure 5.3 indicating the position of F2 if a PCA of sensory analysis was plotted. Here, the positions of the scores for analytical and sensory data were comparable. It therefore appears that the use of analytical data could further separate cultivars such as Kanzi, Pinova Evelina, Dalinette and F3.

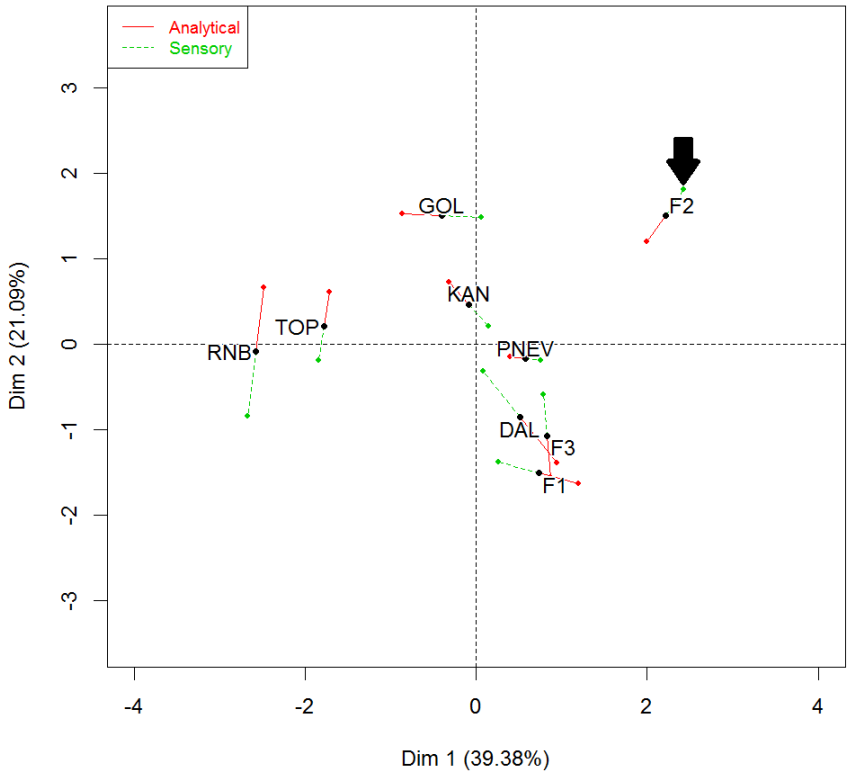


Figure 5.3: Individual factor map (IFM) to illustrate the differences between group variables plotted as single PCA plots (Abbreviations: GOL: Golden Delicious; KAN: Kanzi; PNEV: Pinova Evelina; TOP: Topaz; RNB: Renetta Bianca; DAL: Dalinette).

5.4 Conclusion

This chapter considered the use of two powerful analytical techniques, PTR-ToF-MS and mechanical-acoustic textural measurements, to correlate sensory flavour/odour and texture, respectively. Both techniques showed similar separations between cultivars and associated sensory attributes to instrumental ones indicating their potential to predict sensory attributes. The two techniques also highlighted specific analytical parameters that overlap with sensory attributes and have a potential to be used in predictive models. Comparing PTR-ToF-MS to sensory data, butanoate esters were highly associated with lemon flavour and odour. Pear and banana odours were associated with short ester fragments and acetate esters, namely

hexyl acetate. Three apple cultivars were separated based on their high emission of terpene related compounds. However, there were no sensory attributes that correlated to this. Analytical texture measurements were well correlated with sensory texture. Although sensory hardness was well correlated with texture firmness, the coupled use of mechanical and acoustic texture measurements provided data that more comprehensively explained apple crunchiness. Acoustic parameters were useful in differentiating crunchy cultivars from hard ones which were not crunchy. Titratable acidity was well correlated with sour taste and can be strongly considered for use in prediction models. Sweet taste although negatively correlated to titratable acidity was a difficult parameter to predict as it may be influenced not just by soluble solids content but also by the breakdown pattern of apple flesh during mastication. However, a positive correlation between sweet taste and fruity esters indicated a taste-aroma interaction which should be investigated further using *in vivo* measurements. This chapter has found that specific analytical parameters correlate well with sensory data and indicate the potential to characterize apple flavour using a solely instrumental approach.

The findings of Chapter 3 – 5 have demonstrated the interrelationships between texture and flavour perception. Additionally, an interesting subject addressed within each of these chapters is the nature of the mechanical breakdown of the apple sample during mastication. This has been shown to influence perceived texture and flavour. In order to understand this phenomenon, apple microstructure and morphological properties were studied in the next chapters.

Chapter 6: X-Ray micro-computer tomographic method to visualize the microstructure of different apple cultivars

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6.1 Introduction

The importance of apple texture in relation to eating quality is well documented (Daillant-Spinnler and others, 1996; Jaeger and others, 1998; Harker and others, 2003). At harvest most apple cultivars have a desirable firm and crunchy texture which is used by the industry as an indicator of quality (Johnston and others, 2002; Harker and others, 2008). The texture of apples can subsequently become soft and mealy during storage at a rate that is cultivar dependent and results in a reduction in their quality and value (Harker and others, 1997; Johnston and others, 2002).

Apple firmness has traditionally been analytically assessed using puncture or penetration tests. Recently non-destructive methods to assess texture have been introduced such as the Acoustical Firmness Sensor (AFS, AWETA, Nootdorp, Netherlands) and the Sinclair Internal Quality Firmness Tester (SIQ-FT) (Harker and others, 2008). Underpinning these measurements has been a considerable amount of research investigating the cellular basis of apple firmness. A mature apple fruit consists of parenchyma tissue, vascular veins, cortex and a central core containing seeds. The parenchyma tissues or flesh is the portion usually consumed. Between the cells that make up the parenchyma tissue are intercellular spaces (IS) that have been formed by either the breakdown or dissolution of entire cells (lysigenous IS) or from the separation of cells (schizogenous IS). During prolonged storage, IS can interconnect with other IS which results in the individual spaces increasing in size over time (Esau, 1977). Small and sporadic IS occur in the gaps between at least three intact cells (Pieczywek and Zdunek, 2012), while larger elongated IS form due to a decrease in cell-to-cell adhesion and the interconnection of collapsed cell structures (Harker and Hallett, 1992). IS, therefore occur in a variety of shapes (irregular, elongated, etc.) and range in size from 438-665 μm long and 210-350 μm wide (Reeve, 1953).

Generally fresh firm apples have a compact and tight cell-to-cell adhesion matrix with few/small IS in between cells which are easily ruptured during

consumption. In contrast older apples have larger IS, flaccid cells, reduced cell-to-cell adhesion and reduced water content so that, rather than rupturing, the cells break down into small clumps, during consumption which results in a mealy or floury mouth feel (Harker and Hallett, 1992; Herremans and others, 2013a).

The influence of parenchyma cellular and tissue structure on apple texture and the post-harvest quality of apples has received much attention (Hatfield and Knee, 1988; Khan and Vincent, 1990; Harker and Hallett, 1992). Various techniques have been used to determine cell size and the distribution and size of IS within apples, including a gravimetric method based on Archimedes' principle and a range of microscopy (light, stereoscopic, electron, confocal) based techniques (Bain and Robertson, 1951; Reeve, 1953; Khan and Vincent, 1990; Dražeta and others, 2004; Pieczywek and Zdunek, 2012). The drawbacks of these methods include an inability to measure the depth or height of the cells or the porous regions and the possible alterations to the tissue that can occur during sample preparation. In addition, to enable representative results during microscopic examinations repeated measurements are required to overcome the small tissue sample size observed. The development of a miniaturized desktop X-ray Microcomputer Tomography (µ-CT) scanner has enabled the acquisition of 3D spatial data from intact biological samples up to 60mm in diameter. As little sample preparation is needed prior to µ-CT analysis, the true microstructure is imaged. While µ-CT scanning is limited to a localized region of measurement within the sample it has been used to obtain 2 and 3-D structural data from a wide range of biological materials including mammalian embryos, bone structure, and apples (Sasov and Van Dyck, 1998; Mendoza and others, 2010; Herremans and others, 2013a).

Using µ-CT scanning the microstructure of the parenchyma tissue of two apple cultivars, (Jonagold, Braeburn) has been differentiated based on their porosity which was defined as the fraction of IS against the total sample volume (Mendoza and others, 2007). However, as the apple tissue structure was very complex and irregular, a further study using multi-fractal analysis (MFA) was required to describe the porosity distribution in the tissue accurately (Mendoza and others, 2010). Recently µ-CT scanning has been used to monitor Braeburn Browning Disorder by measuring

porosity and morphological parameters such as the anisotropy and connectivity of the apple parenchyma tissue over a season (Herremans and others, 2013a).

Overall µ-CT scanning has been shown to be a powerful tool to elucidate the microstructure of apples, however, the relationship between µ-CT data and important quality attributes of apples such as texture has not been reported. In the current chapter, µ-CT scanning was used to investigate the microstructure of four apple cultivars (Golden Delicious, Jazz, Fuji and Braeburn) and the data related to their textural characteristics and percentage of dry matter.

6.2 Methods and Materials

6.2.1 Samples

Four apple (*Malus Domestica* Borkh.) cultivars from local orchards were used in this experiment. Jazz, Fuji and Golden Delicious apples were harvested and held at 1°C after harvest for two weeks prior to being stored at 2°C under regular atmospheric air conditions in a conventional cool store for the duration of the experiment. Braeburn apples were kept under identical conditions with the exception that they were only held at 1°C for one week prior to being held at 2°C for the duration of the experiment. All measurements were carried out on the apples at room temperature (20ºC) 100 days after harvest. Three apples per cultivar were chosen for analysis. Regions used per apple are illustrated in Figure 6.1.

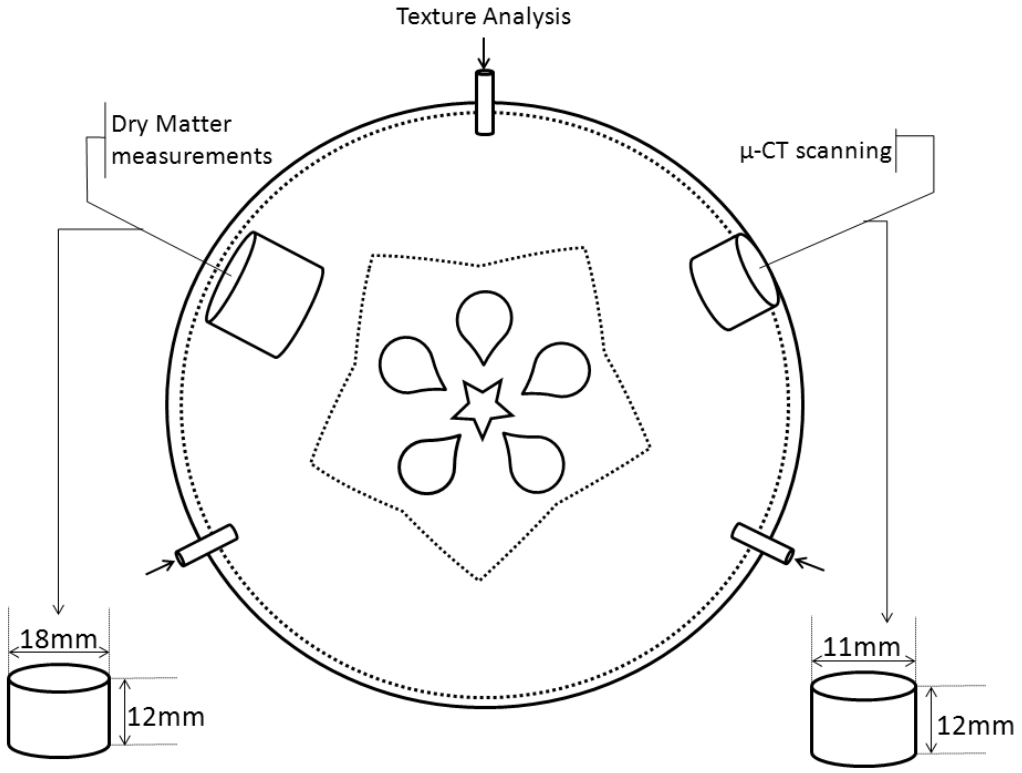


Figure 6.1: A cross-section of an apple illustrating the regions used for experimentation. Texture penetration measurements were carried out at three different regions along the equator on the apple. Two different sized cylinders were cut from an apple used for dry matter measurements (without peel) and µ-CT scanning (including peel).

6.2.2 Texture analysis

Apple texture was measured, by a penetration test, using a Texture Analyzer (TA-HDplus, Stable Microsystems Ltd. Goldaming, UK) fitted with a 2450N load cell and a 6mm cylinder probe attachment. An entire apple was positioned in the centre of the platform and the probe penetrated the equatorial section of the fruit at a speed of 1.5mm/s for a distance of 5mm after sensing a trigger force of 0.25N. For each apple, measurements were carried out in triplicate and three apples from each cultivar were assessed. Locations of measurement is shown in Figure 6.1 The measured parameters were calculated using the acquired force-distance curves defined by Bourne (2002).

Flesh firmness (N): Maximum force recorded over the probe's travel.

Gradient (N/mm): Slope of force curve from the start of the measurement to the maximum force signifying stiffness of skin from beginning until rupture.

Area under the Curve, AUC peel (N*mm): Work needed to penetrate the apple peel.

AUC flesh (N*mm): Work needed for probe to penetrate the apple flesh (5mm).

6.2.3 X-Ray computed micro tomography (µ-CT)

Using the same apples used for textural analyses, samples for µ-CT scanning were obtained by cutting each apple along its medial axis perpendicular to the core axis. Cylinders were obtained using a corer (Figure 6.1). To reduce dehydration the cylinders of flesh, with the peel attached were enclosed in a plastic tube with a 2mm space between each sample. The entire tube was wrapped with cling wrap until analysed. For each cultivar, samples were obtained from three apples in triplicate.

Samples were scanned using a SkyScan 1172 high resolution X-ray µ-CT System (Bruker microCT, Kontich, Belgium). The settings were optimized for clear separation between the apple flesh and IS. The x-ray source was operated at 40keV, without a filter, and with a 0.4 rotation step. A tube of samples (3 cylinders per cultivar) was positioned vertically and measured using the oversize scan mode (2.75 hours / sample). The measured X-ray shadow projections were digitized as 1800x1048pixel 16-bit images and were processed to obtain reconstructed cross-section images using Skyscan NRecon1.5.1.4 software (http://www.skyscan.be/next/nreconcluster.zip). These 16-bit images resulted in 3-D stack of 3550 virtual slices made up of 1360x1360 isotropic voxels (9.89^3 μm^3), where a voxel is a pixel of known depth based on the user set resolution. The images were converted to 8-bit images to give a linear attenuation coefficient gray scale value range of 0-255.

6.2.4 Image segmentation

All image processing was carried out using the ImageJ1.47e software package (Abramoff, 2004). This included 2-D/3-D image rendering and extraction of morphometric data. Image segmentation transforms grey scale images into a binary (black and white) image by selecting a value between [0] and [255] to assign to each voxel as being either part of an IS or apple parenchyma. Image segmentation (Figure

6.2) was carried out on the images (n=3550 per cultivar) using Otsu thresholding (Otsu, 1979). This thresholding technique was used by Musse and others (2010); Herremans and others (2013a) on apples and for other food imaging research (Gonzales-Barron and Butler, 2006; Pareyt and others, 2009; Nashat and others, 2011). A further segmentation step was carried out on these images to remove noise. Outliers with a radius smaller than 2pixels were removed. Each IS was filled using 'fill holes'.

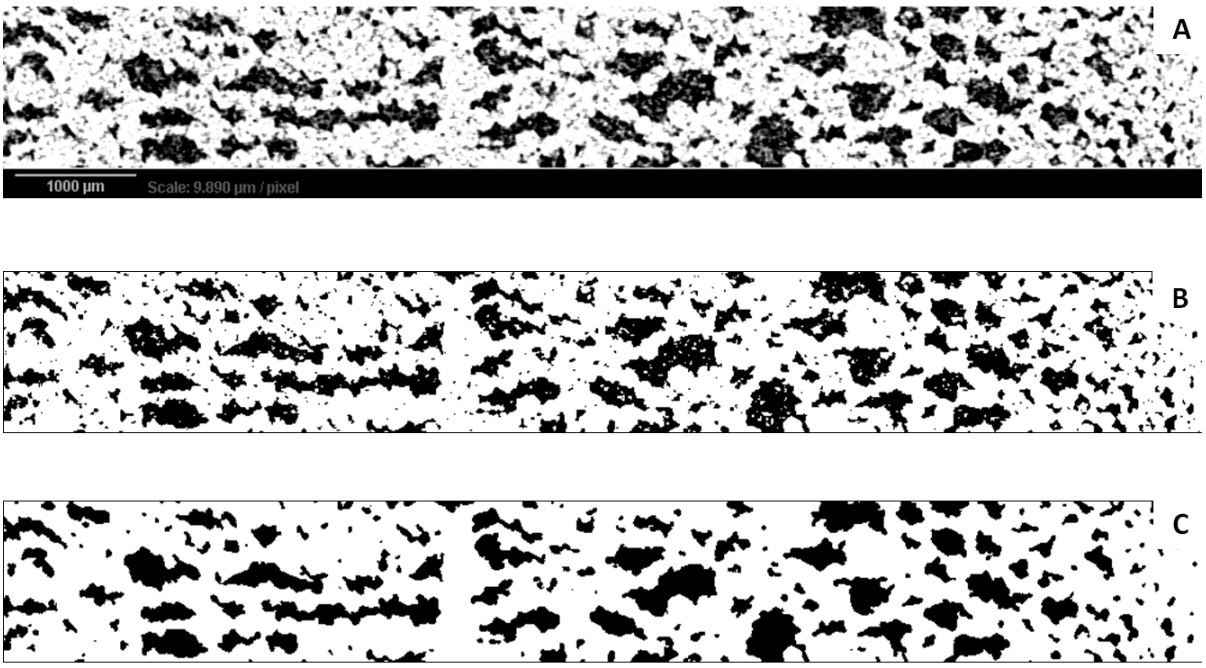


Figure 6.2: The segmentation of the images are shown here in which (A) the original image is converted into an 8-bit image; (B) followed by application of Otsu thresholding; and lastly (C) fill holes and outliers smaller than a 2pixel radius was removed.

6.2.5 Image analysis

Apple microstructure has been well documented by Khan and Vincent (1990) as being anisotropic. This was also shown by Mendoza and others (2010) through their multifractal analysis of apples and Herremans and others (2013a) through their measurements at different depths in the apple. From Figure 6.2, it is clear that the IS near the skin (right side) have a less organized orientation, are smaller and are more closely packed together. In comparison, the IS closer to the core (left side) appear to

be larger and more elongated. As this anisotropy may skew the IS distribution and other effects such as edge effects, artefacts and damage from sample cutting, a 300^3 voxel cube (length per voxel is 9.89µm) was chosen from the most homogenous region of the apple flesh to quantify morphometric parameters.

It has been shown that computed porosity is highly dependent on the resolution used and that a resolution higher than 13.7µm/pixel with a minimum representative elementary volume (REV) of 1.3^3 mm^3 is needed to acquire enough sensitivity to produce reliable results (Mendoza and others 2007). In this study, a resolution of 9.89µm/pixel and a REV of 3.0^3 mm^3 were used for image analysis. In the current research replicates of a single cultivar showed good reproducibility in terms of IS (black particles) distribution and differences could be observed between the cultivars.

Morphometric parameters, surface area (μm^2), volume (μm^3) and porosity (%) were measured using BoneJ (Doube and others, 2010). These parameters are defined as:

Surface area (μm^2): Surface area measured from each IS mesh.

Volume (μm^3): Volume enclosed by each IS surface mesh.

Porosity (%): Amount of calculated IS volume divided by total volume of the selected voxel cube.

The average surface area and volume per IS was calculated by dividing the sum of all measurements by the number of IS measured.

6.2.6 Dry matter

Apple dry matter (%) was measured in triplicate by cutting one cylinder from an apple for a total of 3 cylinders per cultivar (Figure 6.1). The cylinders were macerated in a plastic bag and divided into three drying tins. Dry matter (%) was determined similarly to Section 3.2.3 (Chapter 3) and calculated using Equation 3.1 (Chapter 3).

6.2.7 Statistical analysis

All histograms and graphical images illustrating IS size distribution were drawn using the Statistical Package R (R Core, 2014). One-way Analysis of Variance (ANOVA) was used to investigate the effects of cultivar type on textural and morphometric parameters measured at 5% α-level. Results that were significantly different were separated using Tukey's post hoc comparison testing. These tests were carried out using STATISTICA 8 (Stat Soft Inc, USA). Principal Component Analysis (PCA) was carried out on all significantly different variables using Unscrambler X 10.2 (CAMO, Trondheim, Norway) and in order to interpret relationships between the measured morphometric, textural parameters and dry matter (%) all variables in the PCA were standardized (1/standard deviation).

6.3 Results and Discussion

6.3.1 Intercellular space visualization and textural properties of different apple cultivars

Using the NRecon and ImageJ image analysis software, reconstructed binary images (Figure 6.3) and 3-D geometrical models (Figure 6.4) of the IS structure of Braeburn, Fuji, Golden Delicious and Jazz apple cultivars were obtained. Binary images shown in Figure 6.3 express the IS as black regions and apple parenchyma as white regions. It can be seen the IS appear at random with no distinct pattern. Looking at the images of the cultivars, it is clear Jazz apples contain smaller IS. This suggests Jazz apple tissues remain intact through strong cell-to-cell adhesion. Comparing the 2-D images (Figure 6.3) to the 3-D geometrical models in Figure 6.4 allows the orientation of the IS to be visualized. As with the 2-D images, differentiation between cultivars with the exception of Jazz apples is unclear. However, it did appear from the images that the IS in these apples were clustered with no definite shape or size.

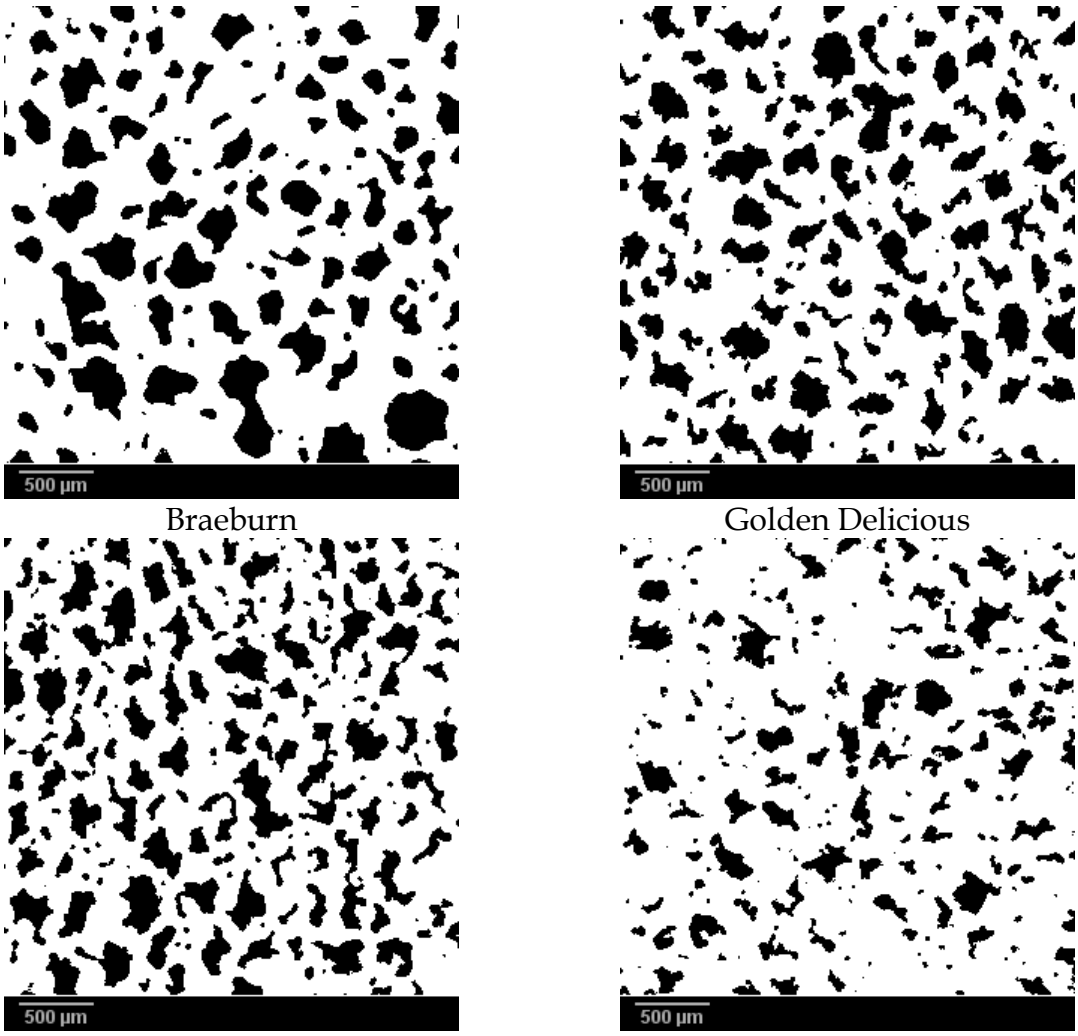


Figure 6.3: Representative segmented binary images of apple cultivars, Braeburn, Golden Delicious, Fuji and Jazz in which the black regions are intercellular spaces and the white regions are apple flesh.

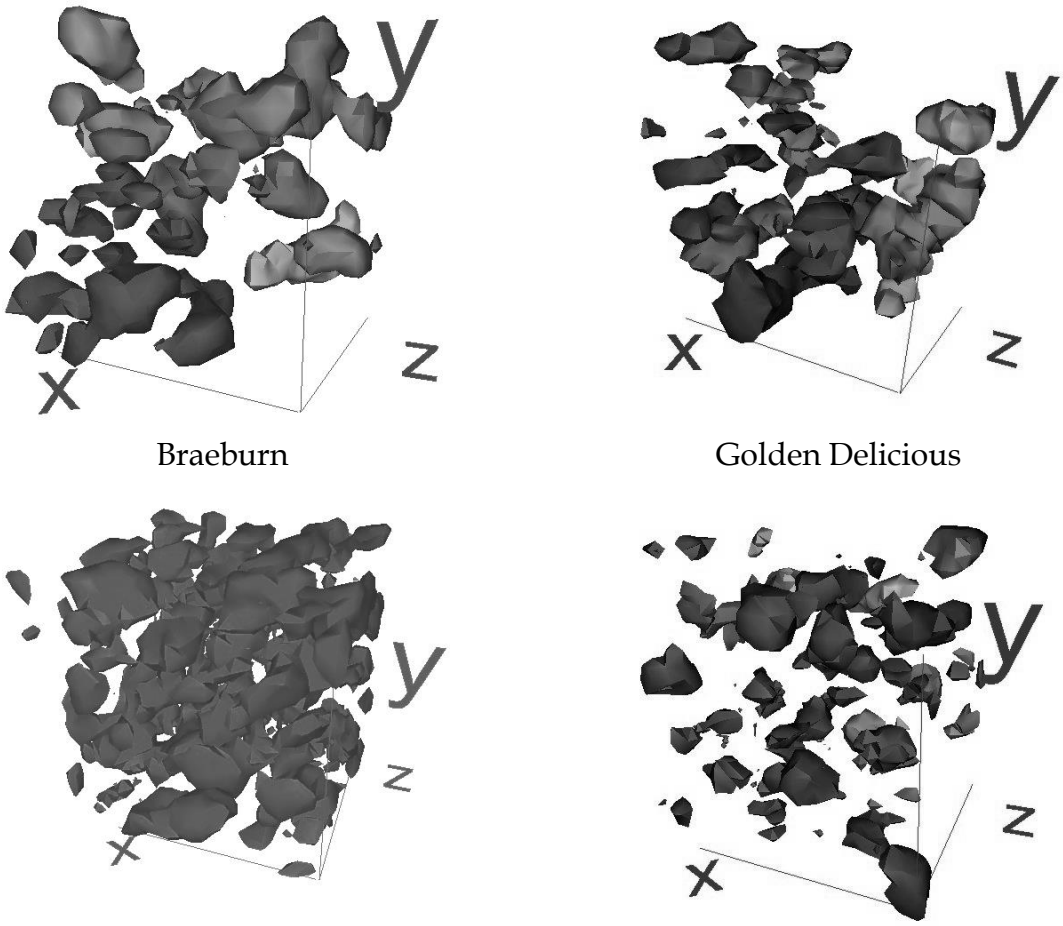


Figure 6.4: 3-D images of apple segments to illustrate differences in intercellular spaces. Images from Braeburn and Golden Delicious apples show that most of the intercellular spaces are elongated in shape. Images from Fuji apples show a largely porous structure. Images from Jazz apples contain the smallest number of intercellular spaces.

To quantify any potential differences in the IS within the different cultivars the images in Figure 6.3 were transformed into numerical data (Table 6.1) and histograms of the distribution of IS volume were graphed (Figure 6.5). The X-axis values of the two visible peak positions were extracted to represent the highest occurring small and large IS volume size. In Figure 6.5 the data is shown for triplicate volumes of the IS measured (3 measurements from the 3 apples) for each cultivar. Each bar height in the histogram represents the proportion of data within each class. A probability density distribution curve that describes the shape of the distribution for the sum is shown in black. Multi-coloured lines on the histogram represent the distribution curve of each sample replicate.

The measured textural parameters (Table 6.1) showed a general trend of Golden Delicious apples possessing the lowest values for all measured texture parameters whereas Jazz apples had the highest values with the exception of AUC peel. Braeburn and Fuji apples had comparable textural parameters and were not significantly different apart from AUC peel. Taking this into consideration, the visualization of the IS size distribution (Figure 6.5) revealed that firm cultivars (Jazz) had a higher proportion of smaller IS as the distribution was skewed to the left hand side. In contrast, in the softer cultivar (Golden Delicious) the IS size distribution was more evenly distributed between large and small IS as indicated by the peaks on both sides of the distribution curve. Although, Fuji and Braeburn share intermediate textural properties their IS size distribution are distinctly different (Figure 6.5). This shows apple flesh firmness is somewhat dependent on the structural arrangement and size of individual IS in apple parenchyma for example while where Braeburn is comparable to Golden Delicious with regards to a higher distribution of large IS though it is significantly firmer. Replicates within each cultivar were slightly different however such variation is expected as apples have been reported to be variable and anisotropic in nature (Khan and Vincent, 1993).

Table 6.1: The measured textural properties, dry matter (%), porosity and extracted morphometric parameters of four apple cultivars (Braeburn, Golden Delicious, Fuji and Jazz). Different alphabets indicate a significant difference between cultivars at a p-value of 0.05 and bracketed () values are calculated standard deviations.

Sample	Flesh Firmness (N)	Gradient (N/mm)	AUC peel (N.mm)	AUC flesh (N.mm)	Average volume per intercellular space (mm^3)	Average surface area per intercellular space (mm^2)	Porosity (%)	Dry matter (%)
Braeburn	23.5 ^{b} (2.3)	20.0 ^{b} (2.8)	52.3 ^{b} (7.6)	48.0 ^{b} (8.4)	$6.8*10^{-3}$ ^{c} ($0.6*10^{-3}$)	$55*10^{-3}$ ^{c} ($1*10^{-3}$)	25.3 ^{b} (0.6)	12.0 ^{b} (0.2)
Golden Delicious	14.2 ^{a} (1.1)	9.8 ^{a} (0.2)	33.1 ^{a} (1.1)	26.6 ^{a} (4.5)	$4.8*10^{-3}$ ^{b} ($1.3*10^{-3}$)	$45*10^{-3}$ ^{bc} ($10*10^{-3}$)	29.8 ^{c} (0.8)	9.8 ^{a} (0.5)
Fuji	28.4 ^{bc} (4.0)	15.7 ^{b} (1.1)	66.5 ^{c} (6.3)	40.3 ^{ab} (6.6)	$2.9*10^{-3}$ ^{b} ($0.3*10^{-3}$)	$32*10^{-3}$ ^{b} ($3*10^{-3}$)	29.3 ^{c} (1)	13.3 ^{b} (0.6)
Jazz	29.8 ^{c} (0.5)	28.2 ^{c} (1.6)	55.1 ^{bc} (1.9)	71.7 ^{c} (6.1)	$1.0*10^{-3}$ ^{a} ($0.3*10^{-3}$)	$13*10^{-3}$ ^{a} ($2*10^{-3}$)	17.0 ^{a} (0.9)	13.0 ^{b} (0.8)

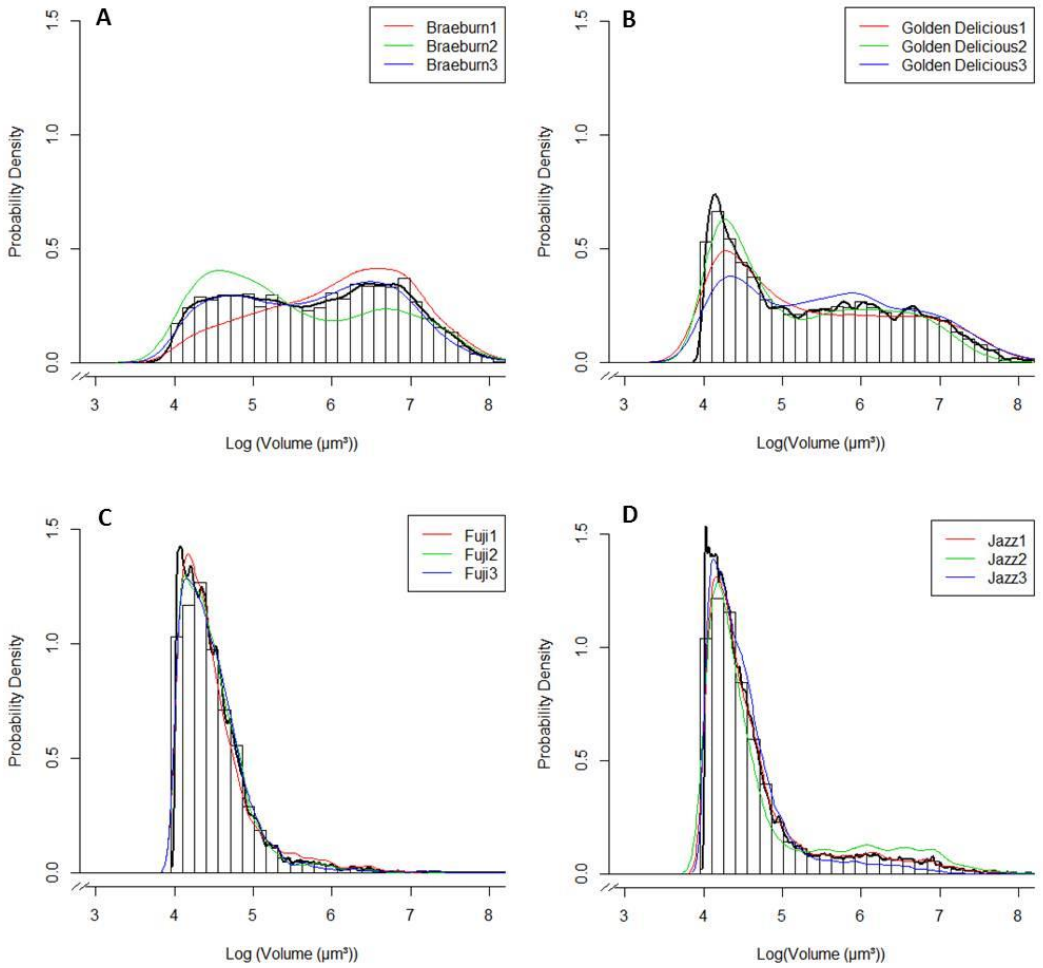


Figure 6.5: The distribution of intercellular space volume (Log volume/µm³) in the apples cultivars: Braeburn (A), Golden Delicious (B), Fuji (C) and Jazz (D) is illustrated using probability density curves. Data from triplicate apples is presented showing that there were slight variations in the data. The average of the data from the three apples from each cultivar measured is shown by the bold black line.

To compare the IS size distribution between cultivars, probability/density distribution curves of the sum of replicates for each cultivar were produced (Figure 6.6). It is important to note, that although there are differences between the distribution curve shapes, all curves peak at similar points along the x-axis. Therefore, the significance of this plot is the intensity of the peaks and not the differences in the peak positions. For example, the two main peaks on the distribution curves for Braeburn and Golden Delicious apples signify a proportion of small ($1.5*10^4 µm^3$) and large ($6.8*10^6 µm^3$) IS in which Golden Delicious contains a higher number of small IS. Braeburn apples appear to have a greater proportion of large IS as illustrated in the 2-D pictures (Figure 6.3).

The twin peaks shown in Figures 6.5 and 6.6 may represent small (schizogenous) and large (lysigeneous) IS (Esau, 1977; Harker and Hallett, 1992; Pieczywek and Zdunek, 2012), where the smaller IS sizes suggests a compact cellular structure with spaces occurring between intact cells (Pieczywek and Zdunek, 2012). The larger IS occur as a result of the breakdown of cell membranes with IS increasing in length and size as the IS become interconnected (Harker and Hallett, 1992).

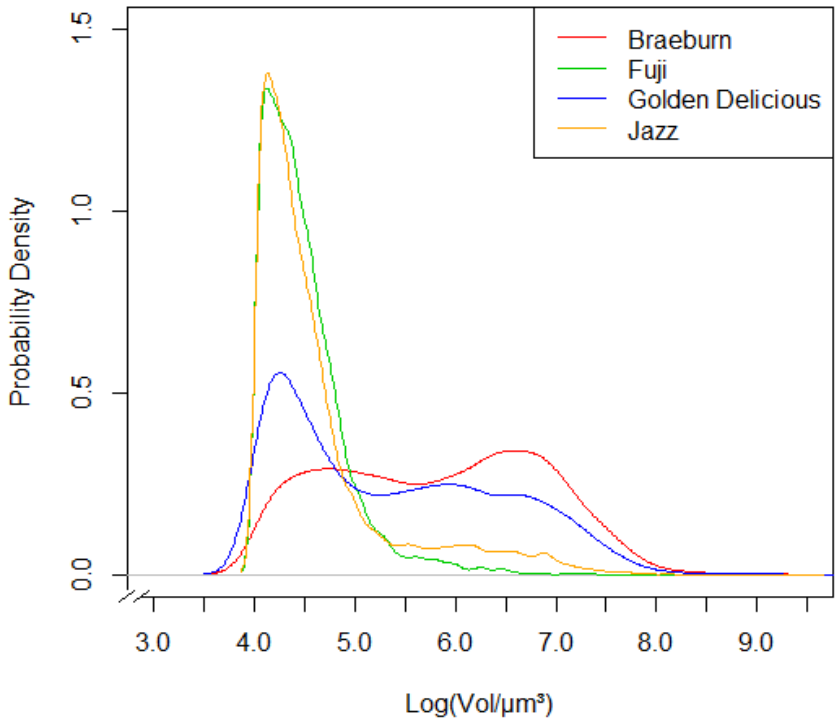


Figure 6.6: Probability density smoothing curves of histograms for the average intercellular space volume distribution for Braeburn, Fuji, Golden Delicious and Jazz apples.

6.3.2 Porosity and morphological properties

Jazz apples had the lowest values for all measured morphometric parameters whereas Braeburn apples had the highest average volume and surface area per IS (Table 6.1). Golden Delicious and Fuji apples were comparable in all morphometric attributes measured including porosity (%). The dry matter (%) of Golden Delicious apples was significantly lower than in the other apple cultivars. Based on the morphometric parameters, the calculated porosity was the highest for Golden Delicious (29.8 ± 0.8%) and Fuji (29.3 ± 1%) followed by Braeburn (25.3 ± 0.6%) and

Jazz (17 ± 0.9%). Earlier studies have reported that the porosity in apples can be as high as 30% and that percentage porosity varies between cultivars (Reeve, 1953; Mebatsion and others, 2006). Previous values reported for the porosity of Braeburn apples of 22.2 ± 2.5% and 21.7 ± 2.9% (Dražeta and others, 2004; Mendoza and others, 2007) are comparable to the data in this study.

Physiologically, a decrease in cell-to-cell adhesion in apples results in the separation of cell columns and an enlargement of the IS which results in increased tissue porosity, a decline in firmness and crispness and a corresponding increase in mealiness (Bain and Robertson, 1951; Vincent, 1989; Harker and Hallett, 1992). A general trend seen in the data indicates that low porosity can be associated with high Gradient and AUC flesh measurements. Flesh firmness increased with an increase of AUC flesh. This data suggests that firm apple cultivars were associated with low porosity and stiff skin, as depicted by high gradient values whereby more force was required to puncture the sample flesh to an equal distance (AUC flesh) compared to the force required for soft cultivars. This trend can be seen in Table 6.1 where Jazz, the firmest apple (29.8 ± 0. N), had the highest gradient values (28.2 ± 1.6 N/mm), the highest AUC flesh (71.7 ± 6.1 N*mm) and the lowest porosity (17 ± 0.9%).

Fuji apples were significantly different to Golden Delicious apples in all textural attributes measured (except AUC flesh) however their measured porosity (%) was not significantly different (29.3 ± 1% and 29.8 ± 0.8% respectively). This suggests Golden Delicious apples may contain localized regions of a few large and interconnected IS while, Fuji apples (Figure 6.3) contain a larger number of small IS, than Golden Delicious apples, that as a sum contribute to porosity (%). The current data on the shape of the IS in Golden Delicious apples is supported by a recent study that reports that the IS in Golden Delicious apples are largely elongated (Pieczywek and Zdunek, 2012). This may also hold for Braeburn apples which had a lower porosity (25.3 ± 0.6%) but the highest average volume and surface area per IS. Overall results suggest apple microstructure and ultimately texture is influenced by both mechanical and geometrical properties.

Golden Delicious apples were the softest apples, had the highest porosity (Table 6.1), an intermediate IS volume/surface area and the lowest dry matter of 9.8%

compared to values of 12.03 to 13.30% dry matter for the firmer cultivars. These results suggest that firm apples such as Jazz have a high dry matter (%) as previously reported by Harker and others (2006). A study from Palmer and others (2010) reported a relationship between dry matter (%) and flesh firmness although this was cultivar specific. In their study Jazz apples that had a high dry matter (%), had a high flesh firmness, presumably due a high proportion of their parenchyma tissue being intact. Golden Delicious apples on the other hand, have been shown in SEM micrographs to have a large amount of exudates on the cell membrane indicating membrane disruption (Varela and others, 2007), which affected flesh firmness and increased IS volume. Therefore whether the parenchyma tissue is intact and turgid or disrupted and flaccid also impacts on firmness.

The comparable porosity of Fuji and Golden Delicious apples, despite Fuji apples being significantly ($p<0.05$) firmer than Golden Delicious apples, can be explained through the cellular packing of the Golden Delicious apple parenchyma tissue resulting in an 'open' microstructure. In comparison, Fuji apples (Figures 6.2 and 6.3) have smaller IS which are less uniformed in size. This pattern of IS packing is reported to maintain crispness and firmness in apples but to decrease the perceived juiciness (Allan-Wojtas and others, 2003).

To further investigate and summarize the interrelationships between all the measured properties, the dataset was subjected to a PCA. PC1 and PC2 accounted for 83% of the total explained variance (Figure 6.7). Overall, cultivars were separated on the basis of texture, porosity and morphological properties on PC1. Golden Delicious and Braeburn apples were associated with morphological properties, porosity and large IS. On the other hand, Fuji and Jazz apples were associated with textural measurements, dry matter (%) and small IS. PC2 associates Golden Delicious and Fuji apples with porosity which was comparable between cultivars (Table 6.1) and Fuji apples were also associated with small IS. This indicates that although the porosity of Fuji apples was similar to Golden Delicious, it consisted of a higher proportion of small IS as also shown in Table 6.1 and Figure 6.3. Interestingly, porosity was negatively correlated with AUC flesh. This correlation suggests

porosity decreases as the amount of force needed to rupture the apple peel and flesh increases.

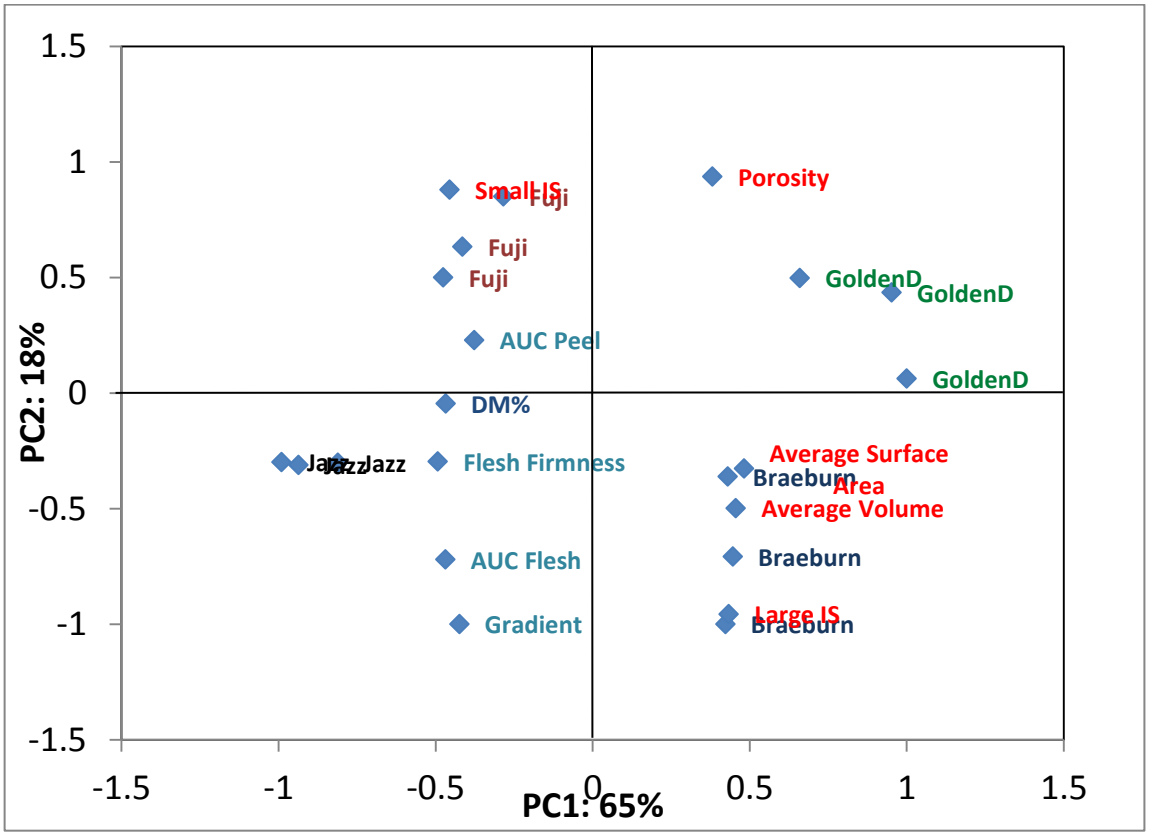


Figure 6.7: Principal components analysis (PCA) on the measured morphological and textural properties and dry matter concentration (%) data grouped by different coloured labels. Here, plotted apple replicates showed repeatability of measurement as they grouped closely together. Abbreviations: Intercellular Space - IS.

Apple texture is influenced by the microstructural organization of the parenchyma tissue. Differences between apple cultivars were visualized using 2-D and 3-D images taken from the µ-CT scanner. While morphometric and textural measurements showed a general trend for firm apples (Jazz) to contain a low porosity (17%) in comparison to soft apples (Golden Delicious) of a high porosity (29.8%), this was not absolute. It is interesting to note, firm Fuji (28.4N) and less firm Braeburn (23.5N) apples showed comparable porosity (29.3% and 25.3% respectively) to that of Golden Delicious apples. By visualizing porosity using IS size range distribution graphs and comparing it on a numerical basis, it was found Fuji apples contained a high number of small IS which as a sum were comparable to the volume of IS in Golden Delicious apples. Conversely, the porosity of Braeburn apples was similar to Golden Delicious apples that contained a high number of large IS. This result suggests that texture is dependent on characteristics that differentiate the cultivars such as the microstructural organization and the distribution, number and size of individual IS in apple parenchyma. The application of µ-CT scanning enabled the visualization of differences in apple cultivars arising from their microstructural organization. This technique may increase the understanding of how apple texture is influenced by their structural organization and how this organization influences eating quality (i.e. break down during mastication).

The use of µ-CT scanning has provided additional information on the microstructure of apples that influence texture. As this study only focuses on apple cultivars at one time point, it would be of interest to follow the development of IS throughout storage. This could also be coupled with the use of post-harvest treatments such as the application of 1-MCP and cold storage which, although retains firm apple texture for longer, has detrimental effects on volatile organic compound/aroma production. Therefore, a comparison in texture, morphological properties and volatile organic compound emission between apples with and

without the application of 1-MCP could contribute greatly to current research. This will be investigated in the following chapter.

Chapter 7: Investigating the effects of cold storage and 1-MCP on the VOC release, texture and microstructure of four different apple cultivars

Chapter 7: Investigating the effects of postharvest treatments

7.1 Introduction

Following harvest, an apples' firm and crunchy texture deteriorate during long term storage (Johnston and others, 2002). This is because apples as climacteric fruit experience a peak in respiration after fruit abscission which simultaneously increases the production of ethylene, a ripening hormone (Barry and Giovannoni, 2007). As the apple fruit sweetens and ester VOCs production increases, excessive softening may occur leading to a reduction in consumer acceptability (Johnston and others, 2002). Therefore, numerous postharvest treatments focused on maintaining apple texture have been extensively studied.

Two commonly used commercial postharvest treatments for apples are cold storage and the use of an ethylene inhibitor, 1-MCP as elaborated in Chapter 2.4. Briefly, the use of cold temperatures during long term storage decreases the rate of respiration in apples. As a result, normal metabolic functions such as ethylene production which are temperature dependent, are impeded (Brady and Morris, 2005). The application of 1-MCP does not decrease ethylene production but, prevents ethylene induced responses by competitively binding onto the ethylene receptor in place of ethylene (Blankenship and Dole, 2003). Although these treatments effectively maintain a firm and crunchy apple texture, VOC production that contributes to overall aroma is also reduced as the involvement of ethylene in the final stages of the VOC biosynthetic pathway is hindered (Schaffer and others, 2007).

Although the effects of cold storage and 1-MCP on IEC, apple texture and VOC composition have been documented (Fan and others, 1999; Johnston and others, 2002; Watkins, 2006), their effects on the microstructure of apple parenchyma is less well known. As mentioned in Chapter 2, the visualization of apple microstructure was initially explored to understand how morphological size and orientation of IS influences texture (Khan and Vincent, 1990; 1993).

An important measurement regularly associated with a soft and mealy apple texture is porosity (Harker and Hallett, 1992). Porosity is a percentage of the total IS

within a volume. It does not reflect the morphology, size or orientation of the IS that has been shown to affect the texture of specific cultivars (Chapter 6). Another aspect to consider is that IS within the apple parenchyma act as a medium for gas transport as within them there is the least resistance for gas exchange (Raven, 1996). Gas transport rates have been investigated based on the diffusivity of O_2 and CO_2. It is often associated with browning disorders due to ethanol build-up causing localised fermentation occurring within pear or apple tissue stored under controlled atmosphere (CA) (Dixon, 2000). The rate of diffusivity is influenced by biological variations in IS volume and size (Schotsmans and others, 2004; Verboven and others, 2008; Ho and others, 2009; Herremans and others, 2013b). Additionally, O_2 and CO_2 gases participate in respiration which is important for normal physiological functions. Thus, respiration metabolism could be potentially indirectly influenced by IS properties (Beaudry, 1999; Ho and others, 2009) and therefore affect mechanical texture properties and VOC emission though this has not been investigated.

Numerous studies have investigated the effects of 1-MCP on texture and VOC composition. However, research on differences in apple microstructure with or without 1-MCP treatment is limited. Hence, this chapter aims to investigate the effects of 1-MCP on apple texture and VOC composition and explore possible interactions associated with changes in apple microstructure over long term postharvest cold storage.

7.2 Methods and materials

7.2.1 Apples

Four apple cultivars (Braeburn, Fuji, Golden Delicious and Jazz) harvested at commercial maturity were sourced from a commercial orchard in Central Otago, New Zealand and stored at 2ºC under regular atmospheric conditions in a conventional cold store. Two 20 kg boxes of apples were obtained for each cultivar. One box of apples acted as a control and the other box was commercially treated

with the ethylene inhibitor, 1-MCP (will be referred to as Smart Fresh™ (SF) from this point on) within 24 hours of harvest before being subjected to cold storage (Table 7.1). For all cultivars and treatments, only apples within the weight range of 165 – 185g were selected for further trials. SF treated and untreated apples were stored in separate cardboard boxes lined with paper pulp fruit trays.

Over the course of the trial, three apples from each cultivar/ treatment combination were removed after either 50, 70, 100, 120 or 150 days and their VOC release, texture and physicochemical properties assessed. In addition on days 50, 100 and 150 apple porosity and structure was assessed using a µ-CT scanner. Apples were brought up to room temperature 24 hours before the analysis. Regions of the apple sampled for the different analysis are shown in Figure 7.1.

Table 7.1: Harvest and Smart Fresh™ (SF) treatment dates for apple cultivars tested.

Cultivar	Harvest date	SF treated
Braeburn	13/04/2013	14/04/2013
Fuji	13/04/2013	14/04/2013
Golden Delicious	20/03/2013	21/03/2013
Jazz	14/04/2013	15/04/2013

7.2.2 Chemicals

Antioxidant dip solution for fresh cut samples was prepared using ascorbic acid (0.2% w/w) (Hawkins Watts, New Zealand), calcium chloride dihydrate (0.2% w/w) (Unilab, Auckland, New Zealand), citric acid anhydrous (0.5% w/w) (Absolute Ingredients Ltd, Penrose, New Zealand) and made up to 100% w/w with distilled water.

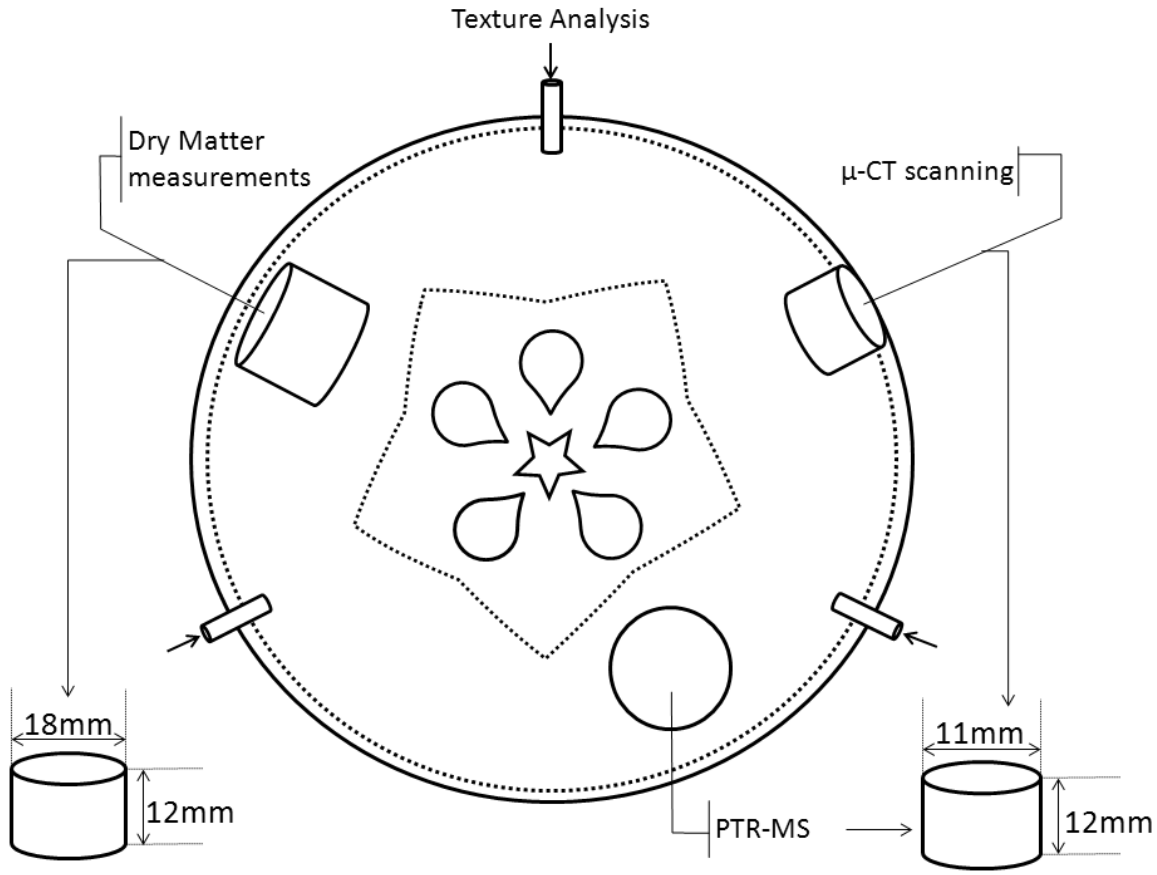


Figure 7.1: Schematic diagram of the apple regions used. A texture penetration test was carried out on three different locations along the equator of the apple. Three cylinders were cut from the apple where the larger one (without peel) was used for dry matter measurements. Two smaller cylinders of equal size were cut for µ-CT scanning (with peel) and PTR-MS measurements (without the peel parallel to the core).

7.2.3 Texture analyser

Apple texture was measured as previously described in Chapter 6.2.2 on the indicated regions in Figure 7.1. The acquired force-displacement curves were used to calculate the following parameters as defined by Bourne (2002).

Fmax (N): Maximum force required to puncture the fruit skin.

Flesh firmness, Flesh.F (N): Averaged force measured after skin rupture

Gradient, grad (N/mm): Stiffness of skin measured as a slope from the start of the curve until Fmax.

Area under the curve, AUC peel (N*mm): Mechanical work needed to rupture the skin taken as the area under the curve of Fmax.

AUC flesh (N*mm): Work measured under the curve after skin rupture.

7.2.4 Soluble solids content and dry matter concentration

The soluble solids content (SSC %) of the apples were determined by Brix° using a hand refractometer (Brix 0-32%, N1, Atago, Japan) on the juice expressed from each replicate from the texture penetration test (Saei and others, 2011). The concentration of dry matter (DM %) was measured as defined in Chapter 3.2.3.

7.2.5 Headspace volatile organic compound measurement

Single apple cylinders were dipped into an antioxidant solution and placed into a 250mL glass bottle (GL45, Duran Group GmbH, Germany) capped with glass fittings fitted into Teflon rings to create an air tight system. They were incubated for 30 minutes at 30°C before measurement. Two polytetrafluoroethylene (PTFE) tubes were fitted into the glass fittings. One was a gas inlet flushing instrument grade synthetic air through an active charcoal filter (Supelcarb®, Supelco, Bellefonte, PA) at a flow rate of 50 standard cm^3 per minute (sccm). The other was connected to the PTR-QUAD-MS (Ionicon Analytik, GmbH, Innsbruck, Austria) inlet (~1m, 1/16" outer Ø Silcosteel™ capillary (Restek Co, Bellefonte, PCA) heated to 80°C during measurement.

The PTR-QUAD-MS conditions were configured in accordance to Biasioli and others (2006). A flow rate of 50 sccm over a mass ion range of m/z 20 – 210 at a dwell time of 100 ms (1 entire spectrum reading = 19 sec) was used. The instrument was operated under the drift tube conditions of 140 Td (Td= Townsend: 10^{-17} V cm^2 mol^{-1}). Each sample was measured for 6 cycles and the mean of cycles 2 - 6 was used for data analysis.

7.2.6 X-Ray micro-CT scanning and image analysis

Image acquisition and segmentation were carried out in accordance to Chapter 6.2.3 - 6.2.4 using the apple cylinder obtained from the apple as shown in Figure 7.1. The BoneJ plugin (Doube and others, 2010) was used to measure morphometric parameters of the IS on the processed 8-bit binary images. These are defined as:

Porosity (%): Volume of calculated IS divided by total volume of selected voxel cube.

Anisotropy: The degree of orientation of the substructures within a REV. The value is reported as isotropic at zero for isotropic samples. An increase in anisotropy brings the value closer to 1 (The data was multiplied by 10 to give positive log values).

Connectivity: The degree to which an IS is connected assuming only one IS is present in the foreground. To do this, the plug-in 'Purify' (BoneJ) which acts as a filtering step is performed to locate all IS within the REV and removes all but the largest interconnected IS present. Next, the connectivity algorithm is performed which calculates the connectivity of the image based on its calculated Euler characteristic.

7.2.7 Data analysis

Headspace analysis

Headspace analysis of the PTR-MS data was converted from cps to concentration (ppbv) in accordance to Lindinger and others (1998). Data processing and the concentration of ethylene were calculated in accordance to Section 3.2.7.

Statistical analysis

A one-way analysis of variance (ANOVA) was carried out to evaluate the effects of treated and untreated cultivars based on time, VOC, texture, morphological and physico-chemical properties. Variables that significantly ($p < 0.05$) differentiated cultivars based on treatment and postharvest storage duration were used for further analysis. To understand the relationship between texture, physico-chemical and morphological properties, Pearson's correlation test at a $p < 0.05$ was also carried out. Both ANOVA and Pearson's correlation calculations were performed using SPSS v20.0 (IBM Statistics, Inc, Chicago, IL). Principal component analysis (PCA) and multi factor analysis (MFA) was performed to understand the interrelationships between the collected data. This comprised of standardised (1/SD) PTR-MS, texture, physico-chemical and morphological properties from Day 50, 100 and 150 measurements. Both PCA and MFA were carried out using the FactoMineR package (Lê and Husson, 2008) in R Core (2014).

7.3.1 Headspace analysis of VOCs from fresh cut cylinders.

Comparing untreated and SF treated apples

A total of 38 VOCs that significantly differentiated ($p < 0.05$) between treated and untreated apples are shown in Table 7.2. The averaged, minimum and maximum VOC concentrations of each m/z for the untreated and treated apples were reported as its tentative identification based on published literature using the PTR-ToF-MS (Cappellin and others, 2012c; Soukoulis and others, 2013; Farneti and others, 2014). The benefit of compound identification using the PTR-ToF-MS has been covered in Chapter 2. The VOC identified included esters, ester related compounds, alcohol, aldehydes and terpenes and were similar to those reported in Chapters 3 – 5. In order to understand the overall effects of SF treatments on VOC production, an averaged concentration of the 5 time points was used (Table 7.2). However, to investigate the efficacy of SF treatments based on the different cultivars, total VOCs based on time were plotted for each cultivar (Figure 7.2) which will be discussed later on. Based on the average concentrations, and supported by the individual measurements at each time point (Figure 7.2 ANOVA $p<0.05$) untreated apples over the 150 day duration of trial emitted VOCs at concentrations at least two times higher than SF apples (Table 7.2), thereby illustrating the efficacy of SF in inhibiting VOC production.

It has previously been reported that the impairment of ethylene related pathways causes a significant reduction in the concentrations of ester compounds but not in the concentrations of aldehydes (Dandekar and others, 2004; Defilippi and others, 2004). In Table 7.2, the majority of the VOCs that approximately halved in concentration in the SF treated apples, compared to the untreated samples were esters, including butyrate (m/z 89), isoamyl (m/z 103), hexanoate (m/z 117) and methyl hexyl (m/z131) related esters, butanal (m/z 73), butyl propanoate (m/z 75), ethyl hexanoate (m/z 145), alcohols (m/z 33, 47, 83, 85) and, ester and alcohol fragments (m/z 41, 43, 53, 55, 57, 61). SF treated apples also showed a slight

reduction in the concentration of specific aldehydes, alcohol and terpene compounds, such as trans-2-hexenal/monoterpene (m/z 81), hexanal (m/z 83), pentanal (m/z 87), farnesene fragments (m/z 95, 123, 135, 137) and α-farnesene (m/z 205). These results were consistent with previous reports (Dandekar and others, 2004; Defilippi and others, 2004). Lurie and others (2002) reported that the VOC composition of SF apples would include more alcohol and aldehydes compared to untreated apples. However, this result was not obvious in Table 7.2 since the averaged concentrations shown are a summation of five time points. It is possible that the VOC recovery of alcohols or aldehydes occurred in SF treated apples after its removal from long term cold storage which will be elaborated on further in Figure 7.5-6.

Table 7.2: A comparison of VOC concentration between untreated (C) and treated (SF) apples

m/z	*Tentatively identified compounds*	*Untreated (C)*			*Treated (SF)*		
		Average (ppbv)	*Min (ppbv)*	*Max (ppbv)*	*Average (ppbv)*	*Min (ppbv)*	*Max (ppbv)*
28	Ethylene	874.9	0.0	3313.3	211.9	0.0	1087.5
31	CH_3O^+	1.9	0.2	5.4	0.3	0.0	2.6
33	Methanol	156.9	24.4	320.5	52.2	10.8	368.9
41	Alcohol and ester fragment	491.4	201.8	1082.5	234.2	92.7	679.3
43	Alcohol and ester fragment	2133.7	662.3	4405.7	620.4	200.9	2514.4
45	Acetaldehyde	9567.7	1709.7	21413.6	4612.5	742.9	12851.1
47	Ethanol	50.0	7.3	117.8	22.9	4.9	86.9
53	$C_4H_5^+$	3.8	1.3	7.9	1.3	0.5	2.5
55	$C_4H_7^+$	94.7	39.3	208.3	35.4	19.1	62.0
57	Alcohol and ester fragment	373.2	180.7	637.9	133.2	34.7	313.9
59	Acetone	40.8	18.6	85.4	22.3	10.2	47.4
61	Acetate related esters	1611.0	425.2	3134.1	399.0	125.2	1605.7
63	Ethylene Glycol	21.6	5.7	54.7	8.7	1.5	21.6
69	Isoprene	5.3	2.4	10.2	3.8	1.5	8.2
71	Alcohol and ester fragment	52.1	9.3	160.4	42.7	13.6	157.3
73	Butanal	5.5	1.2	16.1	2.1	0.6	8.4
75	Butyl propanoate	41.1	7.4	130.3	11.2	0.5	48.4
79	$C_2H_7O_3^+$	3.1	0.3	8.1	0.8	0.1	3.6
81	Terpene related fragment, aldehydes (trans-2-hexenal)	18.2	3.1	54.3	11.9	1.5	40.0
83	Alcohols (trans-2-hexenol, cis-2-hexenol, hexanol)	20.9	9.2	43.4	10.0	3.3	17.8
85	Alcohols(1-Hexanol, Nonanol), ester fragment	21.2	8.1	48.8	5.0	0.7	10.6
87	General fragment (pentanal, 2-pentanone)	1.8	0.7	3.0	1.1	0.3	2.6
89	Butyrate related esters (Ethyl butanoate, Propyl butanoate, Butyl butanoate)	57.7	10.2	298.0	16.4	1.8	99.7
95	Farnesene fragment	2.0	0.0	6.7	1.2	0.0	5.1
99	Aldehydes (trans-2-hexanal), esters (Ethyl hexanoate, Hexyl acetate)	7.4	2.1	19.4	4.0	1.5	8.7
101	Aldehydes(2-Hexanone, hexanal)	1.6	0.5	3.6	0.7	0.2	1.6
103	Esters (Isoamyl esters, propyl acetate, Ethyl 2-methyl butanoate, Methyl butanoate)	23.9	3.1	82.8	12.4	2.2	49.7
109	n.a	2.7	0.5	5.2	1.7	0.0	4.3
117	Esters (Hexanoates, Ethyl 2-methyl butanoate, Isobutyl	42.0	12.7	137.1	13.7	1.1	52.6

m/z	*Tentatively identified compounds*	*Untreated (C)*			*Treated (SF)*		
		Average (ppbv)	*Min (ppbv)*	*Max (ppbv)*	*Average (ppbv)*	*Min (ppbv)*	*Max (ppbv)*
	acetate, butyl acetate)						
121	Acetophenone	1.4	0.0	5.0	0.9	0.0	2.9
123	Farnesene fragment	2.1	0.0	6.3	1.3	0.0	4.6
131	Esters (Heptanoates, methyl hexyl-esters)	3.3	0.2	24.4	1.3	0.0	10.8
135	Farnesene fragment, p-cymene	1.3	0.0	3.8	0.8	0.0	3.5
137	Monoterpenes, farnesene fragment	1.5	0.0	3.8	0.8	0.0	3.0
145	Esters (Ethyl hexanoate, Butyl butanoate)	6.1	1.3	20.3	1.6	0.0	8.6
149	Farnesene fragment, Phenyls (Estragole, Anethol)	4.1	0.2	13.8	1.4	0.0	8.0
173	Decanoates	1.6	0.3	3.8	0.7	0.0	2.7
205	alpha-farnesene	2.5	0.0	9.4	1.4	0.0	6.1

At most time points, the total VOC concentration of SF apples was at least 50% lower compared to untreated apples (Figure 7.2). This is comparable to results reported by Ferenczi and others (2006). Untreated samples for both Braeburn and Jazz cultivars show a significant linear increase in total VOCs over time, which are predominantly driven by the large increase of acetaldehyde (m/z 45) (Figure 7.5). SF treated Braeburn cultivar showed a significant increase in total VOCs after day 100 and slowly approaches the VOC concentration of the untreated samples. The SF treated Jazz on the other hand, showed a strong suppression in total VOCs. Untreated Fuji apples were less than two times higher in total VOC concentration for all time points compared to the SF treated apples with no significant differences in VOC concentrations for any time points. This could be because Fuji does not contain a high concentration of total VOCs and is appreciated for its ability to retain texture over extended postharvest storage (Costa and others, 2011). No significant changes were observed for the untreated Golden Delicious apples however SF treated Golden Delicious samples showed a significant decrease in total VOCs after day 50 and a significant increase after day 100.

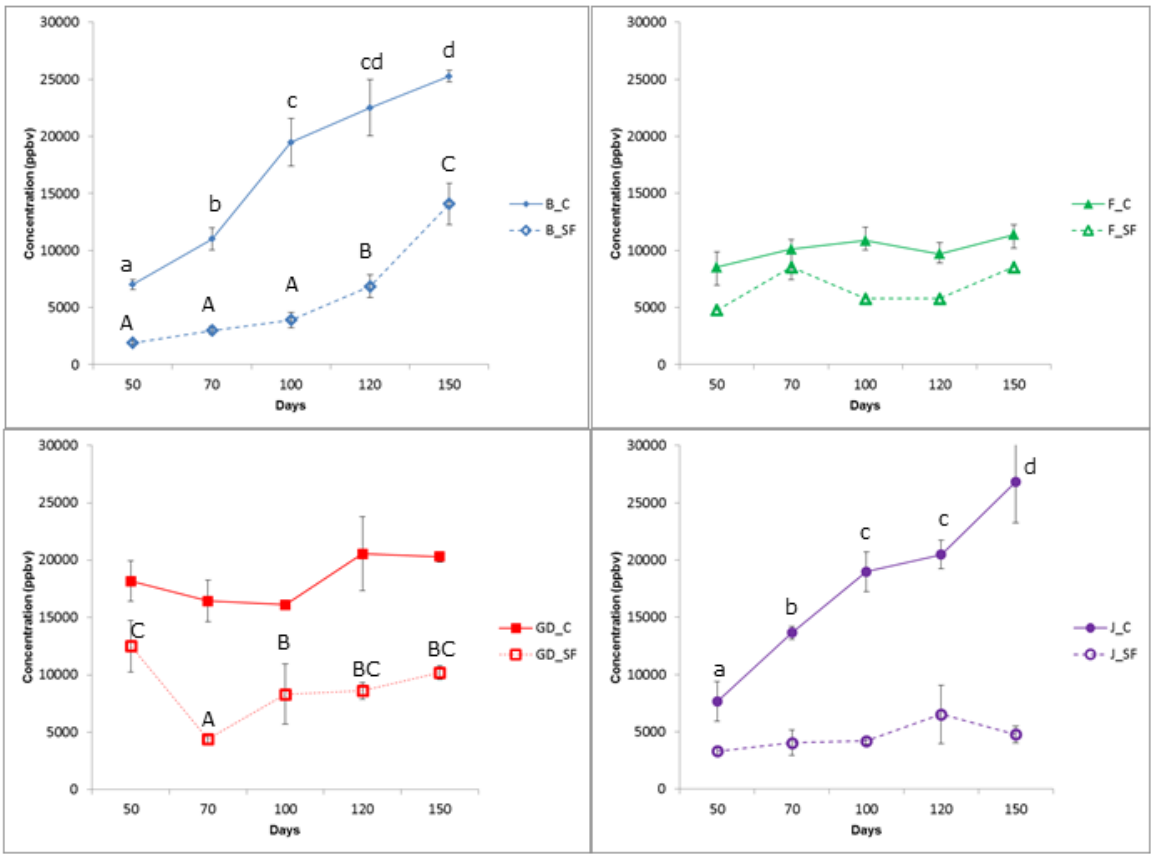


Figure 7.2: Total VOC concentration for all cultivars at 5 different postharvest days. Abbreviations: B – Braeburn; J – Jazz; F – Fuji; GD – Golden Delicious; C – Control (Untreated); SF – Smart Fresh. Different letters were used to indicate only cultivars that were significantly different (P<0.05) between days.

Ethylene emission

Ethylene concentration for the SF treated cultivars was significantly lower compared to the untreated cultivars for all postharvest time points although no significant differences were found between days for all cultivars (Figure 7.3B). As these apples were commercially bred for export and stored similarly, variations in internal ethylene concentration (IEC) is due to genetic differences between the cultivars and is dependent on the rate of aminocyyclopropane-1-carboxylic acid (ACC) synthesis (Costa and others, 2005; Barry and Giovannoni, 2007; Wei and others, 2010).

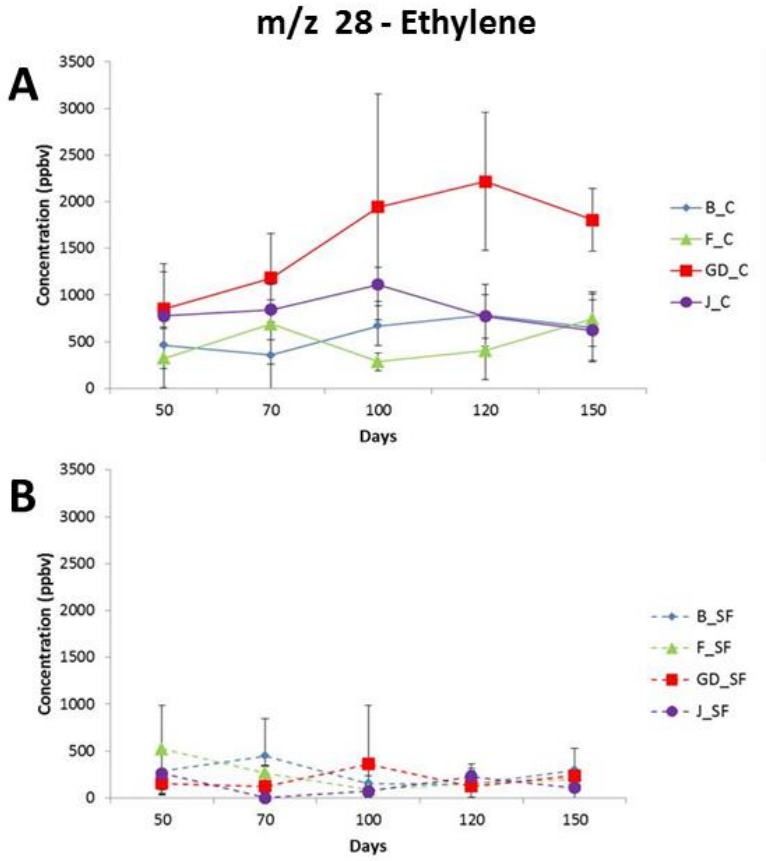


Figure 7.3: Comparison of ethylene (m/z28) evolution (mean ± SD) between untreated (A) and Smart Fresh (B) treated apples during postharvest storage. Abbreviations: B-Braeburn; F-Fuji; GD- Golden Delicious; J-Jazz; C-Control; SF-Smart Fresh.

Ester compounds

The evolution of important esters (m/z 61, 89, 117, 145) that impart fruity flavours during consumption (Chapter 5) was studied over the postharvest storage time (Figure 7.4). In the untreated samples, the Braeburn cultivar was the highest emitter for all ester related VOCs (Figure 7.4A - D left panel). The Fuji cultivar was the lowest for acetate ester related VOCs (m/z 61) but not for butyrate (m/z 89), hexanoate (m/z 117) and ethyl hexanoate (m/z 145) ester related VOCs. Although Golden Delicious was the highest ethylene emitter, this trend was not observed for the ester related compounds. This could be because Golden Delicious produces only intermediate levels of esters as observed in this thesis (Chapter 3-5). Higher levels of ethylene in Golden Delicious is reportedly due to their higher respiration rates in comparison to other cultivars such as Fuji (Chapter 2: Section 2.3.1) (Wei and others, 2010). It could be speculated that ethylene in Golden Delicious favours the regulation

of cell wall-modifying enzymes related to texture softening (Wei and others, 2010). However, more studies need to be carried out in order to understand how ethylene is utilized by Golden Delicious apples (favouring texture softening or VOC production). In comparison to the untreated samples, the SF treated samples showed significantly lower ($p<0.05$) concentrations of ester related VOCs for all cultivars across all time points (Figure 7.4 A-D right panel).

The suppression of ester VOC formation was not absolute as a significant increase in was observed after 120 days of cold postharvest storage for Braeburn and Golden Delicious cultivars. Differences in metabolic rates and substrate availability between cultivars are reflected in the faster recovery (after day 100) of butyrate (m/z 89), hexanoate (m/z 117) and ethyl hexanoate (m/z 145) related esters for Braeburn and Fuji cultivars (Song and Forney, 2008). Conversely, no significant changes were observed for the Jazz cultivar with the exception of m/z 145 for the untreated sample.

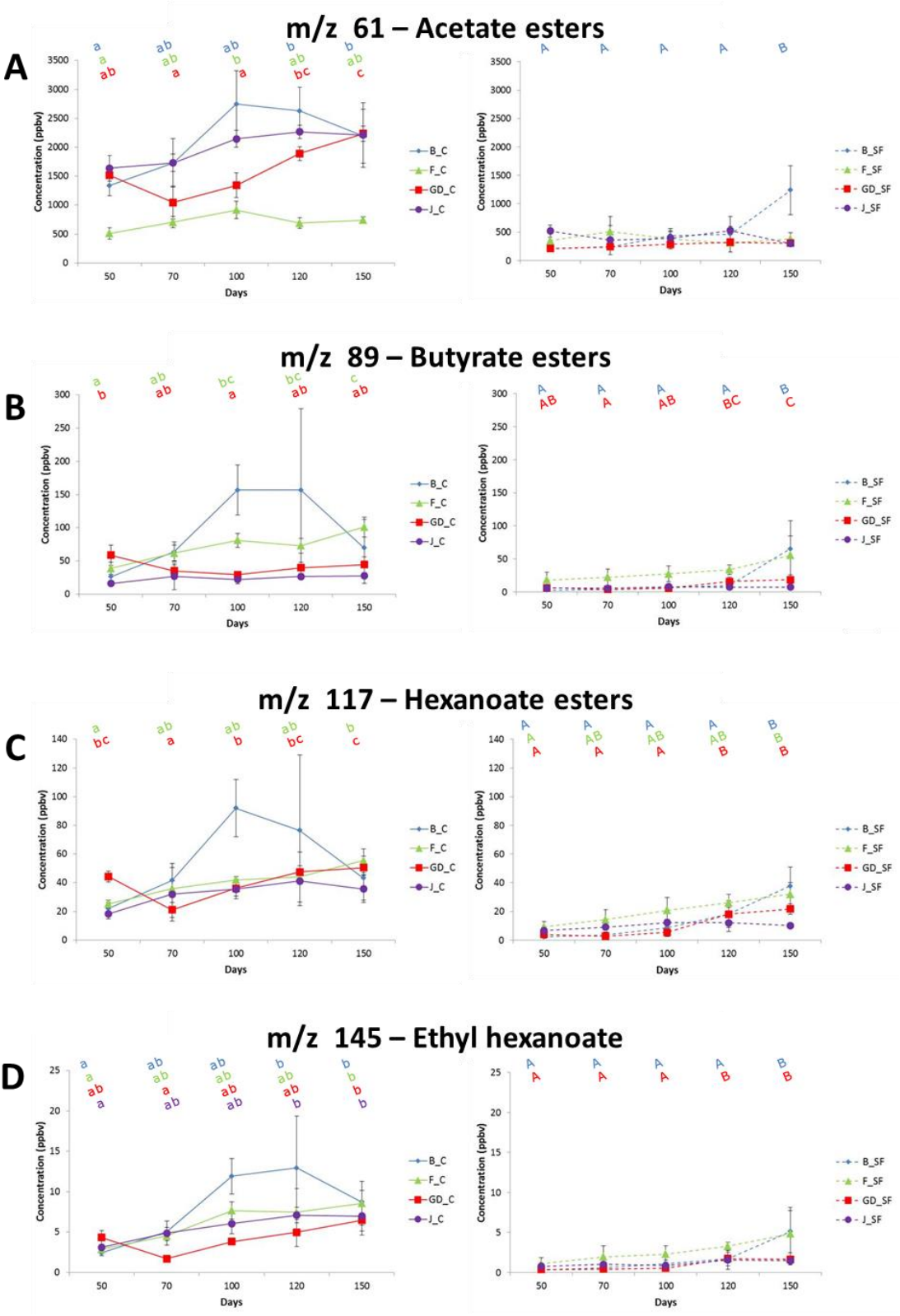


Figure 7.4: Selected ester related mass ions (mean ± SD) for untreated (left panel) and SF treated (right panel) apple cultivars. Where significant changes (P<0.05) between days occur, these are indicated by different letters and colour that correspond to a specific cultivar.

Carbonyl compounds

Although the use of SF was shown to suppress ethylene and ester related VOC emission (previous section), the reduction in carbonyl VOCs was not as pronounced (Figure 7.5). This result was comparable to results Lurie and others (2002) reported who observed a retention of alcohols and aldehyde compound emission in SF treated Anna apples despite a decrease in ester and total VOCs. In the current study, for all cultivars, treatments and postharvest storage days, acetaldehyde (m/z 45) (Figure 7.5A) was the most abundant VOC. A high concentration of acetaldehyde in cut fruit has been consistently reported throughout this thesis (Chapters 3 - 5). Focusing on the untreated samples (Figure 7.4A left panel), only Jazz and Braeburn cultivars showed a significant increase in acetaldehyde production over time. Golden Delicious and Fuji cultivars showed no significant differences in acetaldehyde concentration throughout the storage time. When comparing the increases of acetaldehyde (Figure 7.5) to the total VOCs in Figure 7.2, it was observed the increase in total VOC concentration for Jazz and Braeburn cultivars was predominantly due to the increase in acetaldehyde.

Carbonyl compounds are known to be precursors involved in ester synthesis. Positive correlations were observed ($p<0.05$; mean $r=0.71$) between butanal (m/z73) and butyrates (m/z 89), isobutyls/ethyl 2-methyl butanoates (m/z 117) and butyl butanoates (m/z 145) indicating its contribution to ester synthesis. Hexanal/2-hexanone (m/z101) was also positively correlated ($p<0.05$; mean $r=0.84$) to hexanoates (m/z117) and ethyl hexanoate (m/z145). Both compounds appear to contribute to the formation of esters. However, their involvement maybe impeded in the SF treated samples due to the suppression of ethylene in the final steps of ester production (Figure 7.4) (Table 7.2) (Schaffer and others, 2007).

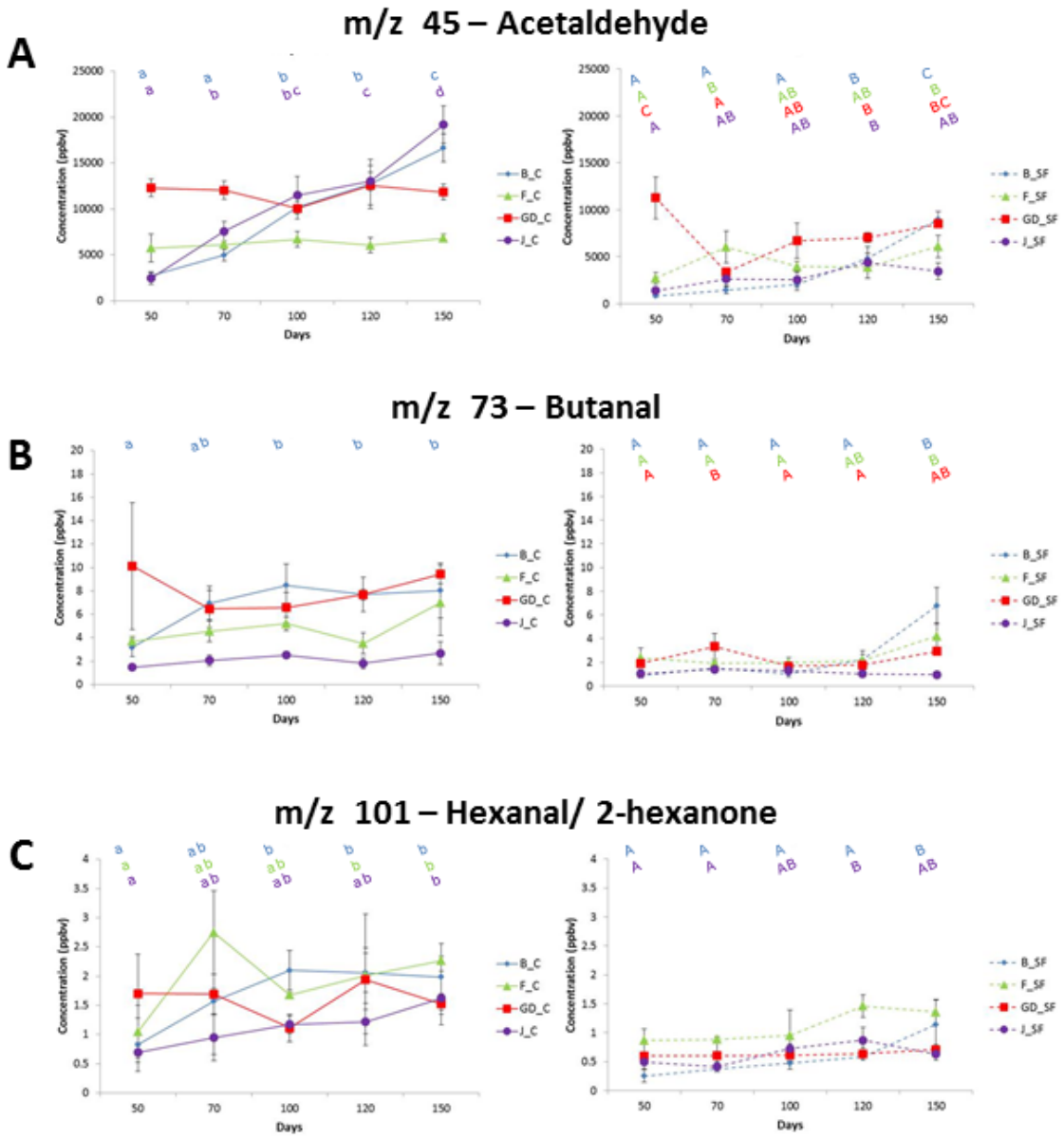


Figure 7.5: Selected carbonyl related mass ions (mean ± SD) for untreated (left panel) and treated (right panel) apple cultivars. Where significant changes (P<0.05) between days occur, these are indicated by different letters and colour that correspond to a specific cultivar.

Alcohol compounds

Ethanol and methanol are commonly produced in apples during ripening (Flath and others, 1967; Knee and Hatfield, 1981). However, the evolution of ethanol and methanol is typically monitored as a spoilage indicator because it is associated with fermentation that may occur if an apple is exposed to anaerobic conditions during long term storage. Anaerobic conditions in apples occur when the rate of respiration is retarded due to low O_2 conditions, which induces an oxidative stress and changes the normal cellular metabolism to that of a fermentation pathway (Dixon and

Hewett, 2001). The significant linear increase of alcohol in Braeburn and Jazz cultivars reaches a plateau after 100 days (Figure 7.6A - B left panel). Golden Delicious cultivars had a slight but insignificant decrease of ethanol between days 70 - 100 followed by an increase. Untreated Fuji cultivars showed a significant increase in ethanol (m/z 47) after 150 days of storage. These findings were contradictory to a previous study carried out by Lee and others (2012) on Empire cultivar apples, who that an increase in methanol concentration was observed regardless of SF treatment while ethanol production decreased (Lee and others, 2012). In this study, both methanol and ethanol increased over time in the untreated samples but was suppressed until a certain time point in the SF treated samples (Figure 7.6A - B right panel) indicating that the application of SF slowed down the increase in ethanol and methanol concentrations. However, these compounds subsequently showed a significant increase after 70-100 days depending on the cultivar.

α-farnesene compound

α-farnesene is a relatively large MW compound which was found to be strongly correlated ($p<0.05$; mean $r= 0.88$) to its smaller fragments (m/z 95, 121, 123, 135) and to monoterpene compounds (m/z81, 137). α-farnesene is an important VOC that imparts a 'green' flavour (Mookherjee and others, 1984). The build-up of α-farnesene during storage is associated to the occurrence of superficial scald spoilage (Lurie and Watkins, 2012). No superficial scald was observed in the chosen cultivars of this experiment as these cultivars are considered have a low susceptibility (Kupferman, 2001). SF treatment decreased the emission of the terpene compound for only the Jazz cultivar (Figure 7.7 right panel). In the case of Fuji, SF treated and untreated apples displayed comparable α-farnesene concentrations. However, the SF treated samples showed an increase in α-farnesene after day 50 which plateaued until day 100 before decreasing at day 150. SF treated Braeburn and Golden Delicious cultivars showed similar trends in which a significant increase in α-farnesene occurred after day 100. No significant changes in α-farnesene was observed for all cultivars with the exception of Golden Delicious which had the highest concentration of terpene compounds at day 50 but, decreased significantly at day 150 (Figure 7.7 left panel).

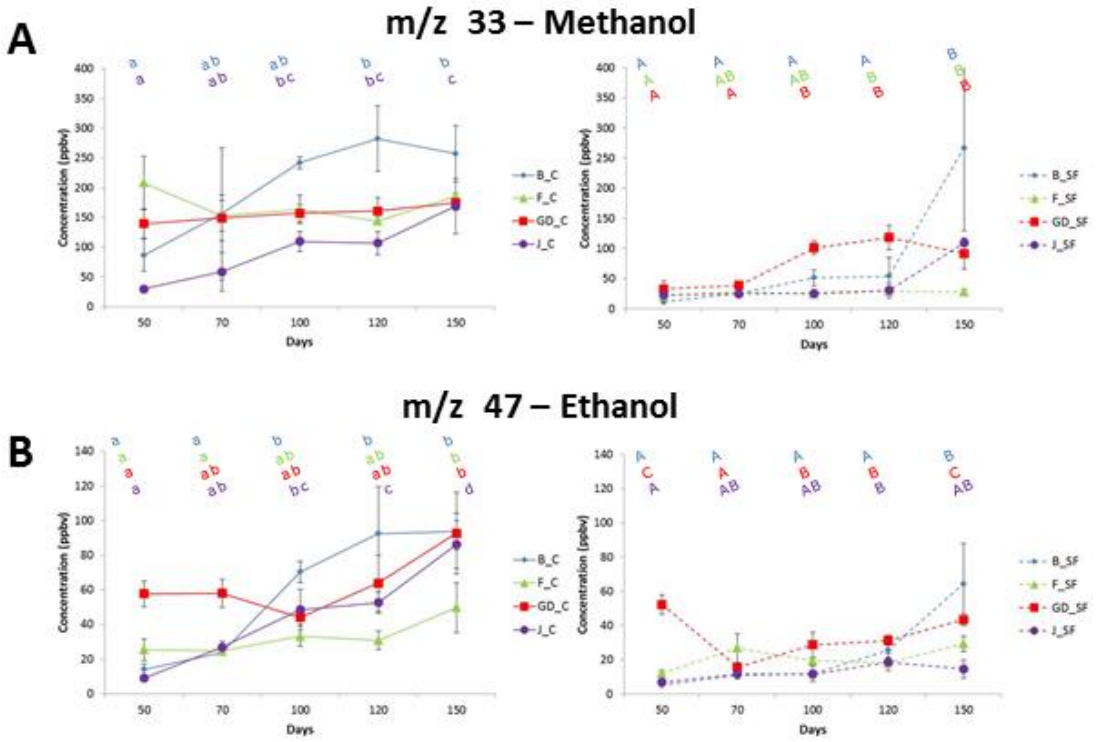


Figure 7.6: Selected alcohol related mass ions (mean ± SD) for untreated (left panel) and treated (right panel) apple cultivars. Where significant changes (P<0.05) between day occur, these are indicated by different letters and colour that correspond to a specific cultivar.

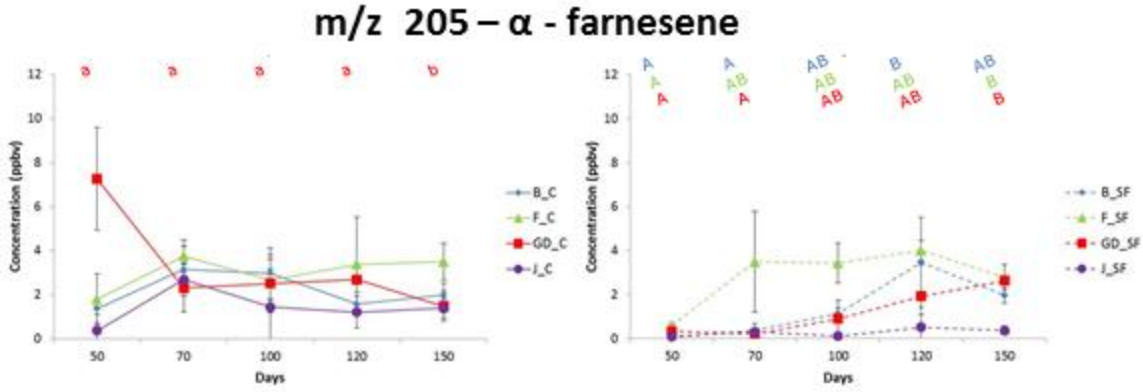


Figure 7.7: a-farnesene (m/z 205) (mean ± SD) for untreated (left panel) and treated (right panel) apple cultivars. Where significant changes (P<0.05) between day occur, these are indicated by different letters and colour that correspond to a specific cultivar.

7.3.2 Texture, physico-chemical and µ-CT results

The data obtained from texture, physico-chemical and morphological properties was subjected to a Pearson's correlation. Here only days that coincided with µ-CT scanning (Day 50, 100, 150) were considered (Table 7.3). Comparing the texture and µ-CT data, porosity was negatively correlated to Fmax (r = -0.691), flesh firmness (r = -0.715), and AUC flesh (r = -0.811). Similar data has previously been discussed in Chapter 6 and the current findings are consistent with previous findings on the undesirability of apples containing a higher porosity due to them having a soft, mealy texture (Harker and Hallett, 1992; Allan-Wojtas and others, 2003). However, this trend may not be generally applicable to all cultivars as reported in Chapter 6 where Golden Delicious and Fuji cultivars were significantly different in texture despite having comparable porosity values. This apparent conflict will be addressed in the next section.

Table 7.3: Pearson's correlation coefficients calculated for physico-chemical, texture and µ-CT data. Highlighted boxes indicate a significant positive correlation (>0.6; a<0.05) (green) and a negative correlation (<-0.6; a<0.05) (red).

	DM	SSC	Fmax	Flesh.f	Gradient
DM	-				
SSC	0.789	-			
Fmax	0.244	0.132	-		
Flesh.f	0.116	0.029	0.900		
Gradient	-0.006	0.001	0.468	0.520	-
AUC.peel	0.316	0.297	0.189	0.014	-0.713
AUC.flesh	-0.123	-0.276	0.773	0.861	0.497
Porosity	0.005	0.187	-0.691	-0.715	-0.484
Anisotropy	-0.156	-0.382	0.435	0.468	0.284
Connectivity	-0.082	0.170	-0.319	-0.257	-0.066

	AUC.peel	AUC.flesh	Porosity	Anisotropy
DM				
SSC				
Fmax				
Flesh.f				
Grad				
AUC.peel	-			
AUC.flesh	-0.188	-		
Porosity	0.183	-0.811	-	
Anisotropy	-0.191	0.654	-0.619	-
Connectivity	-0.057	-0.356	0.493	-0.745

Although the textural differences of some cultivars with similar porosities can be explained by studying the IS size distribution (Chapter 6 Section 6.3.2), how the different sized IS relate to textural stability is still unclear. Additionally, whether large IS occurs as a result of the interconnecting of smaller IS, and how this relates to porosity is unclear. Therefore, to gain further information on the IS orientation and shape, anisotropy and connectivity studies were conducted. These two properties focus on the IS orientation and how much the IS is connected within the selected apple sample. Anisotropy defines the orientation of IS within a selected REV based on the mean intercept length (MIL) method obtained as scalar values between 0 (isotropic) – 1 (anisotropic) (Odgaard and Gundersen, 1993). To understand the concept of isotropy and anisotropy used here, a simple analogy is used. Consider a cube containing different types of solid materials. If this cube contains marbles of similar size, a line that passes through the cube at any orientation would make a similar number of intercepts. This cube can then be said to be isotropic. In relation to apple microstructure, this means the absence or presence of IS along a specific directional axis is symmetrical and could be associated with a few large IS within a selected microstructural REV representing the absence of structures along the directional axis. However, if the same cube contained a bunch of steel rods, the lines running parallel to these rods will encounter fewer boundaries compared to lines perpendicular to the rods. This increases the anisotropic nature of the IS in the cube. The anisotropic nature of the IS within a microstructure increases when a larger chance of intercept may occur. This is represented by smaller IS which are randomly distributed throughout the apple parenchyma. Therefore, a negative correlation between anisotropy and porosity ($p < 0.05$; $r = -0.619$) indicates that cultivars with larger porosity are less anisotropic. This could be associated with IS of larger size. A positive correlation between anisotropy and AUC flesh ($p < 0.05$, $r = 0.654$) suggests that apples which tended to be anisotropic were firmer. This indicates a possible influence of anisotropy on the mechanical properties of apple parenchyma. Connectivity related to the number of connected IS within the microstructure was negatively correlated with anisotropy ($p < 0.05$; $r = -0.745$) and positively correlated to porosity ($p < 0.05$; $r = 0.493$). This showed that apple parenchyma of high connectivity is more porous and less anisotropic in nature. Previously, the textural differences

between cultivars of similar porosities were described subjectively with the use of 2-D and 3-D images (Chapter 6). However, in this chapter a more objective approach with the combined use of anisotropy, connectivity and porosity measurements was used to understand the influence of microstructure on texture which will be elaborated on in Section 7.3.4.

Comparing the texture and µ-CT parameters to VOC release, only two correlations were found. Fmax was negatively ($p < 0.05$; mean $r = -0.65$) correlated to both acetaldehyde (m/z 45) and ethanol (m/z 47) concentrations. This means Fmax decreased at increasing concentrations of acetaldehyde and ethanol which are traits that occur during ripening (i.e. texture softening and increase in VOC) (Power and Chesnut, 1920; Knee and Hatfield, 1981; Mattheis and others, 1991; Bleecker and Kende, 2000; Dixon, 2000). DM was positively correlated to SSC ($p < 0.05$; $r = 0.789$) as SSC is the product of carbohydrate hydrolysis during ripening (McGlone and others, 2003).

7.3.3 Relation between porosity and flesh firmness

In Chapter 6, it was observed that cultivars (Golden Delicious and Fuji) of similar porosity may be significantly different in flesh firmness. However, these results were limited to one time point. This chapter serves to further understand the relationship between porosity and flesh firmness over long term postharvest storage and the effects of SF. A correlation plot on porosity and flesh firmness was used to visualise differences between cultivar and treatments. When focusing on individual cultivars (Figure 7.8), Golden Delicious was clustered away from the other cultivars because it was the softest and most porous cultivar. Jazz, Fuji and Braeburn cultivars were grouped in the lower right hand corner. This indicates Jazz, Fuji and Braeburn were firmer and less porous cultivars compared to Golden Delicious. In order to investigate the effects of time and treatment on porosity and flesh firmness, each cultivar was observed separately. Here, each point represents the individual replicate measured at that time point (Figure 7.9 - 7.12).

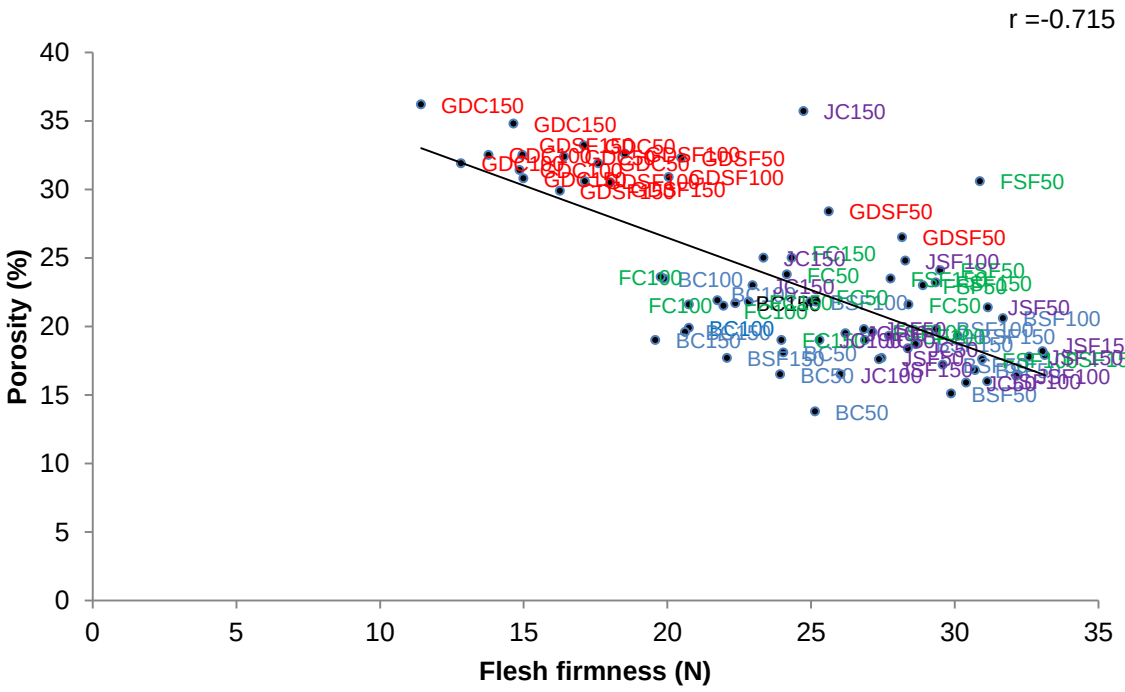


Figure 7.8: Correlation plot of flesh firmness and porosity of all cultivars, days and treatments. Cultivars are separated based on colour where Golden Delicious (GD): Red; Jazz (J): Purple; Fuji (F): Green; and Braeburn (B): Blue.

Regardless of treatment, Golden Delicious showed a negative correlation ($p < 0.05$; $r = -0.774$) between flesh firmness and porosity (Figure 7.9). Generally, the increase in porosity is associated with a decrease in firmness (Chapter 6) and this could be attributed to the presence of large IS (Harker and Hallett, 1992). However, a smaller correlation ($p < 0.05$; $r = -0.452$) was observed for the untreated Golden Delicious. The correlation between SF treated apples increased ($r = -0.779$). This is because SF maintained the flesh firmness of Golden Delicious up until day 50. Without re-application of SF, the flesh firmness dramatically deteriorated and start to follow the trend for the untreated samples (Blankenship and Dole, 2003).

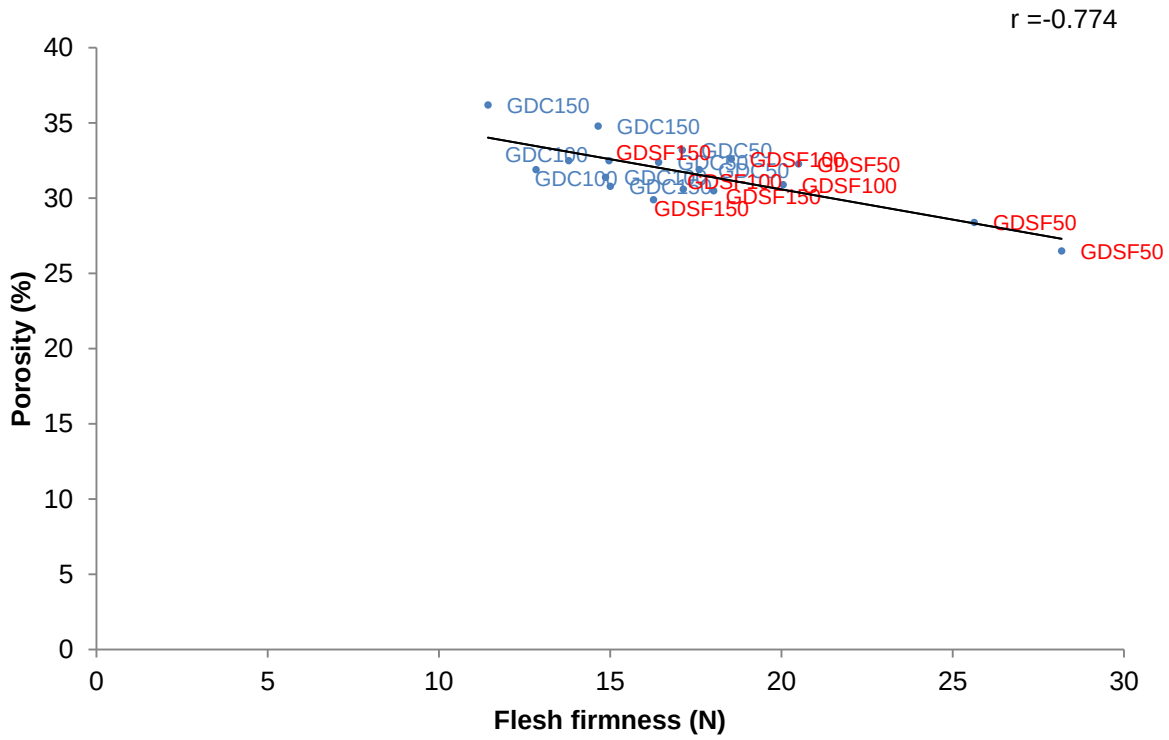


Figure 7.9: Correlation plot of flesh firmness against porosity for Golden Delicious based on day and treatment. SF treated (Red) and untreated (Blue) samples are separated based on colour.

A separation between the SF treated and untreated Braeburn samples were observed although the overall correlation between flesh firmness and porosity was less obvious (r = -0.359) (Figure 7.10). The correlation increased (r = -0.725) only when data points from untreated samples were plotted. This followed the pattern of increasing porosity as the flesh firmness decreases over time. When compared to untreated samples, a low correlation (r=-0.143) for SF treated samples suggests that the application of SF has maintained the apple texture despite an increase in porosity over time.

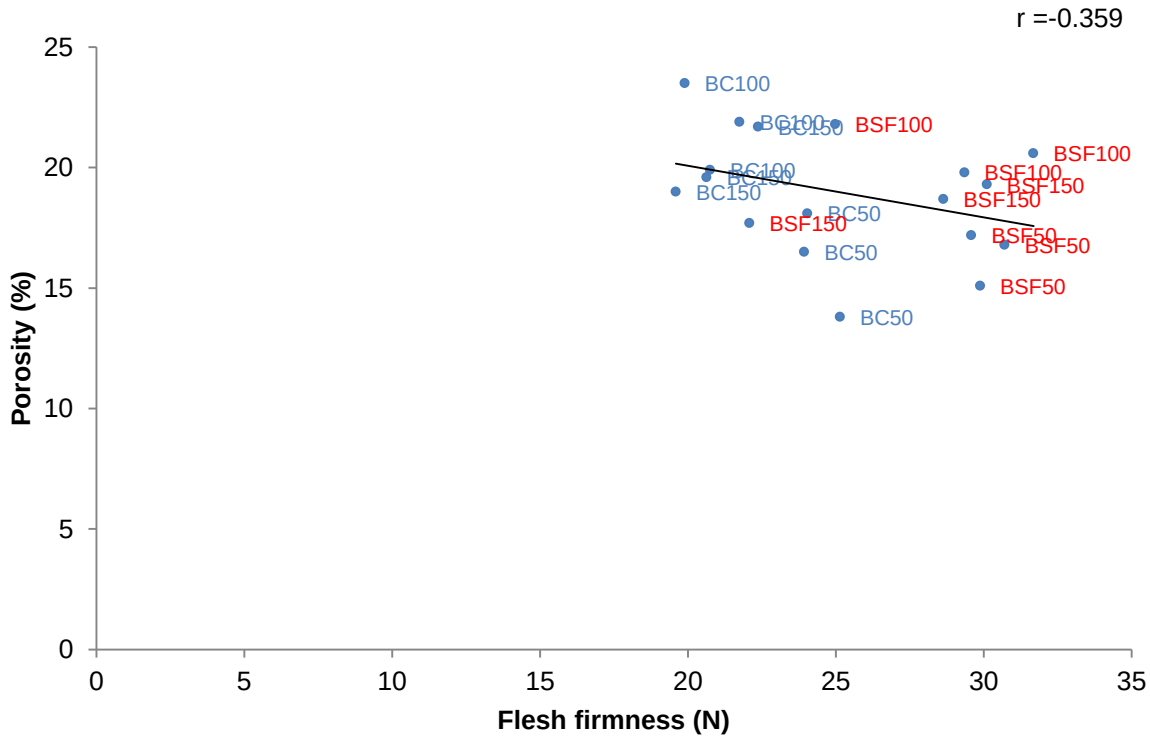


Figure 7.10: Correlation plot of flesh firmness against porosity of the Braeburn cultivar based on day and treatment. SF treated (Red) and untreated (Blue) samples are separated based on colour.

Similar to that of Braeburn, SF treated and control Jazz cultivar samples were separated based on their porosity and flesh firmness correlation (Figure 7.11). SF treated samples maintained flesh firmness better than the untreated samples with a small r = -0.313. In the untreated samples however, a slightly stronger negative correlation between porosity and flesh firmness (r = -0.563) was observed. Jazz cultivars, also known as Scifresh, are good keeping cultivars that maintain a firm texture throughout a long term postharvest storage (White, 2002). Therefore, the last time point at 150 days may not be long enough to observe a significant deterioration in the IS of the Jazz apples.

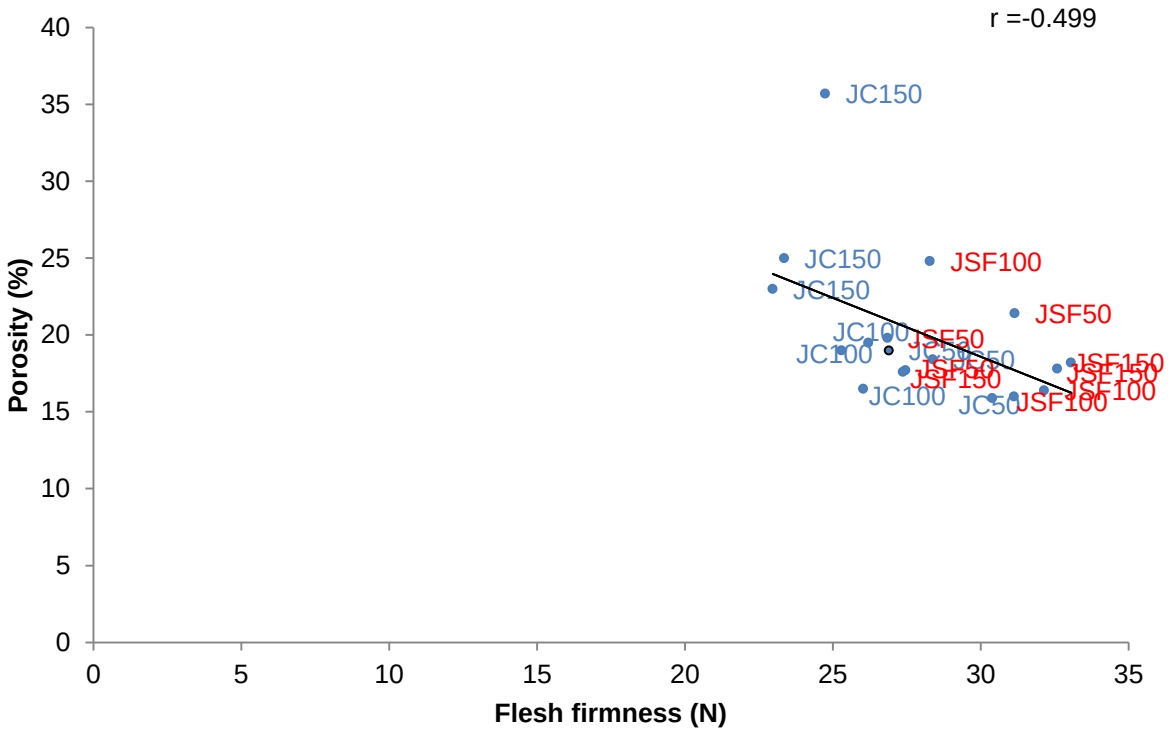


Figure 7.11: Correlation plot of flesh firmness against porosity of Jazz cultivar based on day and treatment. SF treated (Red) and untreated (Blue) samples are separated based on colour.

Compared to the other cultivars, Fuji had no correlation between porosity and flesh firmness. Although the SF treated samples were firmer than the untreated Fuji apples, all samples were comparable in porosity (Figure 7.12). This has been observed in the Chapter 6 and was due to the difference in IS size distribution. High values of porosity within the Fuji cultivar were due to the summation of numerous smaller IS.

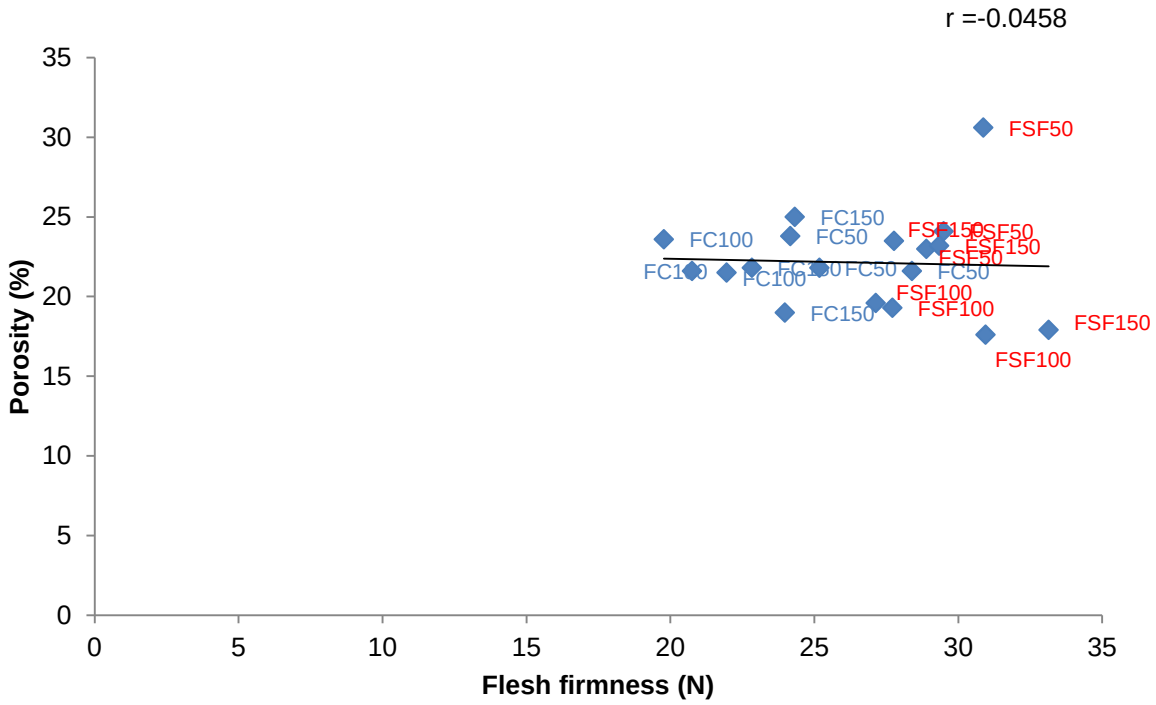


Figure 7.12: Correlation plot of flesh firmness against porosity of Fuji cultivar based on day and treatment. SF treated (Red) and untreated (Blue) samples are separated based on colour.

7.3.4 The relationship between connectivity and anisotropy to porosity.

Apart from IS size distribution, other factors may influence the mechanical properties of the parenchyma. This section will elaborate on the relationship between porosity with regards to the anisotropy and connectivity of the Fuji cultivar. Figure 7.12 shows that although the Fuji samples were of different firmness at different time points; there was little change in porosity. This indicated that there was a change in the IS within the microstructure despite the values remaining constant as demonstrated in Section 6.3.1 (Herremans and others, 2013a). In this section, the correlation between anisotropy and porosity has been plotted to illustrate microstructural differences with regards to anisotropy in the Fuji cultivar despite porosity remaining constant (Figure 7.13). As previously mentioned, anisotropy is based on the MIL defined as the orientation of IS distributed within a selected apple region. Figure 7.13 shows samples at day 50 were more anisotropic regardless of treatment and became more isotropic as the Fuji cultivar matured. This indicates the distribution of the IS of Fuji apples at day 50 were more sporadic within the selected REV and possibly smaller despite similar porosity values. Thus increasing the

probability of the occurrence of intercepts between IS and the cell walls. However, as the fruit matures, the IS size increases as a result of cell walls collapsing thus connecting IS that were previously separated (Varela and others, 2007). This process shifts the IS characteristics to be more isotropic as less intercepts occur.

The calculation of anisotropy based on the MIL could indicate an increase in connectivity between IS. This is because as the IS become more isotropic, the MIL of all vectors are similar. This is illustrated in Figure 7.14 (right panel) where a more isotropic sample will form a more spherical vector cloud. In comparison, samples of higher anisotropy will tend to form an ellipsoid vector cloud indicative of the different vector lengths associated with a higher number of cell-IS intercepts (Odgaard and Gundersen, 1993; Doube and others, 2010). The correlation between anisotropy and connectivity for the Fuji cultivar was negative (r = -0.76) indicating the IS of anisotropic samples are less connected.

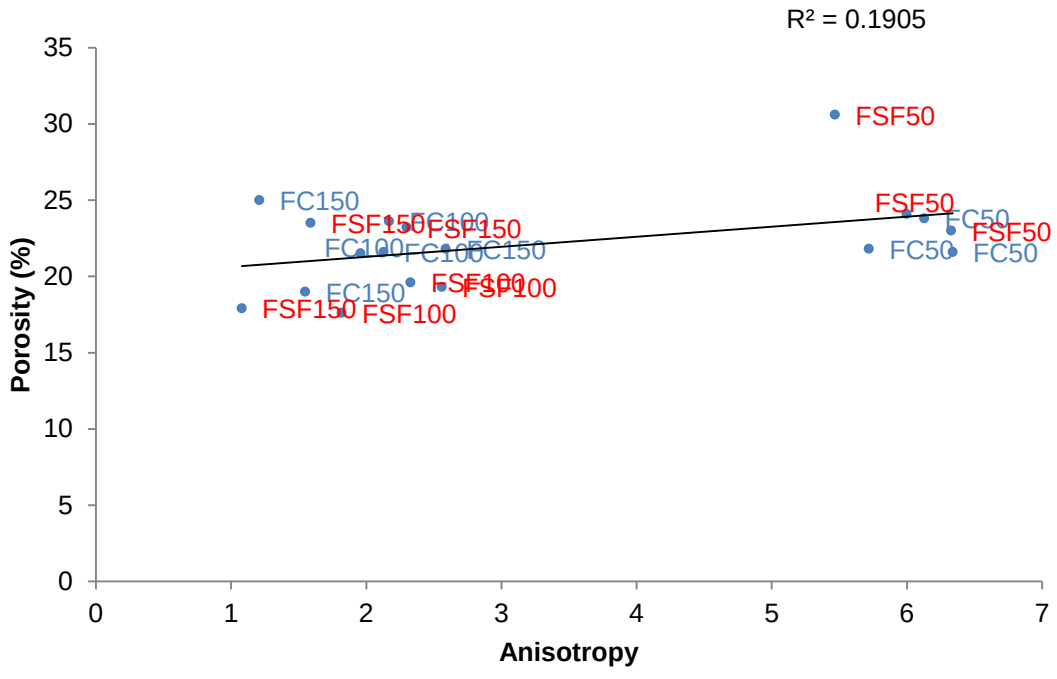
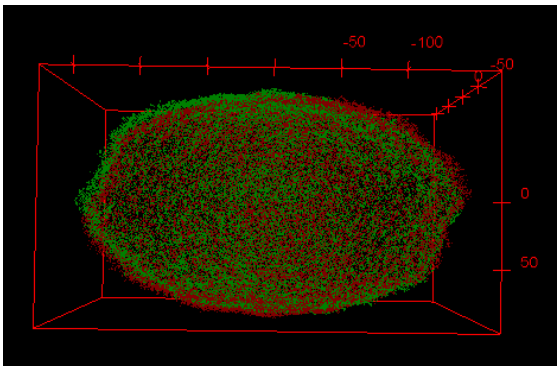
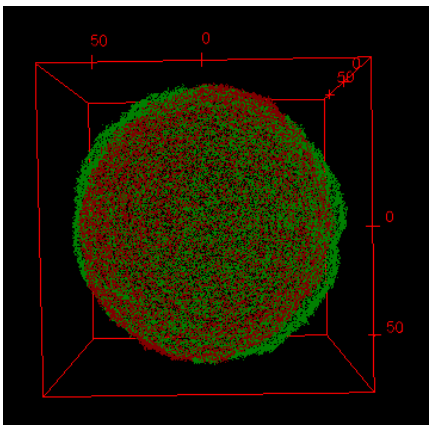


Figure 7.13: Correlation plot of anisotropy and porosity of Fuji cultivar based on day and treatment. SF treated (Red) and untreated (Blue) samples are separated based on colour.



F50C1: Anisotropy - 6.3; Porosity - 24.8%; Flesh firmness - 24.17



F150C3: Anisotropy 2.1; Porosity - 23.6%; Flesh firmness - 23.98 N

Figure 7.14: Graphs of a 3D cloud in which each point represents the vector multiplied by the MIL. Differences in anisotropy within Fuji samples despite the different days and comparable values of porosity and flesh firmness are represented by the different cloud shapes. Colours indicate the vector direction: negative (red) or positive (green).

7.3.5 Understanding the inter-relationships between VOC release, texture, physico-chemical, and morphological properties

To understand the interrelationships between all measured variables a PCA was carried out (Figure 7.15). A total of variance of 66.68% between PC1 and 2 was obtained. PC1 separated the samples based on VOCs and texture properties. Most of the samples highly associated with VOCs were the untreated (C) samples, with the SF treated samples being well associated with textural attributes such as AUC flesh, flesh firmness and Fmax of Braeburn and Jazz cultivars. Positive PC1 was dominated by untreated (C) samples with the exception of Jazz, Braeburn and Fuji cultivars at Day 50. SF treated cultivars were mainly on negative PC1 however, after prolonged storage (Day100 - 150), it appeared that SF treated samples moved towards positive PC1. This was observed for SF treated Braeburn and Fuji cultivars. Anisotropy was associated with textural properties and negatively associated with connectivity and porosity. This means that cultivars which were measured as anisotropic contained IS of irregular sizes and orientation. Porosity and connectivity was well associated with Golden Delicious cultivars, particularly with the untreated samples. Connectivity and porosity was negatively associated to anisotropy and AUC flesh. This indicates that cultivars that were porous contain interconnected IS, which, in turn, decreases the firmness of the cultivar. Although connectivity and porosity were situated on

positive PC1 which is associated with VOC abundance, whether or not these properties influence the release of VOCs is still unclear. This is because of the diversity from the cultivars selected for the experiment. For example, Braeburn cultivars were shown to contain high amounts of selected VOCs comparable to that of Golden Delicious cultivars however, Braeburn cultivars was not strongly associated with connectivity and porosity due to its firm texture. Hence, both connectivity and porosity may influence texture and VOC release. However, the latter is also strongly dependent on substrate availability that is specific to each cultivar for VOC formation (Sanz and others, 1997).

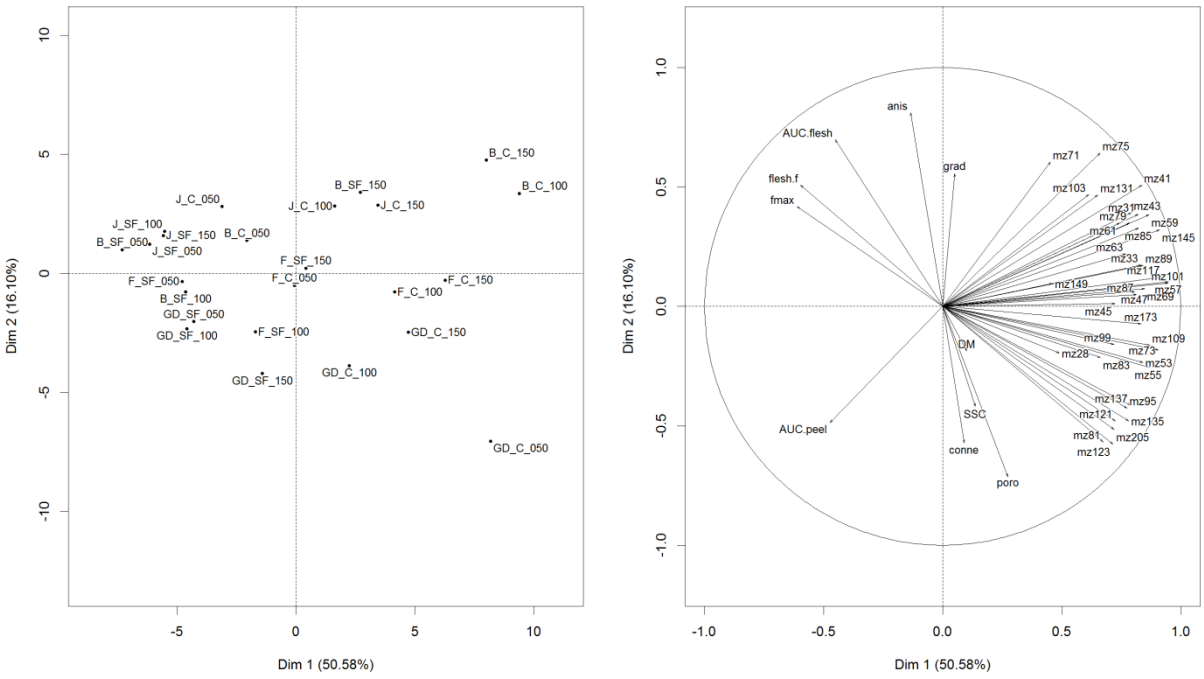


Figure 7.15: PCA of VOCs, texture, physico-chemical and µ-CT data based on the averaged triplicate data for each sample. Abbreviations: B- Braeburn; F- Fuji; GD- Golden Delicious; J- Jazz; SF-Smart Fresh; C- Control/untreated.

Focusing on PC2, cultivars were separated into two groups; Jazz - Braeburn and Golden Delicious - Fuji. Positive PC2 separated the cultivars based on VOC composition and texture. Jazz and Braeburn apples had a firm texture and were more associated to esters, ester related fragments and alcohol (ethanol and methanol) VOCs. Negative PC2 contained Golden Delicious and Fuji that were more associated with terpene related compounds, ethylene, SSC, porosity and connectivity.

7.3.6 MFA

To understand the differences between cultivars at different days based on their treatments two separate MFAs were performed (Figure 7.16). The MFA of the untreated samples explained a larger variance between the samples (79.85%) compared to the SF treated samples (72.45%). As mentioned in Chapter 5, an MFA is a quick way to visualize the behaviour of the samples at a given time point (Figure 7.16). Each dot represents a different time point (red=Day 50, green = Day 100, blue = Day 150). The black point plotted within the individual cultivar samples is the barycentre of all other points. This can be used to evaluate the similarities or differences of these cultivars at a glance. If the coloured dots for a cultivar are close together, this means that large changes were not observed for that cultivar during storage.

Untreated Golden Delicious and Fuji cultivars appeared to be distinctly different during long term postharvest storage. Both C50 and C150 were quite far apart and C100 was at an intermediate point. Braeburn apples showed a larger difference between C50 and C100. C150 however, was more similar to C50. For the Jazz cultivar, C50 was very different whereas C100 and C150 were closer together. This indicates the most change that occurs for the Jazz and Braeburn cultivar occur during day 50 - 100. All four untreated samples were well separated compared to the SF treated samples. The MFA of SF treated samples show Jazz and Braeburn cultivars more similar compared to the other cultivars. SF treatment was also very effective for the Jazz cultivar as no significant change occurred for all the three time points. In general day 50 and day 100 were more similar than day 150 for the SF treated samples.

To understand the agreement between the measured time points, a regression vector (Rv) was used. Here, a larger Rv value indicates that the samples are more similar whereas, a lower Rv value reflects larger differences between samples (Cadena and others, 2013). The Rv coefficient for the untreated samples between days 50 and 100 was 0.73. The coefficient increased for days 100 and 150 (Rv = 0.89). This shows that, regardless of cultivar, significant changes occurred between days 50 and 100 for untreated samples, while changes between 100 to 150 days were less

pronounced. For the SF treated samples, little difference was found between SF50 and SF100 where the Rv coefficient was 0.93. This indicates that changes occurring within that time frame were marginal. However, the Rv coefficient decreased between SF100 and SF150 (Rv = 0.69). This suggests that a larger amount of change occurred during this time which is consistent with the VOC and texture data throughout this study. When comparing both SF and untreated samples, both were similar at day 50 (Rv = 0.81). Based on different time points, C100 was similar to SF150 (Rv = 0.95). This shows that SF treatment can lengthen the shelf life of samples by at least 6 weeks.

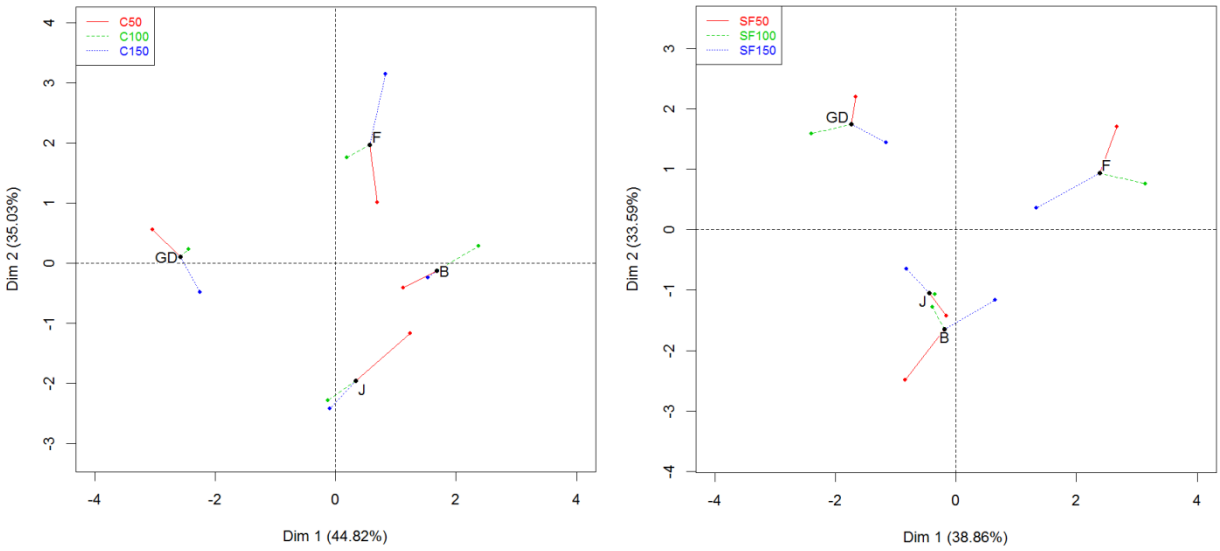


Figure 7.16: Two separate MFAs performed on all collected data to depict individual cultivar differences based on days (50, 100, 150), and compared between untreated (C) and treated (SF) samples.

SF treatment influences the production of VOC and texture retention in apples. The use of SF predominantly suppressed ethylene and ester related VOCs. Generally, SF treated Braeburn, Golden Delicious and Jazz cultivars produced less VOCs compared to the untreated samples at each time point. A minimal difference in total VOC concentration was found between SF treated and the untreated Fuji samples. This suggests that the efficacy of SF is cultivar dependent and best suited for cultivars of high IEC (Golden Delicious). Porosity was negatively correlated ($r = -0.715$) to flesh firmness indicating the influence of microstructure on texture. An inverse relationship between flesh firmness and porosity was observed for untreated Braeburn and Jazz cultivars. This relationship was less pronounced when comparing the SF treated samples. SF treated Golden Delicious samples retained flesh firmness for up to 50 days, after which the texture softened and the porosity increased significantly. This suggests that reapplication of SF is necessary to maintain the texture of Golden Delicious during storage. The Fuji cultivar contained similar porosities for both SF treated and untreated samples even though the former was firmer. This was due to the morphological differences in IS that were characterised as lower in anisotropy and higher in connectivity. This indicates that interconnectivity and orientation of IS can influence texture even if porosity is similar. Porosity and connectivity was associated with the emission of VOCs, demonstrating that specific morphological parameters could be used further to understand microstructural effects on VOC release.

Overall, the largest change in measured attributes for the untreated samples occurred from day 50 and 100 compared to the SF treated samples, which showed the greatest change from day 100 to 150 of storage. The efficacy of SF decreases during prolonged storage suggesting the need for reapplication to maintain the quality attributes of specific cultivars. Nevertheless, SF treated samples at day 150 was comparable to untreated samples at day 100. This underscores the efficacy of SF treatment in retaining apple quality for at least 6 weeks for some cultivars.

Chapter 8: General discussion and future work recommendations

8.1 General discussion

Texture (mouth feel) and aroma (VOCs) are two essential quality traits, which consumers desire in apple cultivars. Although consumers are able to express their texture preferences, they frequently find it difficult to describe their perception of apple aroma. This difficulty is in part owing to the fact that apple aroma consists of more than 300 VOCs (Dixon, 2000). Since a combination of complex sensory interactions between taste, olfaction and trigeminal stimulation shape the overall perception of flavour, both texture and aroma are important drivers of apple quality. In addition, during consumption, the perception of VOCs from apples is influenced by their release which in turn is associated with the mechanical breakdown of apple flesh, underscoring the importance of cross modal interactions between aroma and texture. Therefore, the main objective of this thesis was to understand cultivar-specific differences in texture and aroma, as well as deciphering the interrelationships which contribute to the overall perception of flavour.

Due to the differences in geographical location (New Zealand and Italy) during the experimentation, similarities in cultivar types measured for each working chapter were limited. Therefore, each working chapter within this thesis approached the main objective by characterising each cultivar using its VOC profile, texture, morphological properties (only Chapter 6 - 7) and other standard physico-chemical measurements. The relationship between texture breakdown and VOC release during consumption was studied using either *in vivo* (Chapter 3 – 4) or sensory (Chapter 5) measurements. By individually characterising each cultivar, some cultivars sharing similar characteristics could be grouped and assumptions on flavour release made for cultivars not measured throughout Chapters 3 - 5. The current chapter highlights the important findings of the thesis based on the proposed objectives and then based on the results (generated in the thesis) separates the cultivars into three distinct groups and discuss each group's characteristics in relation to eating quality.

In vivo nosespace measurements have been proposed as a rapid technique to use to study the relationship between texture and flavour without the need for expensive trained descriptive sensory panels. Therefore, the first objective of this thesis was to investigate the feasibility of *in vivo* nosespace and *in vitro* headspace VOC measurements using PTR-MS and to relate the findings to standard evaluations such as textural and physico-chemical properties (Chapter 3 - 4).

In a preliminary study carried out in Chapter 3, PTR-QUAD-MS differentiated the cultivars based on their *in vitro* and *in vivo* VOC profiles. A large difference in VOC abundance and composition was observed between the intact and cut fruit, owing to the increased surface area of the cut fruit allowing for oxidative reactions to occur which created secondary VOCs (Chapter 2). A particularly large increase in acetaldehyde concentrations in the cut fruit could be due to the fruit's response to the mechanical injury (cutting) in addition to the increased surface area (Kimmerer and Kozlowski, 1982). Fewer terpene compounds were found in the cut fruit as they predominantly occur in the apple peel (Guadagni and others, 1971). Additionally, the incubation time used for the *in vitro* cut fruits enabled terpene compounds to reach equilibrium within the headspace which was not possible within the short *in vivo* consumption times. Although the *in vitro* headspace VOCs differentiated the cultivars, the relative differences between the cultivars were not similar for the *in vitro* and *in vivo* measurements for four selected VOCs (m/z 45: acetaldehyde; m/z 47: ethanol; m/z 43, 61: esters/acetate related). This difference may have been due to variability between panellists or due to the absorption of VOCs within the oral cavity (Buettner and Schieberle, 2000). Nevertheless, the relative VOC release profiles between each cultivar were comparable for all panellists (Figure 3.1), suggesting that a similar pattern in flavour release was observed for each panellist. Together, these results demonstrated that VOC emission was influenced by the mechanical breakdown of the apple flesh, and that PTR-MS was a suitable technique for studying interactions between the flavour release and texture.

Further examination of the *in vivo* nosespace data showed its potential to be used to understand differences in the mechanical breakdown of apple flesh. In particular, T_{swal} enabled the differentiation between juicy and mealy apples with a

longer T_{swal} observed for mealy Golden Delicious cultivars compared to juicier Fuji cultivars (Table 3.2). Further, T_{con} could be used to indicate the firmness of a sample. More specifically, firmer samples displayed a longer T_{con}, while softer samples were consumed faster giving them a shorter T_{con}. Finally, T_{max}, although not significantly different between cultivars the trend was for softer cultivars to be lower owing to a faster mechanical breakdown within the oral cavity. In a similar result, van Ruth and others (2004) while also not finding a significant difference in T_{max} reported that T_{max} generally increased at higher mastication rates.

The preliminary study on the *in vivo* and *in vitro* flavour release of six commercially available cultivars (Chapter 3) were promising and formed the basis for an expanded study on a larger set of apples (n = 21) in Chapter 4. Here, a PTR-ToF-MS was used to improve data acquisition and m/z temporal resolution. Cultivars were differentiated into different VOC emitting groups of: A) high in ester-related compounds and low in terpene related VOCs; B) high emitters of only terpene compounds; C) high terpenes and ester VOCs and D) low VOC concentration overall but high in alcohols, and butanoate esters. This data illustrated the diversity of apple aroma as it is influenced by the same VOCs which are present in different concentrations (Dixon, 2000; Aprea and others, 2012; Farneti and others, 2014).

Mechanical and acoustic texture measurements, which displayed strong correlations to sensory crunchiness in previous studies, were also taken (Costa and others, 2011; Corollaro and others, 2013). Soft and firm cultivars were differentiated based on their differences in mechanical firmness. Additionally, firm cultivars were further separated based on their acoustic properties. Initially, all firm cultivars were assumed to be crunchy, however, the results from Chapter 4 implied crunchy apples were firm but firm apples were not necessarily crunchy similar to results reported by Costa and others (2011). Acoustic properties indicative of crunchiness were also correlated to juiciness suggesting the influence of cell wall turgidity in the microstructure where sound is generated. For example during the mastication of crunchy apples, the rupturing of turgid cell walls generates a sound pressure wave which results in the perception of crunchiness (Duizer, 2001). In contrast, soft apples

contain cell walls of low turgidity, which results in flaccid cells that peel apart instead of rupturing. The data also suggested that juiciness is only perceived in crunchy cultivars.

The use of PTR-ToF-MS improved data acquisition by increasing the number of VOCs detected by *in vivo* nosespace by a factor of four compared to the number detected using the PTR-QUAD-MS (Chapter 3). The VOCs were mainly ester related compounds, followed by estragole, hexanal and acetophenone. Again the *in vivo* nosespace parameters did not fully reflect the *in vitro* classification of specific cultivars. However, cultivars were grouped similarly based on their ester related VOCs emission. As expected, cultivars that had high concentrations of terpene compounds displayed low concentrations for all other *in vivo* nosespace VOCs similar to its *in vitro* VOC profile. Nosespace parameters such as T_{con}, T_{swal} and T_{max} followed similar trends to that seen in the preliminary PTR-QUAD-MS study (Chapter 3) and were useful in understanding textural differences between cultivars. T_{con} was longest for firm samples and well associated with high intensities of textural properties. This finding was similar to reported for an earlier study by Sprunt and others (2002), who discovered that firmer gel samples had a longer time to last swallow. T_{swal} was the longest for a soft (Topaz) and firm (FEM11) cultivar. Previously it was mentioned that T_{swal} could be used as an indicator for juiciness. However, the long T_{swal} observed for FEM11 was inconsistent with its classification through instrumental measurements as a juicy cultivar. This difference could be because the instrumental measurement did not adequately reflect the mechanical breakdown of the apple flesh within the oral cavity. T_{max}, which was previously speculated to be shorter for soft cultivars, was shorter for firmer cultivars with short T_{swal}. This could be due to earlier or induced swallowing from the juice expressed from these samples. This observation indicates that juicy cultivars which contained high ester VOCs could be perceived as more flavourful. Similar results for shorter T_{max} have been reported post swallow for carrot pieces (Frank and others, 2012). The results from this study suggest juiciness could heighten the rate of flavour perception as it induces more swallows that decrease T_{max}.

The two studies on *in vivo* and *in vitro* flavour release utilizing both variants of PTR-MS instruments (Chapter 3 and 4) have shown that PTR-MS is a powerful technique that can be used for *in vivo* nosespace studies. However, the release of VOCs into the nasal cavity was not solely dependent on the characteristic VOC composition or the texture of the cultivar. Rather the presence of available juice during mastication was also shown to have an effect on T_{max}. As the instrumental measurement of juiciness may not adequately reflect the perceived sensory juiciness for some cultivars, it would be beneficial to investigate the difference between instrumental and sensory perceived juiciness. Chapter 5 investigated the comparison of analytical and sensory techniques in characterising apple cultivars. This was also carried out in order to gauge the feasibility of using solely analytical measurements to characterise apple cultivars.

The second objective of this thesis was to compare the characterisation of apple cultivars using analytical and sensory approaches (Chapter 5). Instrumental analyses were carried out using two powerful techniques, PTR-ToF-MS and the simultaneous measurements of mechanical and acoustic textural properties. These techniques were chosen to reflect the sensory modalities of odour/flavour and texture. The findings from these trials showed that selected instrumental properties reflected specific sensory attributes and showed a similar cultivar separation (Figure 5.2). This indicates that solely instrumental classification of apple cultivars may be feasible when the sensory approach is not an option. Instrumental texture properties were well correlated with sensory texture attributes. Also, the instrumental textural properties provided additional information by enabling the differentiation of cultivars that were hard but not necessarily crunchy (Table 5.4). Specific sensory flavour and odour attributes such as lemon were well correlated to butanoate esters. Whereas pear flavour was well associated with short chain ester related VOCs and hexyl acetate (m/z 145.122), which was also the sensory chemical standard for pear odour (Table 5.1). Similar to earlier results from the *in vivo* and *in vitro* studies (Chapters 3 – 4), some cultivars were separated due to their abundance of terpene related VOCs but these were not associated with any specific sensory attribute. Titratable acidity was well correlated to sour taste and could potentially be used in prediction models. Although sweet taste was negatively correlated to titratable

acidity and sour taste, it was not correlated to SSC. Nonetheless, a positive correlation between sweet taste and fruity esters suggests a taste-aroma interaction.

Findings on flavour release in apples using *in vivo, in vitro* and sensory techniques enabled the interrelationships between texture and VOC during consumption to be investigated (Chapters 3 – 5). In general, there was a relationship between texture and VOC, whereby firm and juicy cultivars could be perceived as being more flavourful (as indicated by their high I_{max} and AUC values) due to a shorter T_{max} compared to softer cultivars. However, this appears to be true only for cultivars that initially contained a high amount of ester related VOCs.

When predicting sensory attributes, a careful selection of specific instrumental variables (such as titratable acidity and sour taste) could enable the characterization of cultivars using solely instrumental techniques. Although a texture-VOC interaction was not a focus in the sensory studies (Chapter 5), a taste-aroma interaction was observed between sweet taste and fruity esters. In the *in vivo*, *in vitro* and sensory studies on flavour release (Chapter 3 – 5), the characteristic mechanical breakdown during mastication was observed to influence texture and flavour perception. Therefore, the visualization of apple microstructure and its effects on texture was investigated as the final objective of this thesis.

A relatively new technique, known as the X-Ray µ-CT imaging, was used to visualize the microstructure of apple parenchyma (Chapter 6 and 7). This is a powerful technique that can visualise apple samples in their native state, enabling the reconstruction of 3-D images of apple microstructure. Previous studies have focused on understanding apple texture through dissecting the anisotropic nature of apple flesh with the use of a light microscope (Khan and Vincent, 1990; 1993). More recently, 3-D modelling has been used to understand the occurrence of BBD and gas transport systems in apples (Ho and others, 2009; Herremans and others, 2013a; Herremans and others, 2013b; Warning and others, 2014). However, these studies did not explain variations in texture nor the differential release of VOCs based on differences in microstructures.

Chapter 6 pioneered the investigation on the visualization of apple microstructure using μ-CT imaging and its relation to the mechanical textural properties of apples. This preliminary study focused on the influence of apple microstructure on texture using apples stored for 100 days. It was found that porosity was related to flesh firmness, with firmer cultivars being less porous. However, in some cases porosity could not be used as an indicator of texture properties, as porosity is solely the percentage of IS against the total measured volume and does not reflect the size or distribution of IS within the sample. In this study, it was observed that cultivars such as Golden Delicious and Fuji, although similar in porosity, were significantly different in texture (Table 6.1). This appeared to be due to the morphological differences in IS size and distribution. Overall, this investigation concluded that apple texture was influenced by not just porosity but also the different morphological parameters of size and distribution (Figure 6.3 and 6.6). However, to fully understand this phenomenon, it was concluded it would be beneficial to monitor the changes in IS and texture during long term storage. This could be coupled with the use of postharvest treatments such as cold storage and the use of an ethylene inhibitor, 1-MCP to understand the impact of industrial postharvest regimes.

The last chapter (Chapter 7) investigated the effects of cold storage and 1-MCP on the VOC release, texture and microstructure of apples during long term storage (up to 150 days). 1-MCP is commonly used to inhibit ethylene induced processes. This treatment enabled texture to be maintained for longer but often results in apples emitting fewer VOCs, as ethylene is needed in the final stages of ester formation in VOC biosynthesis (Schaffer and others, 2007). Not surprisingly, 1-MCP was found in this study to suppress the emission of ethylene (Figure 7.3). As a result, suppression of ester related compounds was also observed. However, the efficacy of 1-MCP in suppressing the emission of these compounds was cultivar dependent (Figure 7.2). Therefore, 1-MCP treatment may only be useful for cultivars containing high amounts of ethylene (Costa and others, 2005; Barry and Giovannoni, 2007). Fuji cultivars which initially contained fewer total VOCs and less ethylene compared to Golden Delicious, Braeburn and Jazz cultivars was much less affected by 1-MCP application.

Flesh firmness was negatively correlated to porosity and was found to change noticeably in the untreated samples over time (Figure 7.8). Within the experimental time frame, most of the 1-MCP treated samples were less variable in flesh firmness, indicating that a firm texture was better maintained. However, the 1-MCP treatment for Golden Delicious was only able to maintain a firm texture for up to 50 days, after which it rapidly declined (Figure 7.9). Therefore, reapplication of 1-MCP would be useful in maintaining the texture of specific cultivars (Watkins, 2006). Fuji apples displayed similar porosities for both treated and untreated samples, although slight differences in flesh firmness were observed between the two treatment groups. This was due to differences in the connectivity and anisotropy of the Fuji cultivar's microstructure. These two morphological properties were also shown to influence not just texture, but also VOC release. Porosity and connectivity were found to be associated with higher VOC emissions, whereas anisotropy was related to higher values of textural properties. These observations highlight the influence of apple microstructure on both texture and VOC release (Figure 7.15). Lastly, the use of ethylene inhibitors decreased the rate of texture and VOC change for up to six weeks in specific cultivars. Understanding the requirements of cultivar-specific 1-MCP reapplications could be for use in the apple industry. For example, Golden Delicious could benefit from reapplications of 1-MCP to maintain texture; whereas other cultivars, such as Fuji which retain firmness, will be less dependent on 1-MCP treatment.

The main objective of the thesis was to understand the interrelationships between texture and aroma which contribute to the overall perception of flavour. As previously discussed, it is clear both texture and aroma are cultivar-specific. To better understand these differences, cultivars of similar VOC and texture profiles are separated into three distinct groups and discussed accordingly in relation to their eating quality and potential uses.The first group of cultivars were characterised as being firm and crunchy with a low VOC release during consumption. Examples of these cultivars were Fuji (Chapter 3, 7), Granny Smith (Chapter 3) and Pilot (Chapter 4). Based on VOC composition, these cultivars contained low concentrations of ethylene which, in high amounts leads to soft texture and increased VOC production during long term storage (Chapter 2, 7). The characteristics of low VOC

concentrations and firm texture in these cultivars could be due to a low IEC. This was shown in Chapter 7 where the efficacy of 1-MCP as an ethylene inhibitor was not as pronounced on Fuji cultivars compared to other high IEC cultivars such as Golden Delicious (Figure 7.2).

VOCs detected from both the *in vitro* and i*n vivo* measurements on these cultivars were similar. Compared to other cultivars tested, Fuji, Granny Smith and Pilot were lower in maximum intensity (I_{max}) values for the detected *in vivo* VOCs. To understand the rate of flavour release, time to I_{max} was measured. It was initially speculated T_{max} was longer for firmer samples as panellists took longer to breakdown the sample (Table 3.4). However, to help differentiate firm from firm –juicy cultivars, two other parameters were assessed to help to understand textural breakdown during consumption. Here, a shorter time to first swallow (T_{swal}) was indicative of the juiciness perceived in the cultivar. Whereas a longer time for consumption (T_{con}) indicated that the sample was firm and hard needing more time for the panellist to consume. Fuji and Granny Smith cultivars both had a shorter T_{swal} and a longer T_{con} (Table 3.4). Although Pilot had a long T_{con}, it was also longer for T_{swal} which suggests Pilot was perceived as being less juicy compared to Fuji or Granny Smith.

T_{con} for these cultivars could be associated with its microstructure. Though it was previously established porosity is inversely proportional to flesh firmness (Harker and Hallett, 1992), this was not the case for Fuji cultivars (Section 6.3.2). The porosity of Fuji was a summation of numerous small IS compared to softer Golden Delicious which contained large interconnected IS (Figure 6.7 and 7.15). Further investigations showed that the microstructure of Fuji cultivars was more anisotropic in nature which was proportional to AUC flesh (Figure 7.15). Thus, assuming the microstructure of Fuji cultivars in Chapter 3 and 7 were similar; it appears that IS size distribution and orientation can affect textural breakdown during consumption. With regards to general consumption, these are cultivars consumers choose for their texture and juicy mouthfeel where VOC content or flavour is of lesser importance. In addition, due to its low IEC and long storage capabilities, cultivars of similar characteristics to Fuji should be considered as export commodities in the global trade.

The second group of cultivars were classified as soft and mealy apples producing intermediate amounts of VOCs. Golden Delicious (Chapter 3 – 7) and Morgen Dallago (Chapter 3) have been characterised as soft cultivars associated with ethylene, ester and terpene related VOCs. Other soft cultivars such as Topaz, Renetta Bianca (Chapter 4 – 5), Renetta Grigia and Rubens (Chapter 4) were associated to butanoate esters and alcohol related VOCs.

Focusing on instrumental texture data, the above mentioned cultivars were lower in all acoustic and force-displacement parameters (Chapter 3 – 5). An interesting parameter that could be used to dictate the behaviour of flesh rupture upon compression is force ratio. Here, all cultivars had positive values for force ratio which is indicative of a decreased flesh resistance upon probe penetration. This can be used to reflect the breakdown of apple flesh during mastication (Section 4.3.2) and has been associated to grainy and floury sensory attributes (Table 6.2). The behaviour of textural breakdown for cultivars of positive force ratio values was reflected in the *in vivo* results in which soft, mealy cultivars had a longer T_{swal} but shorter T_{con} (Table 3.4 and Table 4.3). Aprea and others (2006a) also found T_{swal} to be longer in thicker custards. Although apples and custard are different matrices, the concept of sample breakdown being required prior to obtaining a bolus for swallowing applies in both studies. The longer T_{swal} was because these cultivars were not juicy enough to induce swallowing before bolus formation. However, after the bolus is formed, a larger amount of sample is swallowed because it is soft and does not require further breakdown (Chapter 2).

The preliminary investigation of *in vivo* and *in vitro* flavour release of six commercially available cultivars proposed T_{max} to be lower in softer cultivars due to a faster mechanical breakdown within the oral cavity (Chapter 3). For these cases, the follow-up study using 21 different cultivars showed Topaz, Renetta Bianca, Renetta Grigia and Rubens (Chapter 4) were discriminated from other cultivars based on their high I_{max} values for butanoate esters and shorter respective T_{max} values. Golden Delicious also showed similar trends with ester related compounds (Table 3.3b and Figure 4.1). In order to understand if differences in microstructure can induce faster mechanical breakdown due to soft texture, μ-CT scanning on the Golden Delicious

cultivar was carried out (Chapter 6 – 7). Although it was previously mentioned porosity alone is not indicative of flesh firmness, in the case of Golden Delicious, porosity was inversely proportional to flesh firmness. This was due to the occurrence of large IS that were highly interconnected. These parameters have been associated with soft texture and increased VOC release (Figure 6.7 and Figure 7.15). This group of cultivars may be more appreciated for its flavour profile rather than its texture as cultivars such as Golden Delicious have shown significant texture softening during long term storage. Rather than marketing these cultivars as fresh eating fruits of inferior texture, these cultivars could be more suited as main ingredients of processed apple products. Apple sauce, apple butter, pies or strudels are some examples in which soft apple texture is acceptable or desired.

The last group of cultivars were characterised as being firm in texture with high VOC emissions. These firm cultivars were attributed into three different VOC composition groups. First, cultivars that emitted high concentrations of ester related compounds (Red Delicious, Jonagold (Chapter 3), FEM4, FEM11, Pinova (Chapter 4) and Kanzi (Chapter 5)); second, cultivars associated with terpenes and aromatic hydrocarbon related VOCs (Dalinette, FEM5, FEM10 and FEM2 (Chapter 4)); and lastly, cultivars that contained high concentrations of both ester and terpene related VOCs (Braeburn (Chapter 4, 7), Fuji Kiku 8, FEM7 and FEM3 (Chapter 4)).

In general, these firm cultivars had a shorter T_{swal}, a longer T_{con} and required more swallows (N_{swal}) to consume. T_{max}, which was previously speculated to be shorter for soft cultivars, was shorter for firmer cultivars with a short T_{swal} and high I_{max} values. This could be due to an earlier or an induced swallow from the juices expressed from these samples during mastication. This observation indicated that juicy cultivars which contained more ester VOCs could also be perceived as being more flavourful. Similar results for shorter T_{max} were found post swallow on carrot pieces (Frank and others, 2012). This was also observed for cultivars that contained high concentrations of ester related VOCs or ester and terpene related VOCs. Cultivars associated with terpenes and aromatic hydrocarbon related VOCs were lower in I_{max} values and had a longer T_{max} for all *in vivo* detected VOCs. This was because VOCs such as terpenes that discriminated these cultivars were not detected

during the *in vivo* measurements. This trend was similar to that discussed for the Fuji cultivars where a longer T_{max} was observed for cultivars that contained low concentrations of detected *in vivo* VOCs.

Juiciness was found to correlate with acoustic properties which suggest that juiciness was perceived only in crunchy cultivars (Section 4.3.2 and Section 5.3.3). This result indicated therefore that apple microstructure influenced sound generation, for example the rupturing of turgid cell walls makes more noise than the peeling apart of flaccid cells. The microstructure of cultivars with a firm texture and high emissions of VOCs such as Jazz were characterised as anisotropic and low in porosity (Figure 7.15). This indicates the presence of smaller IS between the cell walls (Figure 6.3 - 6.4) and low porosity could be a function of turgidity. This is because turgidity, which imparts crispness and juiciness upon rupture is exerted by the intracellular fluids in the cell membranes that pushes against the cell walls resulting in a smaller IS between the middle lamella of adjacent cells (Varela and others, 2007; Rizzolo and others, 2010).

Compared to the first and second group of cultivars, this last group of cultivars had a both good firm texture and a high VOC content. Additionally these cultivars were associated with juiciness and an increased eating quality. These cultivars could be marketed as fresh eating apples or could be considered in juice making or used to produce premium mono-cultivar apple juices or cider.

The findings of this thesis have potential benefits in several practical applications. Collectively, a wealth of VOC fingerprints and texture characteristics for each individual apple variety can be compiled in a phenotypic database. This can serve as standards against which new high quality apples for specific needs, such as fresh eating, cooking or juicing, can be evaluated. In addition, the tentatively identified compounds present could be collected to create a VOC library which can be accessed in future research to characterise new samples or to aid in breeding programmes. The use of PTR-MS measurements can also be incorporated into larger studies to investigate the relationships between genotypes and phenotypes of different apple cultivars with the use of metabolomics. Currently, this has already been established for the Golden Delicious cultivar (Velasco and others, 2010).

By elucidating the different proportions of ester compounds present in the apple VOC profiles, flavour companies can customise artificial flavours to mimic specific cultivars. For example, cultivars that impart high lemon flavour or odour could be created with a higher ratio of butanoate esters (Section 5.3.3).

Lastly, visualization of apple microstructure could be used as a pictorial guide to help understand the basis of textural differences and how they change over time. The efficacy of 1-MCP reapplication on specific cultivars, such as Golden Delicious, has been shown. This would benefit the industry by enabling the texture of Golden Delicious to be maintained during long term postharvest storage especially for export.

The results emerging from this series of chapters have provided an interesting advance towards understanding the interrelationships between texture and flavour. These results could potentially feed into a large database to catalogue the VOC fingerprint and texture characteristics of individual apple cultivars. Ultimately, this could be employed to create high quality apples through the selection of specific phenotypes. Here are some recommendations for future studies that could be performed to expand on this study:

- High variations between panellists during *in vivo* nosespace measurements could be decreased with improved protocols. This could be done by decreasing the number of panellists but increasing the number of replicates per cultivar. Also, the differences in breathing pattern between panellists could be overcome by providing a basic training on breathing which will enable researchers to align their breath profiles decreasing variability. Another option would be to use a model mouth to understand the changes in VOC profile when the apple is masticated. However, there are other less established parameters that need to be considered such as a swallowing effect, which may not be replicated by using a model mouth.
- Although some apple cultivars were characterised by its abundance of terpene compounds, no sensory attributes were associated to these VOCs. This was due to the fact that the cut apple samples used for sensory, *in vitro* of cut fruit and *in vivo* measurements were without the skin. Also, the 30 minute incubation time used for the *in vitro* cut fruits enabled terpene compounds to reach headspace equilibrium which may not have occurred in the short time of consumption. The skin was previously removed for sensory studies as this would have influenced the panellists' decisions. Therefore, differences in the apple skin should be taken into account in a follow-up study using *in vivo*

measurement. This would remove any preconceptions or bias from the panellists.

- The taste-aroma interactions of sweet taste and fruity ester VOCs could be further investigated with the simultaneous use of *in vivo* nosespace measurements and time intensity sensory methodologies. A simplified experiment that removes biological variation within each cultivar would be to obtain samples from a single apple spiked with differing concentrations of specific esters or sugar/acid ratios with replicates measured by the same panellist.
- Apples are biological samples which display variations even within cultivars. It would be beneficial to measure the extent of this variation and whether this affects the extent of cultivar-cultivar differences. This could be performed by measuring several apples from the same cultivar separately in which the apples would be measured as separate samples. These can then be subjected to the process of sensory, PTR-MS, texture and µ-CT measurements.
- The efficacy of 1-MCP was shown to be cultivar dependent. Therefore, a larger study incorporating a selection of cultivars such as those high in IEC, cultivars that soften rapidly and cultivars that maintain its texture during storage is advisable. This would illustrate the benefits and necessity of 1-MCP for specific cultivars.

Chapter 9: Conclusions

This thesis highlighted the applicability of using either the PTR-QUAD-MS or PTR-ToF-MS as a technique to measure *in vitro* and *in vivo* flavour release from fresh cut apples. *In vitro* and *in vivo* measurements characterised apple cultivars based on their distinct VOC profiles, even though the two methods generated different profiles in the *in vivo* measurements due to a smaller selection of VOCs detected using the PTR-QUAD-MS. The data acquisition for *in vivo* nosespace data was improved with the use of a PTR-ToF-MS by increasing the *in vivo* nosespace VOCs detected by a factor of four. Regardless of the technique used, the detected *in vivo* nosespace VOCs were mainly ester related compounds. Terpene compounds were shown to be of importance in the *in vitro* separation of cultivars. Terpenes were not detected in the *in vivo* measurements as the concentrations of the terpene compounds within the oral cavity were too low as they predominantly occur in the apple peel which was removed from the apple portions used. Nosespace parameters such as T_{con}, T_{swal} and T_{max} showed potential to be used to understand the nature of mechanical breakdown during mastication. One of the most noteworthy findings was the change in T_{max} in relation to certain textural properties of specific cultivars. For example, while T_{max} was initially speculated to be faster in softer cultivars due to a rapid increase in surface area during mastication within the oral cavity, T_{max} was also faster for firm and juicy cultivars as they induced a shorter T_{swal} and more swallows, thereby shortening the time to maximum intensity (T_{max}).

It was also found that the careful selection of specific analytical variables could be used to predict sensory attributes such as titratable acidity and sour taste. When comparing VOC measurements, butanoate esters (m/z 103, 117, 131) were well related to lemon flavour and odour whereas hexyl acetate (m/z145) was positively correlated to overall odour indicating its importance in apple base flavour. Instrumental texture was positively correlated to sensory texture attributes and also enabled the separation of cultivars that were firm but not necessarily crunchy. Physico-chemical properties such as TA showed a good correlation between sour taste and could be considered in prediction models. Sweet taste was negatively correlated to TA but not correlated to SSC. However, fruity ester related compounds (m/z 43, 61, 145) were positively correlated to sweet taste suggesting the occurrence

of aroma-taste interactions. These results indicated that instrumental texture is a viable option in place of sensory testing

The use of X-ray µ-CT scanning proved to be a viable tool in visualizing the differences in microstructures of firm and soft apple cultivars. The morphological property, porosity, was observed to be inversely proportional to firmness. However, this was not applicable to all cultivars. Specific firm and soft cultivars were found to contain similar porosities but were significantly different in texture, owing to morphological differences in the size, distribution, connectivity and anisotropy of the IS. VOC release was associated to morphological parameters of connectivity and porosity, indicating a relation between VOC release and apple parenchyma microstructure, which had not been established prior to this study. The use of the ethylene inhibitor, 1-MCP, was found to cause texture to be maintained for longer. However, a suppression of VOC emission was observed. Interestingly, the efficacy of 1-MCP treatment was different between cultivars favouring cultivars of high ethylene concentrations.

The findings from this thesis could be compiled into a phenotypic database of apple cultivars. This would provide a selection for cultivar traits to guide in the development of apples tailored towards specific needs such as juice, dessert apples or fresh eating apples. The attained VOC finger prints of specific apple cultivars could also be utilized by flavour companies to create mono-cultivar artificial flavours. Finally, the visualization of microstructure would provide the industry with a pictorial guide underlying textural change in apples. This would deepen the understanding of microstructural differences, their occurrence and how they affect texture. Lastly, knowledge of the efficacy of 1-MCP on specific cultivars would be useful to extend the shelf life of apples of different genetic background in global trade.

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