Characterisation and Evolution of Organoleptic Properties in *Solanum Lycopersicum* Cultivars

Matthew A. Knight

Ph.D.

Characterisation and Evolution of Organoleptic Properties in Solanum Lycopersicum Cultivars

Matthew Alexander Knight

BSc (Hons)

A thesis in part-fulfilment of the requirements of the University of Northumbria at Newcastle for the degree of Doctor of Philosophy. Research undertaken in the Faculty of Health and Life Sciences

November 2018

Declaration

I declare that the work contained in this thesis has not been submitted for any other award and that it is all my own work. Where metadata are being used for further data analysis, the sources are appropriately acknowledged. I declare that the word count for this thesis is 88,648.

Name: Matthew Alexander Knight

Signature:

Date: 30.11.2018

ABSTRACT

Public dissatisfaction with tomato flavour has resulted in extensive research into methods of chemometric and sensory profiling, crop improvement and novel breeding strategies for future cultivar development. This work focused on characterising the intrinsic content of flavour and taste active compounds, their origin, localisation, evolution and biosynthesis across multiple cultivars using targeted instrumental analysis and non-targeted metabolomics approaches with the aim to better understand cultivar specific clustering driven by genetic differences as expressed by the metabolome.

Metabolomic classification successfully discriminated all 10 cultivars used in this study with 134 discriminant features upregulated in one or more cultivars as determined by ROC. A total of 7 compounds were tentatively identified based on the Sum formula Identification by Ranking Isotope patterns Using mass (SIRIUS) workflow. Those compounds were primarily related to tryptophan metabolism including the flavonones hesperetin, naringenin and prunin. Targeted analysis for taste and flavour active compounds including sugars, nucleotides, amino acids and volatiles was conducted to further understand the inherent biochemical traits that might lead to sensory quality differentiation. Discriminant classification using the volatile constituents alone allowed for a 91.7% successful classification of 252 samples into their respective cultivar groupings, with only a single cultivar proving challenging to classify.

Other than the inherent genetic variation between cultivars, the biochemical changes occurring during ripening is a significant determinant factor of the overall quality of the ripe fruit. Metabolomic profiling of 3 cherry cultivars across different stages of ripening revealed a relationship that was comparable during ripening transition. The transitionary ripening stages, namely the transition from ‘Mature Green’ to ‘Breaker/Turning’ or ‘Orange’ showed the most significant difference and highest degree of flux, due to the rapid conversion of many cellular components and processes. This includes rapid catabolism of GABA (~30% decrease), tripling of glutamate, doubling of the organic acid and sugar contents and the differential patterns of volatile generation. Fruits showed progressive generation of organoleptically important compounds throughout ripening, with less significant differences notable between ‘Table Ripe’ and ‘Light Red’ than in the earlier stages of fruit ripening, however a shift in fruit quality was still notable.

Localisation of compounds in the different parts of the tomato fruit is important in flavour and taste delivery. Hence, the distribution and abundance of flavour and taste active metabolites was determined in three distinct tissues of tomato fruit across three cultivars. This approach complemented the more commonly used homogenisation, enabling further exploration into the roles each tissue plays in the organoleptic profile of fresh tomato. The importance of the locule

fluid, although accounting only for a small percentage of total fruit weight, was highlighted by the markedly increased concentrations of organic acids and umami amino acids, 2-2.5 and 2.3 times greater than that of the flesh, respectively, with comparable sugars. This likely indicates that the tissue is significant to the intense gustatory sensations associated with the first few seconds of fresh tomato consumption.

It is important that investigations in certain tomato fruit biochemical traits are somehow linked with measurable sensory attributes, in order to assess the intensity and acceptability of the cultivars. The distinct chemical profiles for 10 cultivars were then correlated to equivalent sensorial data creating simple binary predictive models. Adjusted R_2 values indicated 93.7%, 80.3% and 82.8% coverage of the populations of data for the models, respectively. Sweetness, sourness and umami all showed very strong, significant relationships following linear regression between the analytical and sensory datasets. Finally, understanding and opinion of fresh tomatoes was elucidated though market research and open-forum sensory analysis. The findings indicated that part of the poor perception of tomato flavour may be due to inappropriate post-point of purchase handling, where 75% of surveyed households still store tomatoes in the fridge which is known to impede the formation of desirable organoleptic components.

ACKNOWLEDGEMENTS

Firstly I would like to thank my supervisor Dr Georgios Koutsidis for his help, support and guidance over the duration of this research. His consistent motivation and enthusiasm was of great help during the more challenging parts of the project. Some of the directions that this study took us in were largely due to the late night problem-solving and brainstorming with him.

I would like to thank Northumbria University, the Technology Strategy Board (TSB) and Biotechnology and Biological Sciences Research Council (BBSRC) for partially funding the project. Thanks also go to my industrial partners on the project Thanet Earth, MMR Research Worldwide and Verivide. Particular thanks go to Joe, Sally, Christine, Mick and Steve for educating me about their various areas of the project and for facilitating much of my own research.

I am extremely grateful to the technical staff at Northumbria for their continued help throughout the project. In particular I would like to thank Dr Karen Haggerty, Vivien Brindley, Sam Bowerbank and Gordon Forrest.

My sincerest thanks go to my friends and colleagues in EBA510, the atmosphere and enthusiasm you have all provided has kept spirits high and provided a lot of well-needed, light-hearted moments throughout this endeavour. Special thanks go to Ross Martinscroft, Dr Hannah Sykes, Rachel Bulmer, Charlotte Marsh, Justin Ma, Dr Alex Twist and Sotiris Kyriakou. You have all provided consistent help, knowledge, encouragement and support, as well as being truly valuable friends.

Finally, my greatest thanks to my family, who have been a constant source of reassurance, support and encouragement. Thank you to my parents for talking through the difficult parts of the study with me and, in many cases, helping me to find the best way to proceed. To my brother, Dom, and friends, particularly Alex and Luke, thanks for the laughs and entertainment over the last few years, as well as helping my through some of the harder times. You have been invaluable.

Hopefully, we all know a little bit more about tomatoes now!

Table of Contents

Table of Tables

Table of Figures

Cultivar Specific Abbreviations

Abbreviation	Full Meaning
ELE	Elegance (Cultivar, Beefsteak)
EL2	Elegance (Cultivar, Beefsteak) : 2nd Crop
TEM	Temptation (Cultivar, Salad)
PIC	Piccolo (Cultivar, Cherry)
ORA	Orange Cherry (Cultivar, Cherry)
SUN	Sunstream (Cultivar, Plum)
AXI	Axiani (Cultivar, Cherry)
CAM	Campari (Cultivar, Salad)
JUA	Juanita (Cultivar, Cherry)
DR2	DR28090TC (Cultivar, Plum)
194	P194 (Cultivar, Salad)
GEN	Genio (Cultivar, Cherry) : Chapter 5 Only
ANG	Angelle (Cultivar, Baby Plum) : Chapter 5 Only
VAL	Valkiria (Cultivar, Salad) : Chapter 5 Only

1 Literature Review

1.1 Origin and Cultivation of Tomatoes

Tomatoes (*Solanum Lycopersicum* Mill.) and other wild species, which originated in South America, are believed to have been consumed as early as 700 AD by Aztec and Inca tribes (BTGA, 2014, Bergougnoux, 2014). Following the colonisation of the Americas, much of the native flora and fauna, including tomato plants were transported back to Europe. Initially, there was much contention as to the edibility of tomato fruits; although many continental European countries had incorporated them into their diets by the end of the 17th century, the British believed them to be poisonous and unsavoury (BTGA, 2014, Petro-Turza, 1986, Bergougnoux, 2014). Due to this, tomatoes were initially cultivated as a decorative climbing plant; it was not until the start of the 19th Century that commercial production of tomatoes as a food crop began. Nutritionally, tomatoes are an excellent source of Vitamin C as well as pro-vitamin A and tocopherols, moreover they provide both dietary potassium and iron. One of the most pronounced nutritional benefits of tomatoes is the high concentration of antioxidant compounds, most notably lycopene, which has been previously heralded as a potent anti-cancer agent. However, numerous peer-reviewed, scientific manuscripts have been unable to find a well-defined link between lycopene or tomato consumption and reduced incidence of cancer (Kirsh *et al.*, 2006, Giovannucci *et al.*, 2002, Giovannucci, 1999, Etminan *et al.*, 2004).

Tomatoes are not best suited to cultivation in the UK. As a crop, they are highly susceptible to variable weather conditions and extremes of temperature, both of which can occur in the UK. The UK growing season for outdoor tomatoes is relatively short, (July-October), but with a poor or variable British summer, much of the commercial crop can be lost. However, controlled climate greenhouses or glasshouses significantly extend the growing period of tomatoes, allowing them to be cultivated from February-November (BTGA, 2014). With the proper application of controlled climate greenhouses, hydroponic cultivation of plants, supplemental artificial lighting and tailored watering and nutrients tomatoes can be grown for 12 months of the year in the United Kingdom (Thanet Earth, 2013). The growth of commercial glasshouse tomatoes requires a delicate balance of many factors to maintain desirable and marketable fruit yields. Glasshouse tomatoes are commonly grown hydroponically, planted in a natural fibre-based soil replacement, such as rockwool (Thanet Earth, 2013, de Koning, 1994). This eliminates many factors which are difficult to control such as pests and bacterial and fungal growth that could have adverse effects on both the plant growth and fruit quality (Thanet Earth, 2013). To ensure an appropriate yield both high volume and quality of fruit the vegetative growth (leaves/stems) and reproductive growth (flowers/fruits) of the plant must be balanced. Commonly this is done by eliminating side shooting and excess vegetation, resulting in the plant diverting more energy and resources to the

production of fruits. However, to ensure that the plant remains healthy, with sufficient photosynthetic potential to allow for the desired yield of fruit, the vegetative growth must be allowed to continue during fruiting (de Koning, 1994). This is particularly important in low light environments, such as unlit glasshouses in the winter months, where reduction in photosynthesis in the leaves may lead to net decreases in available energy and the fruit, or even the plant, failing.

1.2 Global Production of Tomatoes

Tomatoes are a commercially cultivated crop belonging to the Solanaceae family (Selli *et al.*, 2014, Petro-Turza, 1986). The fruit, though it is considered a vegetable in a culinary sense, is a staple crop in many parts of the world, consumed both in fresh and processed forms, such as sauces or pastes. According to data published by FAOSTAT, just under 179 million tonnes of tomatoes were commercially produced annually worldwide in 2016, with a net production value of $64.7 billion (using the 2004-2006 standard international dollar) (FAOSTAT, 2017, Perez-Fons *et al.*, 2014). Data for the year 2016-2017 indicates that China currently produces the most tomatoes annually at 58,489,364 tonnes, followed by India (19,720,000 tonnes), Turkey (12,675,000 tonnes) and USA (11,923,705 tonnes) (FAOSTAT, 2017).

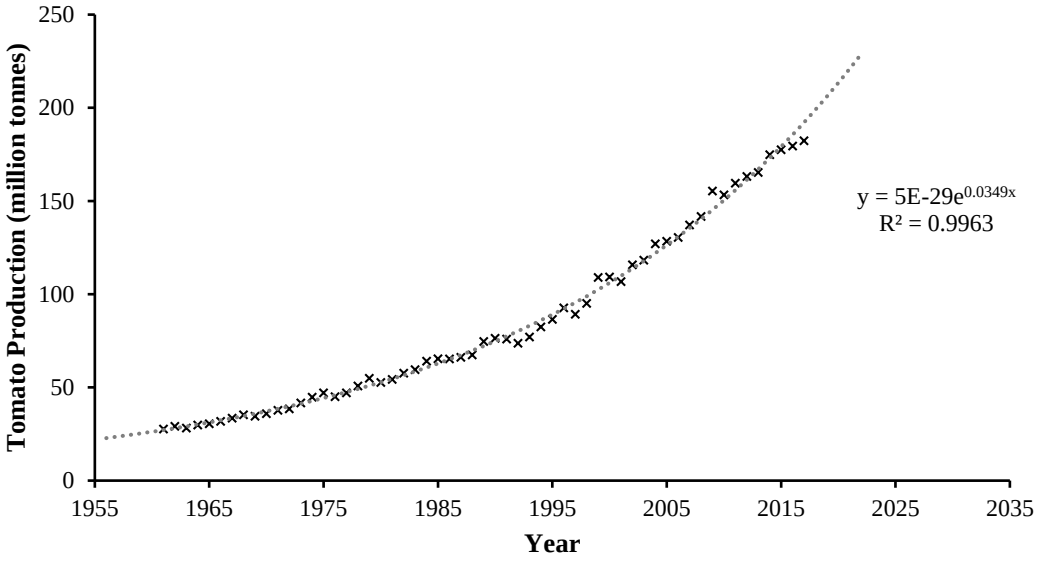


Figure 1.1 - Worldwide, annual production of tomatoes between the years 1961 and 2017. Data shown was retrieved from FAOSTAT records, with 1961 the first available data (FAOSTAT, 2017).

As demonstrated by **Figure 1.1**, an exponential rise in tomato production has occurred between the years 1961 and 2017. Tomato production during the year 2017 was 6.6 times that of 1961, with 182.3 million tonnes and 27.6 million tonnes produced respectively. This increase is not accounted for by the population increase over the same period of 3.08 billion to 7.55 billion for 1961 and 2017 respectively (data not shown) (FAOSTAT, 2017). This indicates that the per capita

demand for tomatoes is increasing year on year, consolidating them as a staple crop for many parts of the world. Furthermore, **Figure 1.2** shows the consumption of total vegetables per capita/annum compared to the consumption of tomatoes from 1965 to 2010 in the UK. Per capita consumption of vegetables has increased over this time period, starting at 64.5 kg in 1965 and increasing to 92.8 kg by 2010. Moreover, tomato consumption not only increased from 9.9 kg to 21.5 kg between 1965 and 2010, but also contributes a higher percentage of total vegetable consumption, increasing from 15.3% in 1965 to 23.2% in 2010 (FAOSTAT, 2017).

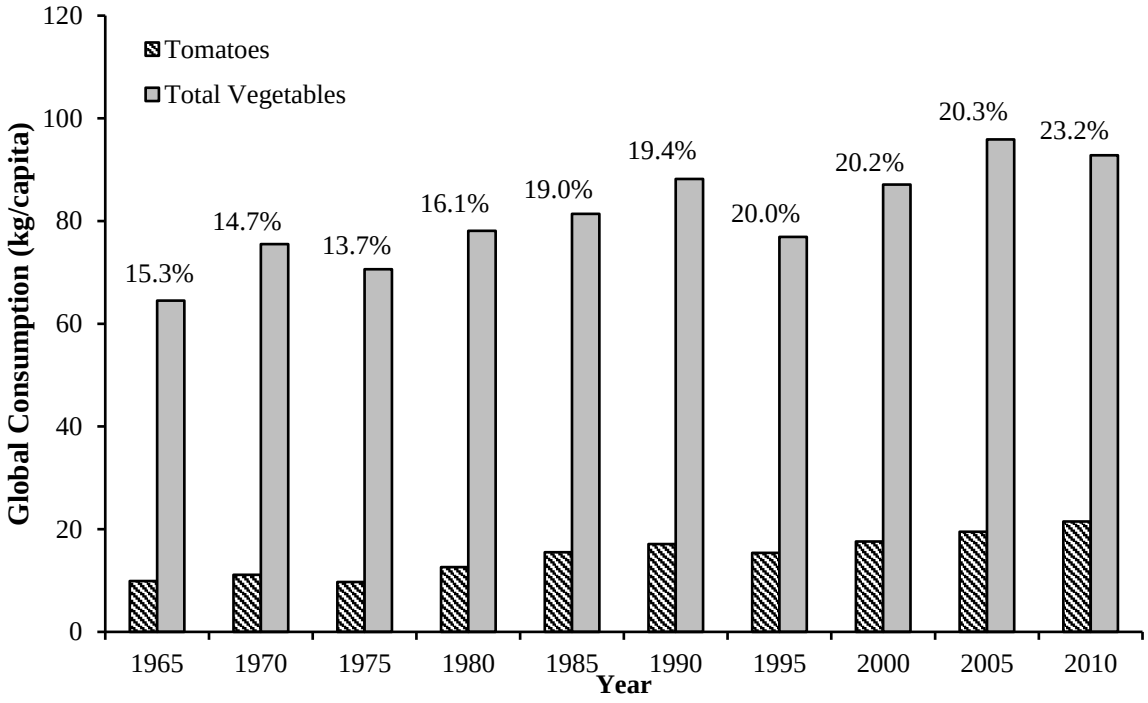


Figure 1.2 - Consumption per capita of tomatoes compared to total vegetable consumption between 1965 and 2010 in the UK. Data labels indicate the percentage of total vegetable consumption accounted for by tomatoes. Data shown was retrieved from FAOSTAT records (FAOSTAT, 2017).

1.3 Consumer Perception of Commercial Tomato Quality

Prior to purchase, consumer perception of tomato fruit quality largely depends on fruit colour and texture, as the flavour and taste cannot be assessed, and undamaged tomato fruits produce limited aroma (Batu, 2004, Radzevičius *et al.*, 2009). Additionally, visual and tactile assessment of fruits are often used as reliable methods of eliminating low quality, under or over-ripe fruits before purchase. Fruits that do not reach the certain sensory thresholds for parameters such as colour or firmness may be unsuitable for sale as fresh fruit and may have to be sold as a lower quality class of product or be discarded. Ultimately, this devalues the crop and reduces profit margins for tomato growers. Therefore, selective breeding of tomatoes has deliberately produced cultivars that produce high fruit yields which are suitable for sale as fresh tomatoes, ensuring that ripening process of the crop is predictable and that the colour, shape and size is uniform and appealing to

consumers (Miller and Tanksley, 1990, Bai and Lindhout, 2007, Tieman *et al.*, 2017). These physiological and morphological changes are commonly referred to as 'domestication syndrome' as they are most brought about by selective breeding of traits considered desirable by consumers rather than those traits that naturally evolve to improve the viability and functionality of the plant in the wild (Miller and Tanksley, 1990, Frary and Doğanlar, 2003). In tomato the extreme level of 'domestication syndrome' has lead to the loss of a significant amount of genetic diversity, with less than 5% of that of wild type relatives (Bai and Lindhout, 2007).

It is a common complaint that supermarket tomatoes have an inferior flavour compared to that of home-grown or greengrocer sourced fruits (Baldwin *et al.*, 2000, Klieber *et al.*, 1996, Maul *et al.*, 2000). The loss or, more accurately, diminished flavour formation is commonly attributed to post-harvest practices, storage and transport conditions and restricted respiration/ethylene production (Boukobza and Taylor, 2002). Cooling of tomatoes is often due to a desire to increase fruit shelf life, allowing fruits to be transported greater distances and sold over a longer period of time, thus increasing profits for the supermarkets. However, during storage and transport at temperatures lower than 10 °C, tomatoes experience chilling injuries (CI), greatly impacting the production of flavour active compounds. Post-harvest temperatures of less than 10 °C were found to have to a marked effect on volatile production, increasing the formation of off-flavours whilst impeding the formation of desirable aromas (Boukobza and Taylor, 2002, Maul *et al.*, 2000). Maul *et al.* states that chilling injury in tomato fruits can lead to delayed or uneven ripeness, fruit softening, reduction in flavour active compounds, external physical damage and increased vulnerability to fungal or bacterial attack (Maul *et al.*, 2000). Furthermore, Maul *et al.* suggests that chilling injuries may set in as high at 12.5 °C, and that the decrease in flavour perceived in supermarket bought tomatoes may be attributed to the post-purchase storage between 3 and 5 °C in a household refrigerator. This suggests that part of the consumer dissatisfaction related to flavour and taste of tomatoes may be a result of refrigeration between purchase and consumption. There have been a number of studies that have claimed that the combined effect of selective breeding of commercially desirable traits and poor post-harvest treatment of the fruits more adequately explains the current poor flavour and crop quality.

One of the most convincing hypotheses for the apparent decrease in overall flavour and aroma quality of fresh tomatoes, is that selective breeding for easier commercialisation has unexpectedly suppressed the flavour potential of commercial hybrids(Klee and Giovannoni, 2011, Saliba-Colombani *et al.*, 2000, Tieman *et al.*, 2012). Characterisation of flavour on a per cultivar basis is a long and expensive process, requiring advanced analytical technologies and sophisticated data analysis techniques to fully comprehend. This, for the most part, is outside of the remit of most commercial breeders, particularly when the intensive nature of new cultivar development and limited crop lifetime is considered (Tieman *et al.*, 2012). As the commercial lifespan of tomato

cultivars is very short lived, both with the aim of invigorating the public interest and seasonal/annual shifts in consumer demand, many cultivars are only commercially grown for up to two years (Thanet Earth, 2013). Consequently, investing in expensive and time consuming chemometric and genetic profiling is not a viable approach for each of the potential new cultivars on offer by breeders. Tieman *et al.* found that although many of the superior alleles responsible for fruit size and sugar/ acid content were preserved between wildtype and commercial cultivars, only 50% of those associated with the formation of desirable flavour and aroma volatiles had been retained in the commercial hybrids. The authors highlight that this reduction would be difficult to discern in new crops as it has occurred through sequential breeding iterations, which initially would have had little impact on flavour, but the effect has been compounded over years of cross breeding (Bai and Lindhout, 2007, Tieman *et al.*, 2012, Tieman *et al.*, 2017). Tieman concludes that manipulation of tomato flavour can be achieved through targeted selection of cultivars presenting upregulated positive alleles whilst also limiting and mitigating the formation of more negatively perceived volatiles.

Due to the climacteric nature of tomatoes, increased respiration and ethylene production is vital for proper ripening and flavour development (Kader *et al.*, 1978, Klieber *et al.*, 1996). Ethylene ($H_2C=CH_2$) is a naturally occurring alkene which is synthesised by all cells of a plant. In addition to affecting fruit ripening, ethylene has been linked to wound-response mechanisms and leaf/fruit senescence (Alexander and Grierson, 2002). The production and expression of ethylene by climacteric plants leads to substantial chemical and physiological changes within the fruit. Many of the positive organoleptic qualities of ripened fruit occur as a direct result of increased ethylene biosynthesis and subsequent ripening; fruit flesh softens, chloroplasts are replaced by chromoplasts to synthesise pigments, bringing appealing new colours to the fruit, flavour and aroma active compounds are synthesised and expressed, and enzymatic starch degradation causes fruits to accumulate sugars (Alexander and Grierson, 2002). Ethylene production is autocatalytic, meaning that its presence stimulates the biosynthesis of further molecules of ethylene (Alexander and Grierson, 2002). In tomatoes, this is particularly apparent at the 'Breaker' stage, when a small proportion of the cells show signs of ripening. Ethylene production then increases in these cells, causing it to diffuse into its neighbours, causing a wave-like effect of ripening across the fruit (Alexander and Grierson, 2002, Bramley, 2002, Brandt *et al.*, 2006, López Camelo and Gómez, 2004).

Aside from storage temperature, packaging (i.e. modified atmosphere packaging (MAP)) can limit fruit respiration and impact proper ripening processes, including volatile formation. Klieber *et al.* found that low O_2 (<3%) packaging delayed the onset of ripening in tomato (*c.v.* 'Bermuda'), reducing the volatile components of the fruits, except for ethanol and acetaldehyde, both of which are considered off-flavours in tomato. It was also noted that high CO_2 (>15%) packaging

environments had no effect on fruit ripening (Klieber *et al.*, 1996). Boukobza and Taylor state that during transport of large quantities of tomato fruit, it is possible that fruits in the bottom or centre of the batch may be subjected to greatly reduced O_2 levels, and subsequently show a decrease in volatile production and flavour development (Boukobza and Taylor, 2002). The authors concluded that tomato fruits that are stressed, either by low temperatures or decreased oxygen levels produced less volatiles than unstressed fruits; this conforms with the findings of Kader *et al.*, Maul *et al.* and Klieber *et al.*

1.4 Sensorial Perception of Foodstuffs

Sensorial perception is paramount to life, allowing organisms to gather information from their surroundings that is relevant to their survival and ability to thrive. The importance of sensory perception in identifying safe, edible foodstuffs cannot be exaggerated as it provides the most reliable way of identifying harmful or hazardous materials. Sensorial information is a key factor in our decisions about choosing foods to consume, initially through visual analysis, followed by aroma, taste and texture-based cues. During consumption, the primary sensorial modalities of gustation, olfaction and trigeminal senses allow for quality and safety assessment of the food. Each of these independent systems contribute to perception and are intrinsically interlinked; it is almost impossible to consume and food or drink without triggering multiple stimulus of these senses. Understanding the contribution of each system to the overall perception of food is vital for the appreciation of the sensorial experience of the consumer, particularly in the sector of new product development or crop improvement.

The independence of flavour and taste has been the subject of significant discussion and investigation for decades (Prescott, 1999, Stevenson and Tomiczek, 2007). Physiologically, the sensorial stimulation provided by flavour or taste-active compounds is only detected by a single sensorial modulus; olfactory detection of flavour and aroma or gustatory detection of the five, principal tastes. However, numerous studies have demonstrated the addition of tasteless, flavour active compounds can alter the perception and intensity of tastes. This effect cannot be adequately explained through the examination of the physiological explanation of sensorial detection, and therefore, probably derives from a combined, psychophysical effect, as a psychological element is likely when the compounds assessed are taken into account. The phenomenon is known as olfactory-gustatory or olfactory-taste synaesthesia and has been widely documented. In a review of sensory synaesthesia by Stenvenson and Tomiczek the phenomenon is explained. The authors note the common use of sensory descriptors for specific odorants or aromas, namely vanilla, caramel, mint and chocolate, all of which are commonly categorised as ‘sweet’, often by inexperienced panellists (Stevenson and Tomiczek, 2007). Olfactory and gustatory synaesthesia has been a method of product perception and taste modulation in the food industry for decades,

becoming all the more important with the rise of reduced sugar, fat and salt foods (Stevenson and Tomiczek, 2007, Belloir *et al.*, 2017). Studies focused on sweetness enhancement have utilised vanillin, caramel-like or fruity aromas to supplement sweetness provided by sugars (Stevenson *et al.*, 1999). However, these flavours are most commonly associated with sweet foodstuffs. This may allow for subconscious, memory-based bias during sensory analysis based on prior experiences, leading to an increase in perceived sweetness in the absence of the stimulus. Numerous food and fragrance companies now advertise "sweetness enhancers" which help to maintain the desired level of sweetness in reduced sugar products or heighten the sweetness provided by traditionally sweet compounds (Belloir *et al.*, 2017). There are two primary modes by which olfaction and gustation may overlap. Firstly, the stimulation of gustatory sensation during orthonasal sampling of specific odorants, as when smelling vanillin or chocolate aromas. Additionally, the retronasal detection of volatile compounds and the additive or suppressive effect on simultaneously stimulated tastes. A direct sensory overlap has not been demonstrated in fresh tomatoes, although it is likely that one exists to a certain extent. Those fruits that present with a stronger fruity flavour and less of the green or off-notes that are characteristic of tomatoes are often rated as being some of the sweetest fruits. Often this is due to increased sugar content, however, there may be a similar synergistic effect between the volatiles complementing the high sugars of the fruits.

1.4.1 Gustation : Five Tastes

Gustation refers to the sensations detected by specialised receptor cells on the surface of the tongue, known as taste buds. Taste buds are located on the circumvallate, foliate, and fungiform papillae areas of the tongue, but the density of taste bud distribution varies singnifcantly (Arvidson and Friberg, 1980, Miller and Reedy, 1990). The taste bud includes three discrete cellular morphologies, commonly referred to as type I, II and III, which can be further classified into glial, receptor and presynaptic cells respectively, however they are all referred to as taste bud cells (TRC) (Chaudhari and Roper, 2010). Each individual taste bud consists of a mixed cluster of TRC. Type I glial cells are thought to be responsible for the detection of salt due to the Na^+ ion channels located in the taste pore, which is open to the internal environment of the mouth and therefore the site of interaction with tastants. Type II cells are responsible for the perception of sweetness, bitterness and umami, however, each Type II cell will only respond to one of these sensations. Therefore, it can be considered that there are three distinct categories of Type II cells each responding to the interaction of one class of compound and only demonstrating a G protein-coupled receptor (GPCR) capable of providing one of these three sensorial phenomena (Chaudhari and Roper, 2010, Chandrashekar *et al.*, 2006). There is no evidence that Type II cells are involved in the sensory perception of salty or acidic sensorial environments. Type III cells are characterised by their ability to detect acidity and carbonation of foods, which separates them from Type I and II cells. Additionally, as they are presynaptic cells, they are

responsible for the transfer of stimulus between Type I, II cells and neurons. Tomchik *et al.* demonstrated that Type III cells generate a broader, less specialised response to each of the five basic tastes, due to the intercellular communication between receptor and presynaptic cells (Tomchik *et al.*, 2007).

Due to the physiology of the taste bud and receptor cells contained within, they are only capable of interacting with, and therefore deriving stimulus from, water soluble components of foods. Insolubilised material is not able to migrate into the taste pore effectively during mastication or able to trigger the response within the TRC or the synaptic impulse required for detection. The process of mastication liberates taste active molecules from the food, dissolving and diluting them in the saliva and allowing migration into the taste pore on the apical tip of the taste buds (Breslin, 2013). Therefore, taste buds are responsible for the sensorial responses to the "five basic tastes", sweetness, sourness, saltiness, bitterness and umami, a savoury, meaty and broth-like sensation. Although this may appear to be a significantly reduced range of detection when compared to flavour and aroma, gustation provides invaluable information about the nutritional composition of the foodstuff and, therefore, about the subsequent benefit of consumption. The intricacies of gustatory stimuli go much further than simple detection, allowing for characterisation, intensity and time dependant metrics to assess the stimulus. For example, a lingering bitterness is immediately noted, as it could be indicative of ingestion of a toxin or harmful contaminant (McLaughlin and Margolskee, 1994, Reed and Knaapila, 2010).

Sweetness is governed by a number of compounds, but the primary providers of sweet sensation in foods are mono- and disaccharides. In addition, certain sugar alcohols and even some amino acids and non-sugar 'sweeteners' trigger a similar sensation. The biological significance of sweetness is simple - sweetness is correlated with glucose or similar compounds that can be converted, within the body to glucose and are therefore directly related to energy intake and availability, which is an important factor in regulating blood glucose levels (McCaughey, 2008). It is also thought that the intensity of sweetness is used as a metric for the estimation of caloric intake, thereby allowing for a subconscious prioritisation of sugar rich foods when calorie starved (Breslin, 2013, McCaughey, 2008). Studies into human type II receptor cells demonstrate that they are able to detect both simple sugars and a number of other, sweet-tasting, small molecules. This includes D-tryptophan and glycine which have both been characterised as presenting a sweet taste; artificial and non-sugar sweeteners such as sucralose and acesulfame-K, finally sugar alcohols such as sorbitol are also detected by the type II TRC. In fresh fruit, including tomato, sweetness is arguably one of the most important gustatory senses (Beckles, 2012, Stevens *et al.*, 1979, Malundo *et al.*, 1995, Gautier *et al.*, 2008, Petro-Turza, 1986). The interplay between sweetness and acidity is one of the characteristically palatable interactions associated with fresh

produce, with a balance of the two sensations being vital for a desirable sensorial experience (Tieman *et al.*, 2012).

Sourness is the detection of H+ ions in the saliva or fluids of the food or drink being consumed. Why humans have evolved to detect sourness has still not been definitively proven. Unlike sweetness, sourness is not a particularly strong marker of nutritional content, apart from the inverse correlation with ripeness. However, sourness can be considered a sensorial marker of Vitamin C content and fermentation in many foods and drinks (Breslin, 2013). Sour stimuli are the result of extracellular pH through detection by acid sensing ion channels (ASIC), although this is not indicative of the presence of specific nutrients (Breslin and Spector, 2008). The binding site for ASIC, initially thought to be proton specific has now been demonstrated to be active at neutral pHs and therefore is capable of interacting with other species in addition to H+ ions (Kress and Waldmann, 2006). As a result, sourness is the inverse of sweetness, with increased or excessive sourness considered a negative trait, commonly resulting from spoiled, contaminated or under-ripe food. However, this does not mean that acidity is a purely negative attribute. Often, the presence of sourness in foods is context or matrix specific, with higher sourness being more acceptable in fresh fruits than it is in fresh meat or nuts. Almost universally, higher acidity levels are tolerated or even preferred when paired with equally elevated sweetness levels, which is a predominant feature of fresh fruit (Gautier *et al.*, 2008, Baldwin *et al.*, 2008, Stevens *et al.*, 1979, Malundo *et al.*, 1995). Even in just the context of fresh tomatoes, the levels of sugars and acids are linked, with cherry tomatoes presenting elevated levels of both sugars and acids, when compared with processing or salad type fruits, yet the ratio between sourness and sweetness remains comparable (Gautier *et al.*, 2008, Malundo *et al.*, 1995).

Umami, the most recently accepted basic taste, is a vital sensory component of various foods and drinks (Yamaguchi and Ninomiya, 2000, Lindemann *et al.*, 2002). Umami is most commonly associated with cooked or aged meat, broths, mushrooms, tomatoes and aged or fermented products such as mature cheddar, Grana Padano, soy sauce and dried fish such as Konbu (skipjack tuna) (Lindemann *et al.*, 2002, Yamaguchi *et al.*, 1971). This is because the compounds that provide the umami stimulus, glutamate, aspartate and specific purinic ribonucleotides, are commonly liberated and concentrated following protein hydrolysis through proteases and/or nucleotide degradation and dephosphorylation during ‘aging’ (Ninomiya, 2002, Yamaguchi and Ninomiya, 2000, Lindemann *et al.*, 2002). The degradation and hydrolysis of proteins during fermenting, aging and cooking leads to an influx of glutamate and aspartate, the two amino acids that convey the umami sensation. Tomatoes have very high levels of free glutamic acid and as well as relatively high levels of aspartic acid. In addition, tomatoes also contain some of the monophosphate nucleotides that contribute to the umami flavour. Of these, only adenosine

monophosphate is present in high concentrations and it contributes a weak umami stimulus when compared to glutamate (Yamaguchi *et al.*, 1971).

Biologically, bitterness is thought to be a marker of toxic or harmful components in food, and therefore, as a taste it is less widely acceptable in many foodstuffs. Due to this bitterness is the least well tolerated flavour by infants and children. However, exposure to bitter foods over time has been demonstrated to increase acceptability, suggesting that the instinctive dislike for strongly bitter flavours can be bypassed through repeated positive experiences (Birch, 1998). Additionally, the number and chemistry of compounds that present with a bitter taste far outweighs those of the other 4 primary senses, so although it is an indicator of negative or harmful compounds, that relationship is not exclusive. There are several foods that are desirable due to their bitterness, with coffee, tea, beer and wine presenting both bitterness and astringency as desirable gustatory attributes. In addition, many vegetables have some degree of bitterness associated with their characteristic flavour, kale, broccoli and radicchio, for example. Tomatoes are not characteristically bitter, although a small number of the metabolites of tomato are known to provide a bitter sensation such as α-tomatine and certain amino acids (Klee and Giovannoni, 2011).

Saltiness is primarily a measure of sodium intake, although multiple other ionic species may also play a role. Sodium is a biological requirement of all life and imperative for the maintenance of ionic and water homeostasis within the body (Lindemann, 1996). Na+ ions are vital components of cellular function and signalling, with sodium gated channels present in a multitude of cellular morphologies across the body (Catterall, 2000). In cuisines around the world the taste of salt is considered desirable; this is in part to do with the taste of salt itself, but also due to the apparent suppressive and enhancing effects it has on other tastes and flavours. In a correspondence by Breslin and Beauchamp it was discussed that the presence and concentration of sodium and other salts in foods moderates negatively associated tastes such as bitterness, whilst enhancing pleasant tastes such as sweetness (Breslin and Beauchamp, 1997). Moreover, Ugawa Konosu and Kurihara found that the presence of NaCl had a strong effect on the perception of some free amino acids. Additionally, it is the salt, glutamate rather than the acidic glutamic acid that is responsible for umami sensation and therefore the presence of sodium ions within food has been shown to increase the intensity of umami in the presence of glutamate (Ugawa *et al.*, 1992).

1.4.2 Olfaction : Flavour and Aroma

Olfaction, in the context of food, is the process by which the volatile aroma compounds of a foodstuff are detected and characterised, both orthonasally prior to consumption, when smelling a product, and retronasally during and after mastication and swallowing (Bult *et al.*, 2007, Verhagen and Engelen, 2006). Olfaction is limited to the detection of volatile compounds, most

commonly with formula weights less than 300 daltons. Typically, food derived odours are the result of compounds with limited water solubility and low polarity, enabling their release from the largely aqueous based food matrix and allowing them to pass retronasally to the olfactory epithelium. Olfaction, although part of the overall multisensorial perception of food, provides the most thorough and matrix specific information about the item. This is due to the capability of the olfactory system to discern the differences between thousands to millions of different odours and at concentrations as low as parts per trillion (Sankaran *et al.*, 2012). Furthermore, unlike other sensorial systems, olfaction can detect a diverse range of analyte chemistries due to the approximately 1000 unique mammalian olfactory receptors (Mori *et al.*, 1999). The actual number of discernible odours is still debated, approximately 10,000 has been the most widely cited figure for decades, but this has been later shown to be a guestimate, rather than a calculated number. However, recent model produced by Bushdid *et al.* calculated the total number of unique odour stimuli that humans can differentiate to be much higher, close to 1 trillion based on the authors predictions (Bushdid *et al.*, 2014). This was based on the premise that both human vision and hearing relies on only 3 detector types to discern between 2.3 – 7.5 million different colours and approximately 340,000 tones (Stevens and Davis, 1938). A parallel was drawn to olfaction, but with limited information available on the physicochemical capacity of the olfactory space it is difficult to prove how many unique olfactory receptors are present. Bushdid *et al.* support their claim with the arguments that the total number of odorous compounds has not yet been determined, but that the number is in the hundreds of thousands. Of those, many are discernible from each other, but the proportion of discrete odour characters between these compounds has also not been empirically demonstrated. Finally, naturally occurring odours are the product of mixtures of volatile compounds, for the most part, and the ratio, proportion and intensity of each component can be capable of significantly altering the perceived aroma. All of these suggest that the power of the human olfactory system has been woefully underrepresented for many years and that our sense of smell is far more adept at characterising and identifying differences than has hitherto been believed.

The process of olfactory detection begins with the introduction of volatile compounds to the olfactory bulb, located superior to the apex of the nasal canal. Neurons travel through to the internal surface of the nasal cavity, in an area known as the olfactory epithelium due to the dense distribution of olfactory receptors. Raised cilla house the olfactory receptors and the G-proteins required for signal transduction, and are covered with a layer of mucus which assists in the trapping and delivery of volatile compounds to the receptors (Lyons *et al.*, 2013). More recently, small, aqueous soluble carrier proteins, referred to as odorant binding proteins (OBPs), have been discovered within the mucus coating of the olfactory epithelium. It is believed that these proteins act as transporters for hydrophobic volatiles, reversibly binding with the odorant before transferring, through the aqueous mucus, to the cilla and sensory receptor cells for

detection (Sankaran *et al.*, 2012). Although OBPs share a significant homology with other biological carrier proteins, their full role and influence in olfaction has yet to be fully elucidated (Pelosi, 1994). Moreover, using targeted insertion of specific P2 odorant receptor genes in mice, Mombaerts was able to determine that specific receptor cells exhibiting the ability to detect P2 were exclusively transferring the signal to glomeruli also expressing the P2 detection gene. The authors inferred that it is likely that single glomeruli are dedicated to receiving only a single receptor signal out of the possible 1000, but that there may be certain cases where multiple receptors stimulate the same response in a single glomerulus, potentially explaining the way mixtures of compounds are detected as both individual odours and as a blend (Belluscio and Katz, 2001, Mombaerts *et al.*, 1996, Mori *et al.*, 1999).

1.5 Organoleptic Quality Aspects of Fresh Tomato Fruit

Consumption of a foodstuff can be defined as taking place out of necessity and for experience, or a blend of both. For necessity we both consciously and subconsciously search for foods that will provide us with our nutritional requirements on a day-to-day basis. However, humans have an additional relationship with food that many other animals don't exhibit, in that we also eat for pleasure and experience. The issues facing the fresh tomato industry are primarily due to the perceived reduction in quality through reduced organoleptic parameters. Nutritionally, tomatoes are just as important as they have always been, but the sensorial experience has suffered through excessive cross breeding, restriction of genetic variability and commercialisation of production, although this in itself is a product of commercialisation and the demand from the consumer for innovation. The difficulty in restoring the desired quality levels of fresh tomato comes from the complexity of their taste and flavour, interactions between stimuli and the multitude of compounds that trigger an olfactory response during consumption.

1.5.1 Sugar Content of Tomato Fruits

Sugars in tomato fruit account for approximately 40-65% of the total dry matter, dependant on cultivar and ripening stage (Petro-Turza, 1986, Balibrea *et al.*, 2006). The predominant sugars are glucose and fructose, although some authors have reported the presence of sucrose at low concentrations (Luengwilai *et al.*, 2010, Beckles, 2012, Malundo *et al.*, 1995, Petro-Turza, 1986). Due to the speed and ease of use, there is an over reliance by commercial tomato growers on total soluble solid (TSS) measurements as an indicator of fruit sweetness/ sugar content. Jones and Scott correlated fruit sweetness of 7 hybrids to both total reducing sugars (RS) and total soluble solids (correlation coefficients 0.38 and 0.40 respectively), and showed a statistically significant correlation at the p=0.01 level. However, the observed relationship was not strong enough to be used as a predictor for total RS content (Jones and Scott, 1983). Kader *et al.* attempted a similar correlation of fruit sweetness to RS content and demonstrated a significant correlation

(0.50) (Kader *et al.*, 1977). One of the primary reasons total soluble solids are not a better predictor of sugar content is due to the large proportion of TSS attributed to organic acids (OA), which are highly variable between cultivars and ripening stages (Beckles, 2012). Beckles noted in their review paper that the composition of sugars is highly cultivar dependant, with high sugar hybrids containing 2-3 times more sugars than regular commercial cultivars. Moreover, that sugar content is inversely proportional to fruit size, leading smaller cultivars such as cherry and baby plum to naturally contain higher sugar levels than larger fruited salad cultivars. Sugar accumulation occurs during the ripening process in tomato fruits with a predictable increase in overall sugar content between mature green (MG) and table ripe (TR) fruits. Commercial tomatoes commonly contain between 3-8% fresh weight (FW) reducing sugars once fully ripe, as shown in a review conducted by Beckles. The study also found data on MicroTom tomatoes, a non-commercial dwarf fruit, which contained only 1.2% FW reducing sugars, which helps to demonstrate the cultivar dependency and range of sugar contents seen in tomatoes (Amemiya *et al.*, 2005, Beckles, 2012).

In terms of flavour potential in fresh tomatoes, sugars are detected by type II TRC during gustation, providing the characteristic sweetness of fresh fruits. Equimolar amounts of each of the two principal sugars produce drastically different gustatory responses with fructose being perceived as being twice as sweet as glucose and approximately 1.4 times sweeter than sucrose, which is commonly used as a baseline for sweetness conversion (McLaughlin and Margolskee, 1994). The relative sweetness of both glucose and fructose is variable and matrix specific. In their second manuscript, Yamaguchi *et al.* noted that the perceived sweetness of the sugars was influenced by both concentration and the presence of other taste-active compounds, namely glycine, DL-alanine, sodium saccharin and sodium cyclamate. The interrelationship between the compounds could be synergistic, additive or suppressive (Yamaguchi *et al.*, 1970). Moreover, sampling temperature has been shown to alter the intensity of the perceived sweetness, primarily at low sugar content, as demonstrated by Bartoshuk and coworkers in their investigations into sucrose sweetness between 4 to 44 °C (Bartoshuk *et al.*, 1982). The perceived sweetness of sugars can also be suppressed by the presence of acids, creating the desirable and iconic sweet sour sensation present in most fresh fruit as well as moderating the intensity of both sensations (Pangborn, 1961, Bonnans and Noble, 1993, Frank and Archambo, 1986, Mcbride and Finlay, 1990, Schifferstein and Frijters, 1991).

1.5.2 Organic Acid Content of Tomato Fruits

Organic acids are the second most abundant constituent of tomato dry matter content, contributing approximately 10-15% (Balibrea *et al.*, 2006, Petro-Turza, 1986). As with the major sugars of tomato fruit, there are two primary organic acids (OA) that are responsible for sourness in fresh tomato, namely citric and malic acids, with the concentration of citric acid being 5-10 times higher

than malic acid. In addition, the presence of other titratable acids at significantly lower concentrations has been previously reported by several authors. Ascorbic, oxalic, fumaric, pyruvic, tartaric and succinic acids have all been quantified in fresh tomato on multiple occasions (Baldwin *et al.*, 1991b, Beullens *et al.*, 2006, Hernández Suárez *et al.*, 2008a, Stevens *et al.*, 1979). However, most authors agree that the overall acidity of tomato fruits is primarily due to both citric and malic acids and that the other organic acids are not present at sufficient quantities to have a significant influence. Therefore, many studies choose to focus on the quantification citric and malic acid when assessing fruit quality (Anthon *et al.*, 2011, Gautier *et al.*, 2008, Hernández Suárez *et al.*, 2008a, Stevens *et al.*, 1979).

The perception of sourness is based on the presence of H^+ ions and therefore, is dependent on the solubilised acids in the matrix, with each acid presenting a specific profile and intensity. In addition, the pH dramatically influences the perceived sourness of an acid, as the higher the pH, the less disassociated protons are available for detection by taste receptors, thereby decreasing the sourness (Hartwig and McDaniel, 1995, Rubico and McDaniel, 1992). Moreover, weak acids such as citric and malic acid do not exist in a fully disassociated state in solution, commonly only 1-2% of the molecules have ionised and released a proton at any given time in solution (Pangborn, 1963).

There seems to be little agreement between previous works as to the relative intensity of organic acids in food. Makhlouf and Blum claimed that in an equimolar comparison of organic acids, citric acid presented with the most intense sour sensation, followed by tartaric, succinic, lactic, acetic and propionic acids in decreasing intensity (Makhlouf and Blum, 1972). When comparing 4 organic acids added to wine, Ough agreed that citric acid presented as the most sour, followed by fumaric and tartaric acids which were both comparable and finally adipic acid, which was significantly less sour (Ough, 1963). Lugaz *et al.* (2005) noted that, when assessed at equal concentrations the order of potency was hydrochloric, citric, malic, lactic then acetic acid. However, when at equal pH this order was reversed, underpinning the notion that matrix specific conditions have a strong impact on the way acids are perceived. The authors also noted that at concentrations of 100 mM and within a range of $pH < pK_a-1$, the acids had not dissociated and released protons into solution and were, therefore, functioning solely as a source of H^+ ions. It is only protons that act as a stimulus for sourness and the compound and anionic group does not interact with TRC directly, as previously postulated by Ogiso *et al.* (Ogiso *et al.*, 2000, Lugaz *et al.*, 2005). Conversely to these findings, Noble *et al.* determined by using binary mixtures of weak acids that citric acid stimulated a reduced sourness response when compared to malic, tartaric and fumaric acids; which were all comparable, lactic acid was considered the most potent (Noble *et al.*, 1986). Based on the findings published thus far, there are many factors that can affect the perceived sourness of organic acids, namely sampling pH, normality, total titratable acidity

(TTA), compound chemistry degree of disassociation and sensitivity of individual participants. Attempts to create a relative sourness/acidity conversion between common food acids have been partially successful, but direct conversion to a 'relative sourness scale', comparable to that created for sweetness is more difficult due to the number of parameters and sampling conditions that have been shown to affect the way acids are perceived (Moskowitz, 1974, Pangborn, 1963).

1.5.3 Umami Components of Tomato Fruits

Since its original discovery in 1909 by Ikeda, and later acceptance as an independent taste by the wider scientific community in the 1980's, umami has become a vital part of our culinary experiences (Ikeda, 1909, Lindemann *et al.*, 2002). The umami stimulus is known to derive from L-α-amino acids such as L-glutamate and L-aspartate amongst a number of monophosphate 5' nucleotides as explored by Yamaguchi and co-workers. In their study, the authors determined the relative umami sensation of seven different amino acids and 22 nucleotides relative to MSG and inosine monophosphate (IMP) respectively (Yamaguchi *et al.*, 1971). Moreover, the conversion to relative monosodium glutamate (MSG) or IMP was possible due to the well-defined ratio between all other umami active compounds, with no significant variability shown for each compound tested. Therefore, the relative umami concentration (RUC) can be determined for the other umami active compounds relative to both glutamate and IMP, both of which represent 1. Moreover, full conversion to 'Equivalent Umami Concentration' (EUC) as first proposed by Yamaguchi *et al.* is possible using the following equation (Mau, 2005, Yamaguchi *et al.*, 1971):

$$Y = \Sigma a_i b_i + 1218\,(\Sigma a_i b_i)\left(\Sigma a_j b_j\right)$$

Whereby, a_i (%) represents the total concentration and b_i representing the RUC of each umami active amino acid (glutamate or aspartate) and a_j (%) represents the total concentration and b_j representing the RUC of each umami active 5'-monophosphate nucleotide (IMP, GMP, XMP and AMP) (Mau, 2005, Yamaguchi *et al.*, 1971).

Fresh tomatoes have been previously shown to be rich in several umami active compounds, primarily glutamate, aspartate and adenosine monophosphate (Oruna-Concha *et al.*, 2007, Boggio *et al.*, 2000, Yamaguchi and Ninomiya, 2000, Ninomiya, 2002). Of these, glutamate elicits 13 times stronger umami sensation than aspartate. Adenosine monophosphate is also not particularly potent in terms of its umami potential, less than one fifth of the intensity of IMP, however it is the only 5'-monophosphate nucleotide present at significant levels in fresh tomato that contributes to the umami sensation (Mau, 2005, Oruna-Concha *et al.*, 2007). In addition, the content of umami components is highly variable, influenced by both cultivar and growth conditions/location. In a paper by Oruna-Concha *et al.* the content of the primary umami components of 13 different tomato cultivars were determined in both flesh (pericarp minus the exocarp) and pulp (seeds and

locular content). The authors determined that the majority of the umami components were localised in the 'pulp' and demonstrated a range of 2.03-16.5 g/Kg of glutamate between Sainsbury's Basics (unnamed cultivar) and Picollo respectively. Aspartate showed a similar variability per cultivar, with 0.22-1.82 g/Kg between Carosel Flavoripe and Picollo. AMP concentration was more stable, between 238-561 mg/Kg between, Growdena, a beefsteak and Picollo a cherry tomato. Unfortunately, the fruit weights and weight distribution across the measured tissue types were not provided, making a mean value per cultivar difficult to calculate. However, this shows the significance of cultivar in the overall umami content and therefore savouriness of commercial tomato fruits (Oruna-Concha *et al.*, 2007). In addition, the authors analysed Elegance tomatoes both grown in the UK and in Italy, where the 'pulp' glutamate (5.59-2.63 g/Kg), aspartate (0.40-0.38 g/Kg) and AMP (437-252 mg/Kg) for UK vs. Italian fruits were quantified. Both glutamate and AMP levels were significantly different between batches, whereas aspartate remained comparable. This indicates that growth conditions, origin and storage may have a dramatic effect on the umami potential of tomato fruits.

1.5.4 Visual Quality Aspects of Tomato Fruit

Typically, a prospective consumer will judge the appeal of tomato fruit based primarily on a visual inspection before purchasing. Therefore, the appearance of tomato fruits is one of the most influential factors in generating sales. Unlike many other fruits, tomatoes have very little discernible odour when whole and un-damaged, so unlike fruits such as melons, mangoes, peaches etc. the fruit fragrance cannot be used to determine ripeness or product quality. This has been combatted by the industry by selling on-truss tomatoes, which contribute a significant tomato-like odour from the vine/truss itself, whilst giving a perceived increase in value and quality. Consumers will consciously, or possibly sub-consciously, estimate certain visual parameters, namely colour, size and damage (biological or physical) before comparing them to their perceived idea of a perfect fresh tomato. Thereby, barring any avoidable visual issues, primarily in physical damage such as splits, cuts, scrapes, scabs or bruises which can be avoided through proper cultivation and post-harvest handling or any biological damage from fungi/mould or bacterial growth, consumers almost exclusively use fruit colour and firmness as methods of determining ripeness prior to sale.

In recent years a number of varieties that do not display the same ripening colouration as common tomato cultivars have emerged onto the market. Before these cultivars became commercially available, red fruits were considered to be the optimum external colour for ripe tomato fruits in the consumer's eyes. However, recently cultivars such as Sungold (Sainsbury's, 2016), mixed baby tomatoes including some yellow and green/red varieties (TESCO, 2016), Amber and Rosso mixed packs (TESCO, 2016) and Golden Cherry (Waitrose, 2016) have been grown on a commercial scale and sold in stores in the UK, these cultivars are considered to be ripe when the

fruit skin is yellow, orange or even has a green/red flush. Furthermore, the influx in anthocyanin production experienced in cultivars such as Indigo Rose or Blue Berries causes the ripe fruit to develop a deep purple/black skin and flesh, these blue, purple or black fruit are not as common in UK supermarkets, but are sometimes sold in 'Heirloom' or mixed selection packs in supermarkets such as Waitrose, LIDL and M&S.

Colour for consumers, from a visual standpoint, is a very difficult to accurately identify or define. This is largely due to the many factors that influence how the human eye interprets colour, from light intensity, vividness of colour, material or surface effects and light artefacts (López Camelo and Gómez, 2004). There is also the issue of describing the observed colour and/or comparing colours from memory to those being assessed; in the case of comparing tomato fruits in a supermarket to those that had been previously consumed and fulfilled the organoleptic quality criteria desired by the consumer. Due to this a 6-10 point colour scale for tomato ripeness is usually used by growers and buyers when selecting fruits of the desired ripeness. This normally encompasses fruits from 'Mature Green' (MG) which spans the final stage of maturation and beginning of fruit ripening, 'Breaker' (BR), the point at which a fruit can achieve ripeness if removed from the plant, up to 'Red Ripe' (RR) or 'Table Ripe' which is considered the perfect level of ripeness for that particular cultivar. The range of ripening stages and fruit colouration is clearly demonstrated in **Figure 1.3** below. The Mature Green stage of fruit ripening covers all fully matured fruits where the entire surface of the fruit is green, this can range from light to dark green and can vary across the fruit. Breaker fruits show the first shift in colouration from green to either white/pale yellow or pink/orange. This colour change should not cover more than 10% of the skins surface. Turning covers fruits where colour change is apparent on between 10-30% of the fruit surface. The red/orange colour begins to intensify past that of Breaker fruits. Pink and Orange fruits are often banded into a single, wide colour category, spanning 30-60% of the fruit surface. Additionally, the red/orange colour starts to become the predominant colour of the fruit. Light Red fruits are fruits where 60-90% of the fruit surface is red, with only a small amount of green/yellow present. As the name suggests, full carotenoid development has not yet occurred, resulting in a desaturated red colouration. The final stage is referred to as Red Ripe and marks the point at which tomatoes should be available to consume. The full surface of the fruit, minus the calyx, is fully red and the colour has intensified past that of Light Red fruits. These stages are applicable to most commercial tomatoes that follow the typical green to red colour change throughout ripening. However, the more niche heirloom varieties and those that ripen at yellow/orange require tailored ripening charts to enable proper harvest, transport and sale of the fruits.

Figure 1.3 – Top depicts the defined stages of ripening in tomato fruits (*cv.* Tomatoberry). MG – Mature Green (1.), BR – Breaker (2.), T – Turning (3.), P – Pink (4.), O – Orange (5.), LR – Light Red (6.) and RR – Red Ripe (7.). In many cases 'Pink' and 'Orange' stages are merged into the same category. The bottom image shows the process of ripening in tomato fruit (*cv.* Gardener's Delight) on truss, in the image fruits range from MG to RR.

To be able to understand fully the relation of fruit colour to overall ripeness, a rigorous measurement system was required. The first implementation of a quantifiable method of colour identification and numerical representation was instated by the Commission Internationale de l'Eclairage (CIE) in 1931, using a XYZ method of plotting colour values and providing a numerical classification for every colour in the human visual spectrum (López Camelo and Gómez, 2004). In 1948, Hunter introduced L*a*b* as axis nomenclature for the description of colour space. This was then incorporated into the existing CIE system of measure in 1976, resulting in CIELAB, which, alongside derivatives, is the primary method and descriptor used for the plotting of colour space today. The LAB system is referred to as colour space due to the tridimensionality used to represent colour on the scale. L can be a value of 0 to 100 and represents black to white respectively. Red and green is measured by the a* axis, red intensity is represented by positive values between 0 to +127, whereas green is represented by negative values between 0 and -128. The b* axis works identically for blue (negative values) and yellow (positive values)

(Tkalcic and Tasic, 2003, López Camelo and Gómez, 2004). Through the use of 3 axis, a 3D representation of colour can be formed which represents every colour perceivable by the human eye.

1.5.5 Flavour and Aroma of Fresh Tomato

Tomato flavour has been studied extensively over the last 50 years, with over 400 volatile components of fresh tomato identified, encompassing hydrocarbons, alcohols, aldehydes, esters, ethers, ketones, acids, phenols and sulphur containing compounds (Petro-Turza, 1986, Selli *et al.*, 2014, Mayer *et al.*, 2008, Buttery *et al.*, 1988, Buttery *et al.*, 1987, Buttery *et al.*, 1970, Baldwin *et al.*, 2000). However, the perceived flavour of fresh tomatoes, a sweet-sour balance with "green" notes, is due to the intricate interaction between these aromatic volatile components and naturally occurring organic acids and sugars (Petro-Turza, 1986, Tandon *et al.*, 2000, Selli *et al.*, 2014). The complexity of this relationship is still not fully understood; however, specific compounds have been identified as 'character impact volatiles" (CIV); compounds that have a significant influence on the perceived flavour of fresh tomatoes. The number of flavour active volatiles is still uncertain and probably fluctuates dependant on the cultivar and cultivation practices. However, most authors agree that the number of unique volatiles that are present at concentrations in excess of their odour detection thresholds is between 15-40 (Krumbein and Auerswald, 1998, Kazeniac and Hall, 1970, Buttery *et al.*, 1988, Buttery *et al.*, 1987, Baldwin *et al.*, 1991b, Petro-Turza, 1986).

The volatile composition is dependent on factors including, but not limited to, cultivar, fruit ripeness, cultivation practices, harvesting, postharvest treatments and storage conditions (Krumbein *et al.*, 2004, Farag and Paré, 2002, Wu *et al.*, 2018, Ruiz *et al.*, 2005, Buttery *et al.*, 1988, Boukobza and Taylor, 2002, Baldwin *et al.*, 1991b). Volatile production in plants is almost exclusively due to chemical or enzymatic cellular reactions, with pathways identified for lipid, amino acid and carotenoid biosynthesis of volatiles, shown below, in **Figure 1.4** (Mathieu *et al.*, 2009, Baldwin *et al.*, 2000, Tieman *et al.*, 2017, Lee *et al.*, 2018, Bauchet *et al.*, 2017). Goff and Klee hypothesised that the volatile components that characterise the positive aromas of foods may be perceived positively due to their nutrient precursors. Essentially, when we smell an appealing fruit or other foodstuff, it is because we subconsciously correlate the aromas to the nutritionally beneficial derivatives (Goff and Klee, 2006). Conversely, there are numerous odours commonly caused by the degradation or spoilage of foods, often perceived as unpleasant and used as an 'early warning system' to prevent consumption of potentially harmful foodstuffs. Therefore, the presence of volatile compounds can be considered as markers for both poor and desirable nutritional and food safety concerns.

1.5.6 Character Impact Volatiles of Fresh Tomato Flavour

More than 80 years ago the first two volatiles, ethanol and acetaldehyde, were identified as being present in tomato fruit (Gustafson, 1934). Further advances in tomato volatile identification were slow, until the introduction of gas chromatography in the 60's, following which the number of identified volatiles increased dramatically to approximately 400 (Petro-Turza, 1986). Of the more than 400 volatile components of fresh tomato flavour, only 20-40 are thought to significantly affect the perceived flavour of the fresh fruit (Krumbein and Auerswald, 1998, Petro-Turza, 1986, Selli *et al.*, 2014, Tieman *et al.*, 2006b). Volatile compounds that significantly alter the flavour of fresh tomato are commonly referred to as Character Impact Volatiles (CIV) or aroma active compounds. Character Impact Volatiles (CIV) are normally determined and characterised by a complementary set of analyses. Firstly, gas chromatography coupled to mass spectrometry is used to detect, identify and quantify the volatiles present in a system. This provides information on the volatile fingerprint of the matrix. However, alone it does not provide adequate data about the flavours present in the sample. To complement this, olfactometry or aroma extract dilution analysis (AEDA) is used for each of the detected volatile components (Krumbein and Auerswald, 1998, Selli *et al.*, 2014). The sensorial profile of the compounds in question is of upmost importance for the perceived aroma of the product, particularly as many compounds exhibit different aromas based on the concentration and medium of evaluation. AEDA can be used to determine the minimum odour threshold per compound, which can then be compared to the amount present in the sample to determine in the odour of the compound could be perceived. By this method, the less than 40 aroma active compounds present in fresh tomato were determined. The remaining volatiles were found to be present at concentrations lower than their detection thresholds and therefore would be unperceivable (Petro-Turza, 1986).

A recent paper by Selli identified 21 aroma-active, CIV of cherry tomato which consisted of aldehydes (10), alcohols (3), ketones (3), lactone (1), sulphur compound (1) and unidentified compounds (3) (Selli *et al.*, 2014). **Table 1.1** lists the character impact volatiles found to be contributing to the perceived aroma and flavour of fresh, cherry tomatoes (Selli *et al.*, 2014).

Table 1.1 - The 21 most aroma-active volatile compounds found in cherry tomato as determined through GC-MS-O and AEDA by Selli *et al*. (Selli *et al.*, 2014).

No.	Linear Retention Index (LRI)	Volatile Compound	Odour Description	FD Factor
1	1057	1-Penten-3-one	Green, Sweet	8
2	1097	Hexanal	Fresh, cut grass	64
3	1135	(*Z*)-3-Hexenal	Green, grassy	1024
4	1170	1-Penten-3-ol	Buttery, pungent	16
5	1224	Unidentified	Green, grass	8
6	1238	(*E*)-2-Hexenal	Green, leafy	256
7	1320	Unidentified	Boiled, chemical	4
8	1340	6-Methyl-5-hepten-2-one	Floral, green	4
9	1377	(*Z*)-3-Hexen-1-ol	Green	8
10	1396	2-Isobuthylthiazole	Fermented	16
11	1435	(*E*)-2-Octenal	Nutty, cooked, raw peanut	8
12	1460	Methional	Potato, pungent	4
13	1472	(*E,E*)-2,4-Heptadienal	Boiled, potato	32
14	1508	Benzaldehyde	Almond, roasty	4
15	1698	5-Ethyl-2(5H)-furanone	Fruity	4
16	1744	Geranial	Citrus, lemon	8
17	1765	(*E,E*)-2,4-decadienal	Fatty, deep fried	16
18	1808	(*E,Z*)-2,4-decadienal	Fatty, fried	4
19	1798	Geranylactone	Fruity, sweet	8
20	1899	2-Phenylethanol	Floral	32
21	2016	Unidentified	Fruity	8

In total, Selli *et al*. identified and quantified a total of 49 volatile components of cherry tomato, many of which had been previously identified in tomato. Of these, only the 21 compounds detailed in **Table 1.1** were considered to impact the aroma of the fresh fruit significantly. Previously, Buttery and Ling had claimed that fresh tomato aroma can be closely replicated with as few as 10 character impact volatiles, namely (*Z*)-3-Hexenal, (*Z*)-3-Hexanol, Hexanal, 1-Penten-3-one, 3-Methylbutanal, (*E*)-2-Hexenal, 6-Methyl-5-hepten-2-one, Methyl salicylate, 2-Isobuthylthiazole and β-Ionone (Buttery and Ling, 1993, Selli *et al.*, 2014). Interestingly, of these; β-Ionone, 3-Methylbutanal and Methyl salicylate were not only not present in Selli's CIV list, but were unreported for all of their samples (Selli *et al.*, 2014).

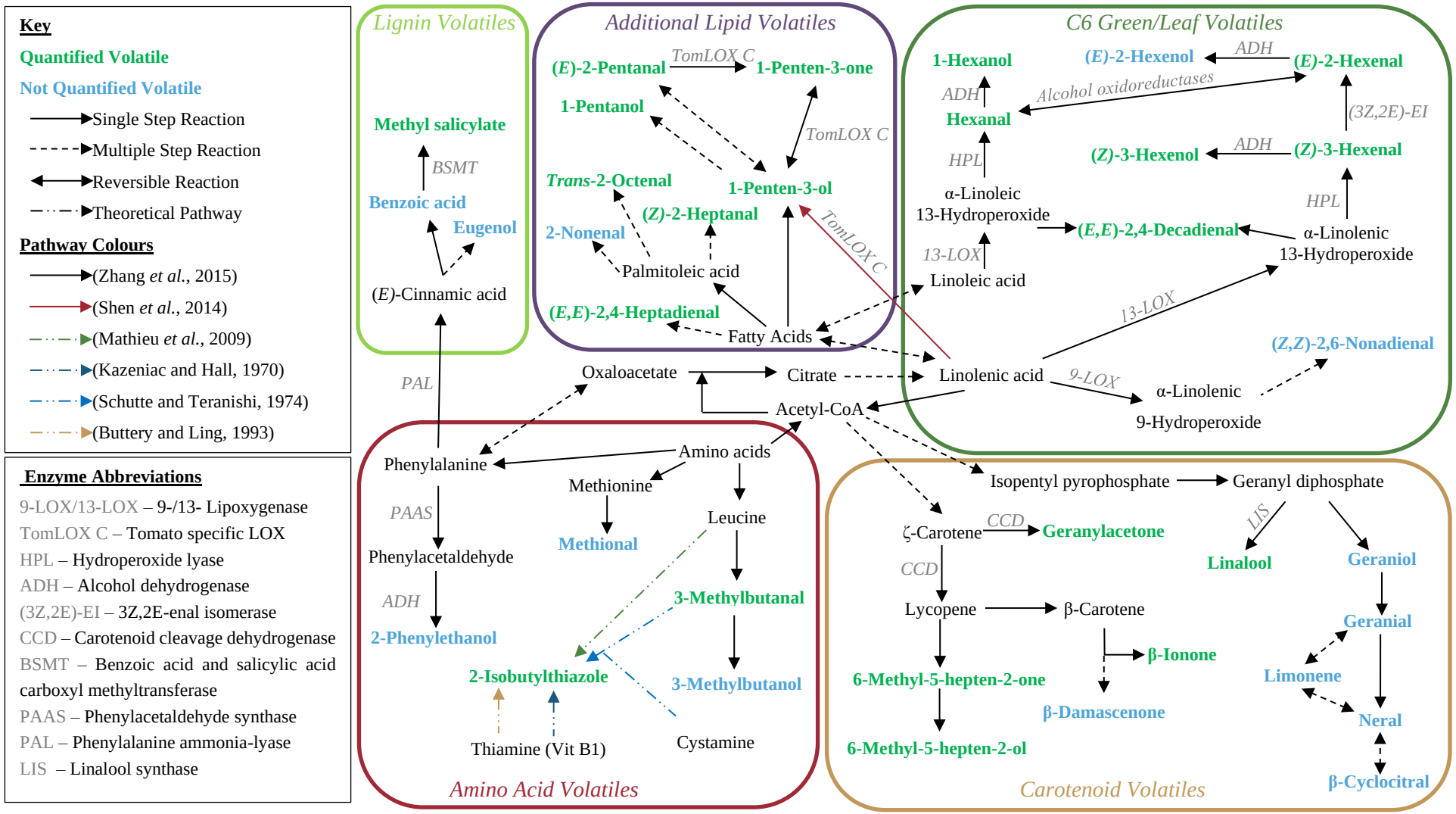


Figure 1.4 - Biosynthetic pathways of the flavour active volatile components of fresh tomato flavour. Adapted from (Zhang *et al.*, 2015). Theoretical pathways for the biosynthesis of 2-Isobutyltiazole from possible precursors are marked by dash/dot/dot lines. At present the route of formation of 2-Isobutylthiazole is unconfirmed.

The routes of formation and biosynthesis of 'character impact volatiles' in tomato are well documented, for the most part. This is due to the importance of understanding the mechanisms and requirements involved in the generation of the primary flavour active compounds, both for the purposes of understanding flavour and as methods of improving it. Many authors have proposed that increasing the levels of flavour active volatiles in commercial cultivars may be the best strategy to combat the perceived loss of quality (Baldwin *et al.*, 2000, Buttery *et al.*, 1988, Mathieu *et al.*, 2009, Tieman *et al.*, 2006b, Tieman *et al.*, 2017). **Figure 1.4** shows the accepted routes of formation of the main, 'character impact volatiles' that have been previously reported, sources of the biosynthesis routes are indicated in the key. The one unconfirmed formation route is that of 2-isobutylthiazole, which has been the subject of intrigue for decades, but at the time of this work, appears to have no confirmed pathway. This list isn't comprehensive, as some compounds are reported in some studies, but omitted in others; those that are represented are the most commonly reported volatiles at levels sufficient to impact the overall aroma and flavour.

Lipid derived volatiles are some of the most important compounds responsible for the flavour of fresh tomatoes. They can be separated into six carbon, linoleic or linoleic acid derived green/leaf volatiles and a wider complement of volatiles derived from various fatty acids and enzymatic processes. Due to the importance of the six carbon volatiles they are discussed in depth in the following section, so this section focuses on the remaining lipid derived volatiles. The formation of C5 volatiles, 1-penten-3-one, *cis*-2-pentenal and 1-penten-3-ol, is not as well understood. A pathway involving the oxygenation of 13(*S*)-hydroperoxy-9(*Z*),11(*E*),15(*Z*)-octadecatrienoic acid (13-HPOT) by LOX and subsequent β-scission was proposed by Salch *et al.*, but the mechanism was not elucidated (Shen *et al.*, 2014, Salch *et al.*, 1995). The study conducted by Shen *et al.* determined that the proposed pathway was correct and that the C5 volatiles are formed from further action of 13-LOX on 13-HPOT and was independent of HPL. Moreover, in 'knockdown' HPL tomato plants there was a decrease in C6 aldehydes, increasing the available substrate for further 13-LOX oxygenation of the hydroperoxides and yielding increased amounts of C5 volatiles (Shen *et al.*, 2014). The method by which 13-LOX interacts with 13-hydroperoxides of either linoleic or linolenic acid was not well described by Shen and coworkers. The formation of double bond containing aldehydes such as decadienal, nonadienal and heptadienal in tomatoes does not have been thoroughly discussed. Grosch and Schwartz showed that 2,4-decadienal formed from linolenic acid in ^{14}C spiked cucumbers from a cucumber LOX isoform. However, Leflaive and Ten-Hage also demonstrated its formation from arachidonic and eicosapentaenoic acid in algae, along with 2,4-Heptadienal (Leflaive and Ten-Hage, 2009).

The lipid derived volatiles are almost exclusively products of the action of the initial lipolytic acyl hydrolase(LAH)/ lipoxygenase (LOX) pathway. Fatty acids are liberated from membrane bound phospholipids, by either the enzymatic action of LAH or tissue damage/disruption. LAH works by hydrolysing glycerolipids, commonly phospholipids, LAH hydrolyses the ester bond between glycerol and the fatty acids. This initiates the action of LOX, substrates of which contain multiple *cis* double bonds, which cleaves the carbon backbone of linolenic (18:3) and linoleic (18:2) acid at either the 9^{th} carbon, by 9-LOX or the 13^{th} carbon, by 13-LOX. The resulting fatty acid 13-hydroperoxides, are then oxidatively cleaved by HPL to form the C6 aldehydes, hexanal, *cis*-3-hexenal and *trans*-2-hexenal (Shen *et al.*, 2014). Moreover, the action of alcohol dehydrogenase on these aldehydes results in the formation of 1-Hexanol, (*E)*-2-Hexenol and (*Z*)-3-Hexenol. The second route, through 9-LOX, appears to be less active and produces less volatiles than the 13-LOX. The resulting fatty acid 9-hydroperoxide of linolenic acid yields (*E,E*)-2,4-nonadienal, which is not particularly prevalent in tomato

Many of the fruity flavours and aromas involved in fresh tomato flavour can be attributed to volatiles derived from carotenoid breakdown or through sequential enzymatic reactions of isoprenoid precursors, namely isopentenyl pyrophosphate (IPP) and geranyl diphosphate (GPP). Those derived from isoprenoids such as IPP include geraniol, geranial, limonene, citral and cyclocitral. They are characterised by floral and fruity aroma profiles and tend to occur in low concentrations in fresh tomato. Carotenoid derived volatiles in tomato comprise β-Ionone, geranylacetone, 6-methyl-5-hepten-2-one and 6-methyl-hepten-2-ol (Tieman *et al.*, 2006b, Lewinsohn *et al.*, 2005). In the study conducted by Lewinsohn *et al.* the volatile profile of several types of tomato were comparted to the native carotenoid levels in an attempt to identify possible routes of biosynthesis of the volatiles. It was noted that wild type, red skinned tomatoes, contained approx. 85 and 10 % of total carotenoids, in acyclic lycopene and bicyclic β-carotene respectively. Complementary to this, wild type tomatoes accumulated higher levels of the acyclic volatiles, such as 6-methyl-5-hepten-2-one and geranylacetone, than the monoterpenes geranial, citral and the mono-cyclic, β-ionone (Lewinsohn *et al.*, 2005). Lewinsohn *et al.* suggest that many of the carotenoid derived volatiles, do not have just one possible precursor, but that they can form from a range of the tetraterpinoid pigments between phytoene and β-carotene in the carotenoid synthesis pathway, as shown in **Figure 1.5**. This is in agreement with the formation pathways first proposed by Zhang *et al.* and shown in **Figure 1.4** above where alternative routes of formation are proposed for certain volatiles. In a study by Vogel *et al.* the volatile complement of tomato mutants showing altered carotenoid development was determined. The mutants included a *yellow-flesh* mutant lacked phytoene synthase-1 (PHY-1) gene, responsible for the condensation of two molecules of geranylgeranyl phosphate into phytoene, which is the precursor of all other carotenoids in tomato. This cultivar was shown to produce significantly lower levels of all carotenoid derived volatiles due to complete interruption of carotenoid biosynthesis. Tangerine

tomatoes are unable to isomerise *tetra-cis*-lycopene to all-*trans*-lycopene, resulting in orange flesh and resulting in suppression of cyclic carotenoid volatiles, but a concomitant increase in linear volatiles such as geranylacetone. Similarly, 'Old Gold' lacks the ability to β-cyclise all-*trans*-lycopene, again resulting in elevated linear and suppressed levels of cyclic volatiles (Vogel *et al.*, 2010).

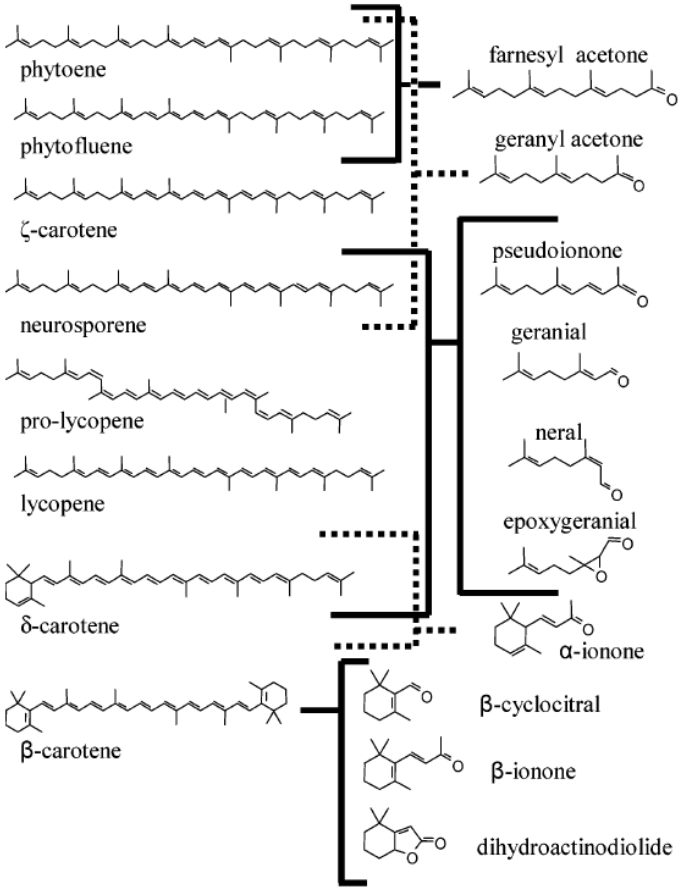


Figure 1.5 - Proposed precursors of carotenoid derived volatiles in tomato fruit (Lewinsohn *et al.*, 2005)

Of the amino acid derived volatiles, isovaleraldehyde (3-methylbutanal) and 3-methylbutanol are believed to be derived from leucine, 2-methylbutanal from isoleucine, 2-phenylethanol from phenylalanine and methional from methionine (Baldwin *et al.*, 2000, Buttery and Ling, 1993).

1.5.7 Proposed Biosynthetic Pathways of 2-Isobutylthiazole

Of all the volatiles that are widely accepted as influencing the perceived aroma/flavour of fresh tomato fruits, 2-Isobutylthiazole is the only one where the precursors and route of biosynthesis has not been determined. Suggestions for precursors and intermediates have been proposed by several authors, but, as of yet, the pathway has yet to be elucidated. There are three main

propositions for the precursors of 2-Isobutylthiazole which are visualised in **Figure 1.4** - amino acids (primarily Leucine), conversion of 3-Methylbutanal via cystamine or Thiamine (Vitamin B1).

Speculation surrounding the routes of formation of 2-isobutylthiazole has been ongoing for close to 50 years, which no definitive route/pathway, enzymes or QTL having been revealed. Initial characterisation and confirmation of 2-isobutylthiazole using synthetic standards seems to have been done by Buttery and Ling in 1970. The authors commented both the importance of 2-isobutylthiazole in tomato aroma as well as the high degree of variance in its abundance between cultivars (Buttery *et al.*, 1970). Additionally, it was noted that 4,5-thiazoles have been confirmed as precursors of thiamine, with the incorporation of sulphur, most likely from a reaction with methionine. Although this wasn't strictly proposed as a mechanism of formation for 2-isobutylthiazole, the basis for its possible formation from thiamine/methionine was presented. Buttery and Ling also suggested amino acids, specifically Leucine or Isoleucine as possible precursors for 2-Isobutylthiazole, in later investigations into volatile biosynthesis in tomato (Moretti *et al.*, 2002). The possibility of branched chain amino acid precursors, such as leucine and isoleucine, was investigated by Kochevenko *et al.* in 2012 through supplementation with ^{13}C labelled branched chain amino acids (BCAA) (Kochevenko *et al.*, 2012). The authors concluded that there was little evidence for BCAA involvement in the formation of 2-isobutylthiazole or any of the other volatiles that were monitored. However, branched chain keto acids were linked to the increase in volatile production. Transamination of suitable amino acid precursors, potentially leucine, to α-keto acids and subsequent addition of a thiazole, potentially through cyclisation of cystamine, may be the true route of formation. The involvement of cystamine has also been previously proposed by Schutte and Teranashi in their review on sulphur containing volatiles. The authors make particular comment on the formation of 2-isobutylthiazole within intact fruits, a unique phenomenon, not seen in other thiazole containing compounds which are formed through processing, particularly high heat treatments (Schutte and Teranishi, 1974). Schutte and Teranashi refute the biosynthetic pathway via Thiamine proposed by Kazeniac and Hall, noting that thiazoles derived from thiamine are more commonly substituted at the 4- and 5-positions, and not the 2- which would be required for 2-isobutylthiazole formation. Instead the authors propose that the reaction of the abundant, leucine-derived, volatile isovaleraldehyde, 3-methylbutanal, and cystamine would likely produce the desired result. The proposed route is visualised in **Figure 1.6** below.

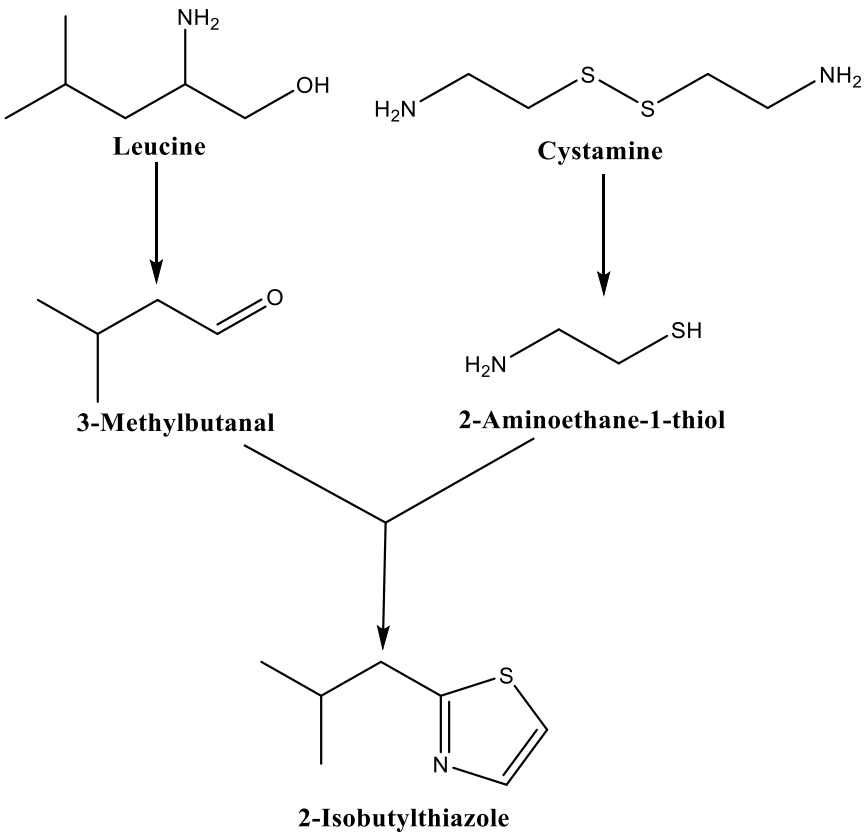


Figure 1.6 – Biosynthetic route for the formation of 2-isobutylethiazole from the leucine derived volatile 3-methylbutanal and cystamine, as proposed by Schutte and Teranishi (1974)

Of all of the proposed pathways, this seems the most likely to yield 2-isobutylthiazole, however the enzymes and genes involved in the pathway are as yet unknown. Additionally, there appears to be no published studies that took Schutte and Teranashi's advice to pursue further understanding and confirmation of this biosynthetic route using radio labelled ^{14}C isovalealdehyde. Fernandez and coworkers were able to chemically synthesise thiazoline and a number of thiazole containing, aroma active volatiles based on the proposed biosynthetic route put forward by Schutte and Teranashi. Although 2-isobutylthiazole was not one of the end products of their work, this suggests that the proposed route is capable of yielding the compound (Fernandez *et al.*, 2002).

1.5.8 Enzyme Cascade and the Formation of C6 Volatiles through Tissue Disruption

Six carbon (C6) 'leaf' aldehyde volatiles, primarily hexanal and *cis*-3-hexenal, which are generated through the sequential lipoxygenase (LOX) and hydroperoxide lyase (HPL) pathway are largely responsible for the characteristic 'green' notes in tomato flavour (Riley and Thompson, 1998, Selli *et al.*, 2014, Petro-Turza, 1986, Baldwin *et al.*, 2000). The C6 aldehydes are known to form due to autoxidation of fatty acids, but are primarily formed during polyunsaturated fatty acid metabolism, particularly from linoleic and linolenic acids, which are relatively abundant in fresh tomato fruits as shown in **Figure 1.7** (Feussner and Wasternack, 2002, Riley *et al.*, 1996).

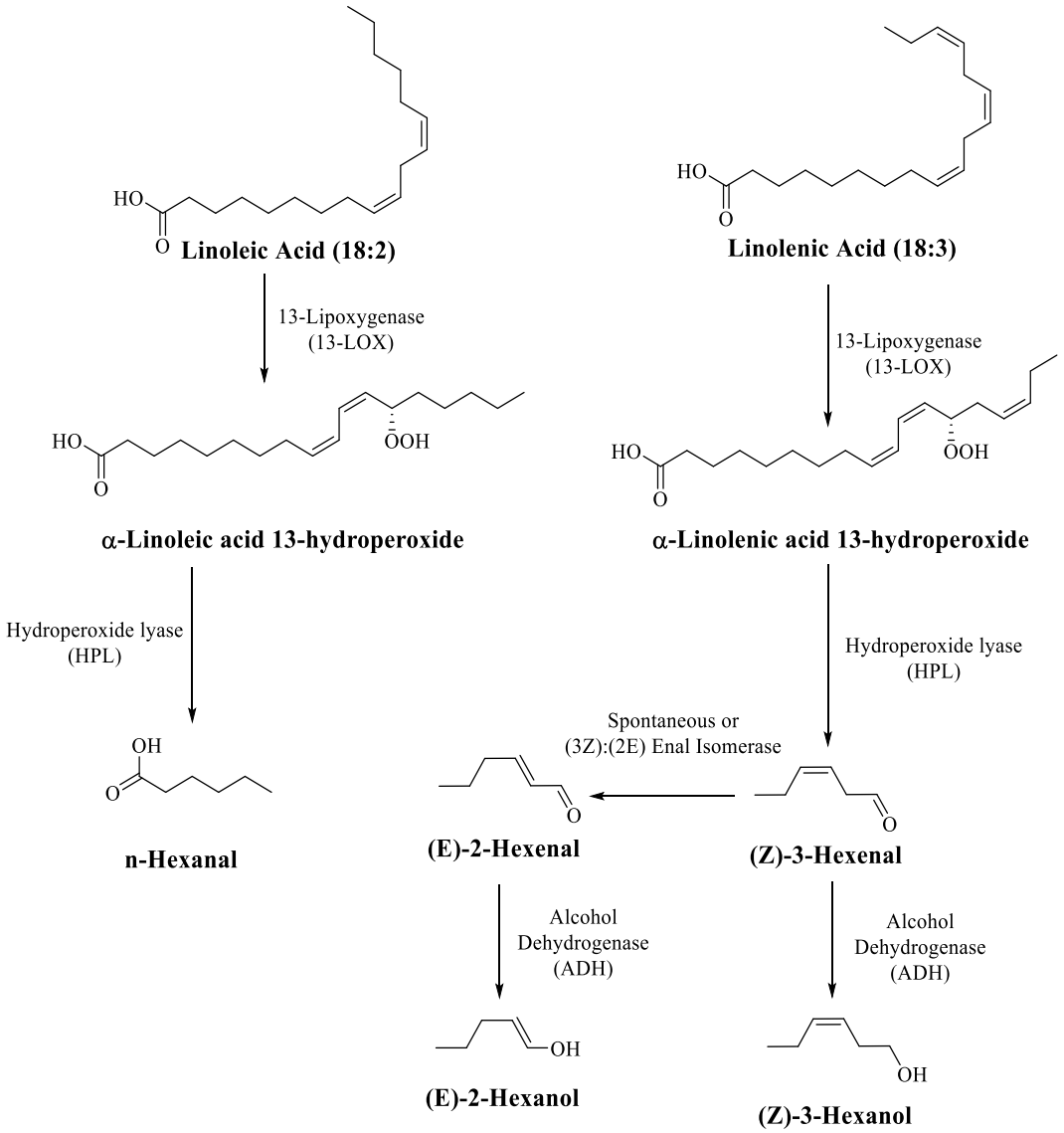


Figure 1.7 - Enzyme-catalysed, metabolism of polyunsaturated fatty acids (PUFA) in plants by the sequential action of 13-lipoxygenase (LOX), hydroperoxide lyases (HPL) and alcohol dehydrogenase (ADH) resulting in the formation of C6 aldehydes and alcohols commonly referred to as leaf aldehydes.

Most lipoxygenases show preferential enzyme catalysed metabolism of free fatty acids, rather than those in the phospholipid bilayer, preventing degradation or alteration of cellular membranes during the process (Feussner and Wasternack, 2002). Furthermore, by separating the skin, outer flesh and inner flesh of tomato fruits Hatakana *et al.* were able to show that most of the LOX activity occurs in the outermost layer of flesh, adjacent to the skin. It was claimed that stressors such as wounding, tissue damage and UV irritation increases LOX action and subsequent formation of C6 aldehydes; this has been subsequently confirmed both in tomato (Hatanaka *et al.*, 1992, Riley and Thompson, 1998, Riley *et al.*, 1996) and other plants. However, it is also believed that some degree of LOX/HPL occurs in whole, undamaged fruits as both Hatakana *et al.* and Riley and Thompson detected low levels of hexanal and *cis*-3-hexenal/ trans-2-hexenal respectively during their study into volatile formation in ripening tomato fruits. Furthermore, it

was seen that the naturally occurring levels of both aldehydes was not affected by fruit ripeness, as both green and red-ripe tomatoes had comparable baselines when enzymatic action was inhibited with $CaCl_2$ (Riley and Thompson, 1998, Hatanaka *et al.*, 1992).

Riley and Thompson identified that these aldehydes are generated upon tissue disruption or damage, and not during fruit ripening. Furthermore, it was seen that ripe, red tomatoes had a circa 4-5-fold higher capacity to generate volatile C6 aldehydes than under-ripe, green fruits. It was further speculated that it was not the levels of endogenous hexanal and *cis*-3-hexenal that increased with fruit ripeness, but the activity of LOX and HPL, and therefore the potential for their generation, possibly increased during fruit development and ripening (Riley and Thompson, 1998). Riley, Willemot and Thompson had previously identified a 2-fold increase in microsomal LOX activity between the mature green and breaker stages of tomato fruits, hypothesising that this may account for the increases in volatile aldehyde generation as tomato fruit ripen (Riley *et al.*, 1996).

1.5.9 Aroma and Flavour Profile of Tomato Character Impact Volatiles

The flavour and aroma of fresh tomato is due to the complex complement of volatile compounds, both present in tomato and synthesised in response to tissue damage or disruption. Due to the number of volatiles present, molecular chemistries, biosynthesis pathways, precursor compounds and aroma profiles it is hard to fully define tomato aroma. **Table 1.2** below lists most of the volatiles thought to impact the flavour and aroma of fresh tomato together with their aroma profiles, odour detection thresholds and dilution factors where available. The odour detection values have been collected from multiple sources and determined using trained sensory analysis panels. The value represents the minimum parts per billion of the target volatile in water that is required for 50% or more of the panellists to detect it and is a common measure of the efficacy of odours and aromas. Flavour Dilution factors (FD) are commonly associated with aroma extract dilution analysis (AEDA) using gas chromatography olfaction (GC-O). Hereby, the odourant is sequentially diluted and passed through the GC-O system with a selection of sensory panellists able to smell the resulting aroma. As the level of dilution increases the intensity of the aroma decreases until it is undetectable. The last detectable value can then be determined from the range of panellists. The aroma profiles are sourced from a reputable aroma database, The Good Scents Company, designed for the perfume and flavour industries. The character of the compounds in question was provided in aqueous media, and at a level of dilution that allowed for good aroma profiling and reduced the aggressiveness of certain aromas.

One of the more predominant aromas of fresh tomato is the green/grassy odours that form from lipid oxidation. Although the comparison is made between green and freshly cut grass, green aromas are often considered synonymous with tomato-like flavour and aroma during sensory

analysis due to the importance of lipid oxidation products in the overall character of fresh tomato. The most abundant of these are the C6 aldehydes and alcohols that derive from linolenic and linoleic acids. Hexanal, *cis*-3-hexenal, *trans*-2-hexenal and *cis*-3-hexanol are commonly reported as being present at levels above their odour detection values in fresh tomato. Each of these compounds provides a subtly different aroma profile, however they share the common link of presenting a green/grassiness as a primary note as shown in **Table 1.2**. Although the C6 aldehydes are responsible for the majority of the green aroma present in tomato, a number of other compounds also contribute to the sum total of the experience. Of these only 2-isobutylthiazole, 6-methyl-5-hepten-2-one and geranylacetone are not lipid oxidation products. The leucine derived 2-isobutylthiazole has a very different aroma profile to the C6 aldehydes, presenting a more spicy/peppery and capsicum-like aroma to complement its green notes than the grassier lipid derived volatiles. The two carotenoid derived volatiles present with green as a back note, with a more fruity primary character. Unfortunately, due to the varying odour detection thresholds, abundance, volatility and aroma character of each of these volatiles it is not feasible to calculate a "total green" flavour or aroma for the fruits and therefore the component parts must be assessed individually.

The more fruity and floral aroma and flavour of tomato fruits is largely provided by the volatiles derived from carotenoid catabolism or degradation. One of the reasons that under-ripe fruits are as not appealing to the consumer is due to the lack of carotenoids available both for colour and the formation of sweet/fruity/floral volatiles. The precursors of these compounds have been previously illustrated in **Figure 1.5**, showing that a number of aroma active volatiles derived from carotenoids form throughout the carotenoid biosynthesis pathway. These compounds can be broadly split into light/citrus/fruity volatiles formed from carotenoid precursors such as geranyl diphosphate and the sweet/fruity/woody volatiles that from directly from carotenoid breakdown. Many of the descriptors used for the fruity and floral volatiles are accurate for the compound in isolation, but little of that character is discernible in fresh tomatoes due to the variety of other aromas.

The remaining volatiles largely provide more earthy/woody/vegetable-like off aromas, which is paramount to the overall tomato flavour. Although many of the earthy or mouldy off-notes are unpleasant in isolation, they are a counterpoint to the high, green and fruity notes provided by other volatiles, helping fresh tomatoes to retain their savoury flavour. This is particularly apparent in the amino acid and lignin derived volatiles, although off-notes are also contributed by lipid and carotenoid derived volatiles.

The complexity of aromas provided by the volatiles present in fresh tomato is paramount to the overall experience and flavour of the fruits. Although tomatoes contain some aromas that are not

characteristic of overall tomato flavour, they are an important component of the experience as a whole. The intensity of the green aromas acts as a counterpoint to the light and sweeter, fruity and floral notes. Additionally, the woodier, earthier aromas, help to provide a deeper flavour and supress some of the fruity, sweeter aromas. This synergism and combination helps to balance the culinary position that tomatoes are in where they are a fruit that commonly used as a vegetable, requiring traits and flavours of each.

Table 1.2 – Odour profile of each of the volatiles shown in **Figure 1.4** many of which are considered to be Character Impact Volatiles in fresh tomato aroma and flavour. Odour detection thresholds shown in ppb and medium of analysis, (aq.) – aqueous, n/a – not available. Flavour Dilution (FD) factors are presented as those represented by various studies. Source of the value is indicated by superscript characters with full references shown at the bottom of the page.

Compound	Odour Strength	Odour Profile	Odour Detection Threshold (ODT) ppb (*aq.*)	Flavour Dilution (FD) Factor
Amino Acid Derived				
2-Methylbutanal	High	Musty, chocolate, nutty, malty, fermented	0.2 [d]	
3-Methylbutanal	High	Ethereal, aldehylic, chocolate, peach, fatty	0.2 [a]	128 [g]
3-Methylbutan-1-ol	Medium	Fusel, alcoholic, pungent, ethereal, cognac, fruity, banana, molasses	250 [a]	
2-Phenylethanol	Medium	Sweet, floral, fresh, bready, rose, honey	1000 [a]	
Methional	High	Musty, potato, tomato, earthy, vegetable, creamy	0.2 [b]	256 [g]
2-Isobutylthiazole	High	Green, wasabi, privet, tomato leaf, earthy, vegetable, metallic	3.5 [a]	4 [g]
Lignin/Cell Wall Derived				
Eugenol (Nat.)	Medium	Sweet, spicy, clove, woody, phenolic, savory, ham, bacon, cinnamon, allspice	6 [b]	
Benzoic acid (Nat.)	Low	Balsamic, urine	n/a	
Methyl salicylate	Medium	Sweet, root beer, wintergreen, aromatic, phenolic, camphoreous	40 [a]	16 [g]
Carotenoid Derived				
6-Methyl-5-hepten-2-ol	Medium	Sweet, oily, green, coriander	2000 [b]	
6-Methyl-5-hepten-2-one	Medium	Fruity, apple, musty, ketonic, creamy, cheesy, banana	50 [a]	4 [h]
Geranylacetone	Medium	Fresh, green, fruity, waxy, rose, woody, magnolia, tropical	60 [a]	8 [g]
β-Ionone	Medium	Sweet, fruity, woody, berry, floral, seedy	0.007 [a]	
β-Damascenone	High	Woody, sweet, fruity, earthy, green, floral	0.002 [b]	4 [g]
S-Linalool	High	Sweet, floral, petitgrain, lavender	6 [b]	16 [g]

Geraniol	Medium	Floral, sweet, rosey, fruity and citronella-like with a citrus nuance	40-75 [f]	
Geranial	Medium	Citrus, lemon	32 [b]	8 [g]
Limonene	Medium	Citrus, orange, fresh, sweet	10 [c]	
Neral (Citral)	Medium	Sweet, citrus, lemon, lemon peel	30 [f]	
β-Cyclocitral	High	Tropical, saffron, herbal, clean, rose, sweet, tobacco, green, fruity	5 [b]	
Lipid Derived				
Hexanal	High	Green, fatty, leafy, vegetable, fruity, clean, woody	4.5 [a]	2048 [g]
1-Hexanol	Medium	Pungent, ethereal, fusel, oily, fruity, alcoholic, sweet, green	500 [c]	
cis-3-Hexenal	High	Green, fatty, grassy, weedy, fruity, apple	0.25 [a]	4096 [g]
trans-2-Hexenal	High	Green, banana, aldehydic, fatty, cheesy	17 [a]	16 [g]
cis-3-Hexenol	High	Green, grassy, melon rind, pungent	70 [a]	4 [g]
trans-2-Hexenol	High	Fresh, fatty, green, fruity, vegetable, leafy, herbal	400 [f]	
(*trans*,*trans*)-2,4-Heptadienal	High	Fatty, green, oily, aldehydic, vegetable	137 [e]	8 [g]
(*trans*,*trans*)-2,4-Decadienal	High	Oily, cucumber, melon, citrus, pumpkin, nutty	0.07 [f]	32 [g]
trans-2-Heptenal	High	Green, fatty	13 [a]	4 [g]
1-Penten-3-ol	High	Pungent, horseradish, green, vegetable, tropical, fruity	400 [b]	16 [h]
1-Penten-3-one	High	Pungent, ethereal, peppery, garlic, mustard, onion	1 [a]	128 [g]
trans-2-Pentenal	High	Pungent, green, fruity, apple	1500 [d]	
trans-2-Octenal	High	Fresh, cucumber, fatty, green, herbal, banana, waxy, green, leafy	3 [c]	8 [h]
Nonanal	High	Waxy, aldehydic, citrus, fresh, green, lemon, peel, cucumber, fatty	0.08-0.1 [f]	4 [g]

All odour profiles sourced from The Good Scents Company with those from newer published works prioritised (www.thegoodscentscompany.com). Aqueous profiles were prioritised over those in alcohol/glycerol where possible as they are more comparable to a tomato-based evaluation medium.

a - Buttery *et al.* (1987), b - Buttery *et al.* (1990), c - Buttery *et al.* (1970), d - Baldwin *et al.* (2000), e - , f - Leffingwell (2018), g - Krumbein and Auerswald (1998), h - Selli *et al.* (2014)

1.5.10 Organoleptic changes throughout Fruit Development and Ripening

As is exclusively true of fleshy fruits, tomato fruit ripening is primarily used by the plant as a method of seed dispersal (Klee and Giovannoni, 2011). The appearance and organoleptic qualities of these fruits change throughout ripening to provide a more palatable and appealing, edible fruit for animals to consume, thereby ensuring thorough seed dispersal for the plant. This is often achieved through decreased acidity, increasing sugar content, colour changes and softening of the cell wall (Klee and Giovannoni, 2011).

As with all fruits, tomatoes undergo drastic physiological and chemical compositional shifts as the fruit grows and matures throughout ripening. Tomato fruit are climacteric; and, as such, ripen due to the effect of the plant hormone ethylene, which is produced and released by the fruit as ripening commences. The production and expulsion of ethylene by the fruits is accompanied by an increase in respiration as the fruit begins to physically and chemically alter (Alexander and Grierson, 2002). Typically, ripening is initiated in a small number of cells. As the initial cells increase the production of ethylene it diffuses into neighbouring cells, which in turn stimulates and initiates the process of ripening. In tomatoes on the vine, ripening often begins around the calyx, and spreads down the fruit in a 'wave' inciting complete fruit ripening. Over the course of ripening, chloroplasts are converted to chromoplasts and the chlorophyll they contain is degraded and replaced by carotenoids and tocopherols which are synthesised during ripening (Marano and Carrillo, 1991). The catabolism of chlorophyll leads to a reduction in the intensity of green colouration in the fruits, often appearing white/pale at the mature green stage, as carotenoids accumulate the fruit pass into the 'Breaker' stage, by which they are a green flushed yellow colour. Shortly after the 'Breaker' stage is reached, the remaining chlorophyll is degraded and the carotenoid composition is the primary factor driving the colour; by this stage, the transition from yellow to red is relatively linear. Due to the predictable colouration of fruits across varying ripening stages, it is the primary attribute used by both the industry and consumers to assess the palatability and quality of the fruits. Although colour alterations are the most obvious change that occurs within ripening fruit, from a quality standpoint they are some of the least important, as they mainly provide information on the other ripening induced aspects that result in edible fruits. Fruit firmness is also drastically altered as fruits reach maturity. Cells begin to modify and catabolise parts of the cell walls, reducing the structural integrity of the cells and resulting in softening of the fruits. Unlike colour formation, degradation of the cell walls continues past the desirable level of ripeness for consumption of the fruits, leading to an unpalatable degree of fruit softening at the end stages of the process when fruits are considered over ripe. Due to this, fruit texture is a better measure of over-ripe fruit than colour is.

Flavour is a vital characteristic for all foodstuffs, many consumer complaints in recent years have identified poor flavour of fresh tomatoes. Many of the compounds that are involved in fruit quality

and flavour as generated or accumulate due to fruit ripening and therefore the process of ripening has a direct impact on the end quality of the fruits. Ripening has been previously shown to determine the final concentration of sugars, organic acids, free amino acids and volatile compounds all of which have a direct influence on the organoleptic quality of the fruit (Gautier *et al.*, 2008, Oms-Oliu *et al.*, 2011, Wu *et al.*, 2018, Baldwin *et al.*, 2000, Sorrequieta *et al.*, 2010). Sugars have been previously shown to accumulate as tomato fruits begin to ripen, reaching peak levels in table ripe (TR) fruits. Gautier *et al.* noted a 3.4 fold increase in the reducing sugar content of Cervil cherry tomatoes from approximately 14.5 g/Kg to 50 g/kg between mature green (MG) and table ripe (TR) respectively. The authors also noted that the total titratable acidity was highest in MG tomatoes at ~115 meq H^+/Kg, decreasing by 28% during ripening to 83 meq H^+/Kg in TR fruits (Gautier *et al.*, 2008). Sugar accumulation is in part due to import into fruits during ripening and maturation and partly due to starch degradation and resulting release of glucose and fructose (Ho, 1988). The reduction of citric and malic acids can be attributed to cellular respiration including the citric acid cycle (TCA) which involves the consumption of citrate and malate, and the conversion of citric and malic acid to sugars through gluconeogenesis (Anthon *et al.*, 2011). The conversion of acids to sugars through gluconeogenesis is the most likely route for the decrease seen during fruit ripening.

The translocation, specifically accumulation or reduction of compounds within ripening tomato fruits has been previously studied (Arias *et al.*, 2000b, Betancourt *et al.*, 1977, Damon *et al.*, 1988, Robinson *et al.*, 1988, Valle *et al.*, 1998, Yelle *et al.*, 1988). As many authors have shown, many compounds involved in the flavour of fresh tomato - organic acids, sugars and amino acids, are transported, via the phloem, to the fruits during maturation and ripening. Furthermore, several papers have investigated the changes in chemical composition in fruits that are removed from the truss at mature green stages, as opposed to field ripened fruits (Arias *et al.*, 2000b, Betancourt *et al.*, 1977, Kader *et al.*, 1977). There is some disagreement between authors as to the impact of off-the-truss ripening as opposed to in-field ripened tomato. Raffo *et al.* found that "Naomi" cherry tomato fruits that reached full ripeness on the plant had significantly higher levels of lycopene and slightly higher levels of both β-carotene and α-tocopherol. However, they also reported that the water-soluble antioxidant activity decreased due to the reduced concentration of a number of phenolic compounds, including chlorogenic and caffeic acids (Raffo *et al.*, 2002). Kader *et al.* found that when studying "Cal Ace", "Cherry", "Calmart" and "Early Pak 7", there was a significant reduction in reducing sugar content in fruits picked at both "Mature Green" or "Light Pink" and ripened off the plant when compared to those removed from the plant at "Table Ripe". Additionally, "Cal Ace" fruits showed a significantly lower concentration of titratable acids in fruits ripened off the vine (Kader *et al.*, 1977). This agrees with the findings of a paper published by Betancourt *et al.* in the same year, where it was seen that both the sugar and titratable

acid content was reduced in “Ace 55” and “Rick High Sugar” fruits removed from the plants at a ‘Breaker’ stage as opposed to field ripened fruits (Betancourt *et al.*, 1977).

During its vegetative stage, before the set of the fruit, tomato plants amass energy in the form of stored carbohydrates through photosynthesis in their leaves. Sucrose, the primary photoassimilate, is slowly transferred from leaves to fruits as the ripening process is initiated; however, tomato fruit have very low, if any sucrose (Beckles, 2012, Baldwin *et al.*, 2008, Damon *et al.*, 1988, Petro-Turza, 1986). This is due to intracellular invertases hydrolysing the sucrose to produce glucose and fructose, both of which are abundant in ripe tomato fruits (Wang *et al.*, 1993). As discussed by Walker *et al.*, there is an inverse relationship between the rate at which the plant transfers sucrose from the leaves and the concentration of hexose sugars in the fruits (Walker and Ho, 1977, Wang *et al.*, 1993). Walker *et al.* further noted that the size of the fruit had an inverse relationship on the amount of sucrose transported from the leaves; this seems accurate as cherry tomatoes, by weight, can contain 2-3 times more soluble sugars (glucose, fructose etc.) than beefsteak or salad types. The authors, whose study involved exposing tomato plants to C^{14} CO_2 to monitor the transfer of C^{14} photoassimilates between their origin in the leaf and several parts of the plant, including the fruit, also identified C^{14} malic and citric titratable acids between the leaves and fruits of the plants. This indicates that the titratable acids that amass in the fruits during ripening are also metabolised in the leaves and transferred through the phloem to the fruits, rather than being metabolised in the fruits themselves (Walker and Ho, 1977). Baldwin *et al.* noted that, during ripening, fumaric and malic acids decreased as the fruit reached the final stages of ripeness, whereas citric, oxalic and succinic acids were all seen to increase, which is in agreement with previous findings by Davies, who noted a decrease of malic and concomitant increase of citric acid during ripening (Baldwin *et al.*, 1991b, Davies, 1966). This increase in citric and decrease in malic acid does not appear to be absolute, as previous studies have noted a decrease in both acids between the MG and TR ripening stages (Raffo *et al.*, 2002). Additionally, it was seen that amino acids, specifically glutamic and aspartic acids, both of which are key umami flavour components, were synthesised and transferred into the fruits throughout ripening. Walker and Ho’s study alone provides a wealth of information regarding the synthesis, transport and concentration increases of many flavour active components in tomato fruits throughout ripening (Walker and Ho, 1977).

Volatile formation throughout tomato fruit ripening has been previously studied (Buttery and Ling, 1993, Buttery *et al.*, 1970, Buttery *et al.*, 1987, Buttery *et al.*, 1988, Kazeniac and Hall, 1970, Riley and Thompson, 1998, Riley *et al.*, 1996, Baldwin *et al.*, 1991b). It has been widely reported that a large proportion of tomato fruit volatiles increase significantly from the onset of ripeness until the fruit reaches full maturity. This is probably due to an increase in many of the precursors of these volatiles, which also amass during the ripening process; these range from

carotenoids and free amino acid, both of which have been shown to increase in the fruits throughout ripening. In particular, carotenoids are not present in 'Mature Green' tomato fruit, so carotenoid derived volatiles such as Geranylacetone, 6-Methyl-5-hepten-2-one, 6-Methyl-5-hepten-2-ol and β-Ionone, which are known carotenoid breakdown products lack a precursor and therefore are of very low abundance in fruits that have not yet reached the 'Breaker' stage (Baldwin *et al.*, 2000).

Baldwin *et al.* monitored the concentrations of 5 volatiles in ripening tomato fruits and noted that four of the five, including *cis*-3-hexenal, *trans*-2-Hexenal, Acetaldehyde and *trans*-2-*trans*-4-decadienal increased significantly over the course of fruit ripening (Baldwin *et al.*, 1991b). Riley and Thompson showed that 'Mature Green' tomato fruits produced little to no volatile C6 aldehydes upon maceration and incubation, where 'Ripe Red' fruits showed approximately a 5-fold increase. They hypothesised that this was due to less lipolytic acyl hydrolase (LAH) being present in green fruits, which led to less linoleic and linolenic fatty acids being liberated from membrane phospholipids. Subsequently, the lack of substrate for the lipoxygenase (LOX) and hydroperoxide lyase (HPL) pathway resulted in supressed formation of C6 aldehydes (Riley and Thompson, 1998).

1.6 Omic Studies and Metabolite and Biomarker Identification

Tomatoes have been the target of quality focused research for over 50 years, with studies characterising flavour, taste, nutrition, optimum growth conditions and disease resistance of the fruit (Kader *et al.*, 1978, Petro-Turza, 1986, Quinet *et al.*, 2006, Tieman *et al.*, 2017). Many of the findings of these original studies were performed using out-dated techniques and reduced instrument sensitivity. With the expansion of –omics technologies the potential for both more detailed and intricate study of biological systems is possible. Additionally, advancements in scientific technology and analytical instruments enables the accurate quantitation of greater numbers of compounds in single analytical runs. Due to the broad spectrum and hierarchical nature of omics technologies they are very complementary, often referred to as system biology. This enables the sequential correlation between the genome, transcriptome, proteome and metabolome of organisms for the most comprehensive picture of the biochemistry of matrix.

1.6.1 Application of Metabolomics to Biological Systems

Metabolomics, otherwise known as small molecule analysis, is a comparatively new field of –omics technologies capable of providing comprehensive profiles of all primary and secondary metabolites in a biological system (de Vos *et al.*, 2007, Moco *et al.*, 2006, Wishart, 2008). Metabolomic applications are extensive; assisted by the ability to quantify large numbers of small metabolites (<1500 Da) per analyses, metabolomics has found its place in human, animal,

microbial and plant-based studies (de Vos *et al.*, 2007, Wishart, 2008). As with many emerging technologies, metabolomics is still being honed to provide the most comprehensive picture of biological metabolomes as feasibly possible. To do this, several technologies are being utilised as methods for metabolomic data collection, primarily LC-MS, GC-MS and NMR spectroscopy (Wishart, 2008). Further expansion of the technology is likely, in part due the refinement of the analytical processes, but also due to the establishment of multiple online, open access metabolomic databases (Moco *et al.*, 2006). The population of online databases and resources is of paramount importance for the evolution of the field and for the better understanding of the primary and secondary metabolites in each system. Due to the chemical diversity of most biological systems, it is almost impossible to get a fully comprehensive profile of a biological system using a single analytical technology and, therefore, it is not uncommon for multiple analytical and sample preparation routes to be used to provide as much in-depth information as possible. Initially, metabolomics was confined to clinical and pharmaceutical applications, however, with the technology advancing, various industries, including food and agriculture, have begun exploiting it as a method of metabolomic fingerprinting, quality assurance and product authenticity analyses (Wishart, 2008). The dynamic interplay between genomics, transcriptomics, proteomics and metabolomics now allows relationships between variations in the proteome to be tied to the resulting shift in the metabolome, elucidating the effect of specific genes and enzymes on the production of metabolites within the system (Tikunov *et al.*, 2005).

The application of metabolomics can be classified into profiling for targeted studies and fingerprinting for untargeted approaches (Dunn and Ellis, 2005). Profiling, otherwise known as targeted metabolomics, involves the collection of quantitative or semi-quantitative data on a narrow band of preselected metabolites of interest. Often this approach is used to focus on the metabolic shift in a class of compounds or the effects of external factors on specific pathways or biosynthesis routes. Fingerprinting, is a broad spectrum, untargeted approach to metabolite analysis. Rather than focusing on a specific group of compounds or certain pathway, as in profiling, a snapshot of as much of the metabolome as possible is acquired. This is more commonly used as a method of differentiation between sample groups through data reduction and visualisation techniques, such as principal component analysis (PCA) or correlative approaches such as partial least squares (PLS) or least squares regression analysis (LSRA).

There are two primary forms of metabolomic analysis, targeted and untargeted (Cevallos-Cevallos *et al.*, 2009, Dunn and Ellis, 2005, Weckwerth, 2003). In reviews published by Weckwerth and Theodoridis *et al.*, it is claimed that, in the spirit of other –omics techniques, metabolomics, by definition, must be an untargeted and comprehensive snapshot of a biological system, proposing that targeted approaches are not true –omics (Weckwerth, 2003, Dunn and Ellis, 2005). However, both have their uses in food science and plant-based analyses, having

entirely different functions and aims. With targeted metabolomics a limited number of total metabolites are the focus of the analysis; this will generally require identification and quantification of compounds, both through MS library identification and authentication and calibration using pure standards (Cevallos-Cevallos *et al.*, 2009). Due to the quantitative nature of targeted metabolomics it is known as metabolomic profiling. Commonly, targeted metabolomic analyses are used to monitor classes of compounds and biosynthetic pathways in biological systems through accurate quantitation of the resulting metabolites and intermediates. Conversely, untargeted metabolomics is a large-scale 'snapshot' of all metabolites present in a biological system and is commonly referred to as metabolomic fingerprinting (Cevallos-Cevallos *et al.*, 2009, Wishart, 2008). Due to the volume of data collected during untargeted analysis, multivariate data analysis coupled with data reduction and visualisation techniques, such as principal component analysis (PCA) or partial least squares regression (PLS), can be used for class-based discrimination and classification purposes (Dunn and Ellis, 2005, Hall, 2006, de Vos *et al.*, 2011).

1.6.2 Metabolomics of Tomato Fruits

Since the emergence of efficient metabolomic workflows, the metabolome of tomato fruit has been extensively studied, occupying the niche of a model fleshy fruit (de Vos *et al.*, 2011). Due to the economic importance of tomato fruits much of the research has been focused around organoleptic and nutritional research, as this is of significant commercial and cultivatable value to the industry (de Vos *et al.*, 2011, Tieman *et al.*, 2017, Wang and Seymour, 2017). Following the domestication of tomato plants, which has produced numerous cultivars with specific organoleptic and nutritional characteristics, ~95% of the wild type genome has been lost (Perez-Fons *et al.*, 2014). As the final quality aspects of the fruit are directly related to the metabolites and, therefore, intrinsically linked to the genome of the cultivar, it is advantageous to be able to identify fluctuations and variations the in the metabolome of new cultivars. From this, it may be possible to elucidate which metabolites contribute to the desirable or undesirable characteristics present in the fruit, and which genes and enzymatic pathways are responsible for their production (de Vos *et al.*, 2011, Perez-Fons *et al.*, 2014, Causse *et al.*, 2004, Causse *et al.*, 2002, Fulton *et al.*, 2002). **Table 1.3** below highlights some of the previous metabolomic work on tomato fruits that has been conducted in the last 15 years. A range of technologies has been employed including LC-MS, GC-MS and NMR for untargeted profiling studies. Many authors have focused on small elements of the overall metabolome, such as isoprenoids or organoleptic components.

Table 1.3 – Examples of previous studies that have investigated the tomato metabolome, either using targeted or non-targeted metabolomic workflows. Several studies have employed a number of complementary technologies to acquire as comprehensive a coverage as possible over the detected metabolites.

Author	Summary
Moco *et al.* (2008)	Integration of NMR and LC-MS based metabolomics workflow on 50 cultivars using pooled samples. The different selectivity of the analytical methodologies enabled a wide coverage of detected metabolites. Inter-correlation between both technologies was successful in revealing multiple highly correlated features.
Tikunov *et al.* (2005)	Large scale SPME-GC-MS profiling of tomato volatiles between 94 tomato cultivars. Tomato fruits were pooled to create representative samples per cultivar. Strong correlations between specific volatiles/groups of metabolites were observable.
Moco *et al.* (2006)	The study created pooled tomato samples from a combination of 96 different cultivars and turning, pink and red ripe fruits, so achieve broad tomato, rather than cultivar specific metabolite coverage. In addition, a metabolomic database for tomato was created and the data from this work was made publicly available.
DiLeo *et al.* (2011)	Ten replicate fruits of three cultivars were assessed by non-targeted NMR profiling. Weighted Correlation Network Analysis (WGCNA) was used to identify relationships with the most abundant detected metabolites in the different cultivars.
Gómez-Romero *et al.* (2010)	LC-MS and UV profiling of the metabolites of three tomato cultivars. The authors tentatively identified 135 compounds, with 21 being the first reported instance in tomatoes. Many of the compounds were phenolic, including hydroxybenzoic or hydroxycinnamic acids, phenylacetic acids, flavanoids and glycosides.
Bino *et al.* (2005)	Investigation into the secondary effects of *hp* mutant tomato fruits that are known to increase lycopene formation. Through LC-MS and GC-MS based metabolomics some of the associated upregulated compounds, including isoprenoids and multiple volatiles. Many of these also provide health benefits alongside the touted increased lycopene concentration.
Oms-Oliu *et al.* (2011)	Five replicate fruits of eight different ripening stages of *Plaisance* tomatoes were profiled by GC-MS to identify shifts in primary metabolite concentrations. Many of the metabolites that were significantly in flux over the ripening period were related to organoleptic quality, including amino acids, organic acids and sugars alongside compounds that are the precursors for important volatile constituents.

1.7 Hypothesis

Due to the natural variability of tomato cultivars it is predicted that the chemical and sensorial profiles will be as distinguishable as the cultivars themselves. The difference in chemical composition of organoleptically impactful compounds and other secondary metabolites will allow for the classification and discrimination of different tomato cultivars. In addition, the stage of ripening and tissue of tomato fruits will display observable differences, allowing for discrimination. Due to the distinct chemical composition of different cultivars, there will be strong correlations between certain aspects chemical profile and organoleptic assessments.

2 Methods and Materials

2.1 Tomato Fruits for Chapters 3, 4, 6, 7 and 8.

Trusses of 10 cultivars (Elegance-ELE/EL2, Temptation-TEM, Sunstream-SUN, Orange Cherry-ORA, Picollo-PIC, Campari-CAM, DR2, 194, Juanita-JUA, and Axiani-AXI) of commercially grown tomatoes were sourced from Thanet Earth Marketing (Kent, UK) over several sampling periods, each lasting for 5 weeks. Representative images of each of the cultivars can be seen in **Appendix 1**. The winter harvest of fruit took place February-March 2015 and the summer harvest took place in September and October 2015. Trusses were hand-picked on Monday mornings and transported by courier to Gilden Photonics in Glasgow for Hyperspectral Imaging. Following this they were transported by courier Northumbria University where they were held at 15 °C in MIR-154 cooled incubators (Panasonic Biomedical, Loughborough, UK) until they were either snap frozen and extracted or subjected to destructive analyses (Instron, pH and Brix), all samples were imaged prior to any analysis. Fruits selected for chemical analysis were removed from the vine, weighed and snap frozen. Salad varieties were cut prior to snap freezing, ¼ of each fruit was used for Elegance and ½ of each fruit for Temptation, Campari and 194, fruits were frozen within 5 seconds of the initial cut. All other cultivars were frozen whole. Samples were snap-frozen in liquid nitrogen (LN_2) then removed and placed into a Model A11B analytical mill (IKA, Staufen, Germany) where they were homogenised for 5-10 seconds. The homogenate was transferred to 50 mL falcon tubes and stored in a -80 °C Forma 88300V63 freezer (Thermo Scientific, Langenselbold, Germany) until required for analysis. The following analyses were perform on these fruits for the associated chapters.

Table 2.1 - List of analyses performed on the 11 cultivars for Chapters 3, 4, 6, 7 and 8.

Analysis	Analytical Instrument
Digital Imaging	DigiEye
pH and °Brix	pH meter and refractometer
Sugars	Plate reader (UV)
Organic acids	Plate reader (UV)
Amino acids	GC-MS (EZ:FAAST)
Nucleotides	HPLC-UV
Volatiles	Headspace SPME GC-TOF-MS
Metabolomic profiling	Q-Exactive LC-MS
MS2 fragmentation	Q-Exactive LC-MS

2.2 Tomato Fruits for Chapter 5.

Fresh salad (cv Valkiria), baby plum (cv Angelle) and cherry (cv Genio) tomatoes were sourced from a local supermarket in 2016 for sensory analysis and 2017 for chemical analysis. Representative images of each of the cultivars can be seen in **Appendix 2**. Tomatoes were cut in half with a scalpel, with care taken not to crush the pericarp of the fruits and the locule fluid was isolated from the gel and seeds using 1.2 mm stainless steel mesh, this process took less than 5 min per cultivar. The locular fluid was transferred into Eppendorf tubes and immediately snap frozen in liquid nitrogen and stored at -80 °C until required for analysis. The pulp and seeds were separately snap frozen and ground into a homogenous powder in a Model A11B analytical mill (IKA, Staufen, Germany). The frozen homogenate was then transferred to 50 mL centrifuge tubes and stored at -80 °C pending extraction and further analyses.

Table 2.2 – List of analyses performed on the 3 cultivars for Chapter 5.

Analysis	Analytical Instrument
pH and °Brix	pH meter and refractometer
Sugars	Plate reader (UV)
Organic acids	Plate reader (UV)
Amino acids	GC-MS (EZ:FAAST)
Volatiles	Headspace SPME GC-TOF-MS

2.3 L*a*b* Colour Analysis by DigiEye

Trusses were inverted and imaged using a DigiEye digital imaging system (VeriVide, Leicester, UK) using a mounted Nikon D7000 SLR digital camera (Nikon, Melville, NY). The system used diffuse lighting settings and a pale blue backboard for all images. Calibration of the system was performed using a DigiTizer version 3.41 colour chart (VeriVide, Leicester, UK). Images were saved as uncompressed, .TIFF, RGB files. Following this, L*a*b*, Chroma and Hue values were extracted using the DigiPix software (VeriVide, Leicester, UK) for DigiEye using a custom filter designed to sample primarily red colours and eliminate artefacts on the fruit, such as surface reflectance and scabs/abrasions.

2.4 Whole Fruit pH

Samples previously punctured on the Instron were subsequently analysed for pH using a S220 SevenCompact pH meter with an InLab Solids Pro probe with automatic temperature control (Mettler-Toledo, Leicester, UK). The pH probe was calibrated using the supplied

calibration solutions at pH 4.01, 7.0 and 10.0 prior to use. The probe was then inserted into the locular cavity of the fruit, amongst the seeds and the pH taken.

2.5 Soluble Solids as % Brix

Following pH analysis each fruit was crushed and the juice separated from the seeds and flesh. 3-4 drops of the juice was transferred to a HI-96801 digital refractometer with automatic temperature compensation capable of measuring 0-85% Brix (Hanna Instruments, Leighton Buzzard, UK) and analysed. Brix measurements were given to 2 decimal places and as % soluble solids.

2.6 Extraction of Nucleotides, Sugars, Organic Acids and Free Amino Acids

A method adapted from that used by Oruna-Concha *et al.*(Oruna-Concha *et al.*, 2007) was used for the extraction of nucleotides and amino acids. Frozen, homogenised tomato (500 mg) was weighed into 2 ml Micro-centrifuge tubes. Hydrochloric acid (1 mL, 0.01 N) was added to the vial and the sample shaken using a SI-0266 Vortex Genie 2 (Scientific Industries Inc, Bohemia, NY) with attached horizontal microtube holder at speed setting 9 for 20 min. The sample was then centrifuged for 10 min at 10,000 g in a Harrier 18/80 refrigerated centrifuge (MSE, London, UK) at 4 °C. Following centrifugation the supernatant was transferred to a fresh microtube and the extraction was repeated with the addition of a further 1 mL of HCl to the tomato solids in the original microtube. Following the second extraction, the supernatants were combined, lyophilised and resuspended in 400 µL HCl and frozen at -20 °C pending analysis. Samples were slowly passed through a 13 mm, 0.22 µ regenerated cellulose syringe filter that had been preconditioned with deionised water prior to instrumental analyses. Extraction efficiencies of each of the analytes of interest were calculated to be >95%.

2.7 Quantitative Analysis of Nucleotides by HPLC

An aliquot (400 µl) of extract from the above procedure was transferred to a 1 mL disposable syringe attached to a 17 mm, 0.22 µm regenerated cellulose filter. The sample was passed through the regenerated cellulose filter, drop wise, and collected in a clear autosampler vial with a 300 µl insert and stored at -80 °C until required for analysis at which point it was held at 7 °C in the autosampler. Analysis was performed using a Dionex UltiMate 3000 HPLC system attached to an RS Diode Array Detector (Thermo Scientific, Langenselbold, Germany). Samples were injected onto an Acclaim AmG C18 (3.0µ, 3.0 x 150mm) (Thermo Scientific, Langenselbold, Germany) with a AmG C18 guard column, maintained at 17 °C. Eluents were 30 mM phosphate buffer, 15mM triethylammonium acetate and 0.1% formic acid at pH 5.3 (A) and methanol (B).

Prior to analysis, the column was equilibrated in 100% A for 600 min to allow consistent interaction with the ion-pairing reagent. The analytical method, including an equilibration step is shown in **Table 2.3** below.

Table 2.3 Multi-step gradient of the analytical HPLC method for the determination of nucleotides and nucleosides.

Time	Eluent A	Eluent B	Curve
-5.00	100	0	5
0.00	100	0	5
7.00	100	0	5
18.00	80	20	9
23.00	25	75	5
25.00	25	75	5
28.00	100	0	5
30.00	100	0	5

Individual nucleotides were identified by comparison to the pure standard (Sigma-Aldrich, Gilligham, UK) and quantified using the absorbance at 254 nm.

2.8 Quantitative Analysis of Amino Acids by GC-MS

A 100 µL aliquot of the supernatant collected from the nucleotide and amino acid extraction was derivatized using the EZ:FAAST amino acid derivatization kit (Phenomenex, Torrance, CA). The resulting sample was analysed on a Agilent Technologies 6890N Network Gas Chromatograph coupled to a 5973N Mass Selective Detector and GC Sampler 80 autosampler (Agilent Technologies, Santa Clara, CA) using the method detailed by Elmore *et al* (Elmore *et al.*, 2005).

2.9 Quantitative Analysis of Volatiles by HS-GC-TOF-MS

Volatile analysis was carried out using a 7890A GC System (Agilent Technologies, Santa Clara, CA) coupled to a Bench TOF-dx (ALMSCO International, Llantrisant, UK) and Combi PAL System (CTC Analytics, Zwingen, Switzerland). Homogenised, snap-frozen tomato (500 mg ± 25 mg) was weighed into amber SPME vials and enzymes were deactivated using a 25% NaCl solution at pH 2.0 using 0.05M H_3PO_4. Volatile extraction was performed through agitation (40 °C, 40 min, 250 rpm, 5 s on/2 s off) during which a 65 µm PDMS/DVB fibre (Supelco Analytical, Bellefonte, USA) was inserted into the vial headspace for 1 min. Volatiles were thermally desorbed at 250 °C for 5 min onto a 60 m VF-WAXms capillary column (250 µm i.d., 0.25 µm film thickness). The GC oven was set to 40 °C, held for 5 min, following a temperature

ramp at 4 °C/min to 180 °C and held for 1 min, finally the temperature was increased at 20 °C/min to 260 °C and held for 5 min. The helium flow was set at 1 mL/min throughout. The mass spectrometer was operated at positive, full scan mode (m/z 30-450). The detector voltage was optimized and set between 2,000-2,150 V, depending on the analysis date. Electron impact (70 eV) mass spectra were acquired within m/z 45–460 at a scanset rate of 1.98 Hz. Data analysis was performed using Enhanced ChemStation (Agilent Technologies, Santa Clara, CA). External calibration was performed for each of the CIV of interest, all standards were sourced from Sigma-Aldrich. Calculated Linear Retention Index (LRI) values for the studied analytes can be seen in **Table 2.4** below.

Table 2.4 – Observed Retention Time (RT) and calculated Linear Retention Index (LRI) for the volatile compounds under investigation in this study. Values marked with an asterisks are tentative due to low detectability of the nonadecane peak required for LRI calculations.

Analyte	RT	LRI
Isovaleraldehyde	6.93	881
1-Penten-3-one	9.95	991
Hexanal	12.17	1,053
trans-2-Pentanal	14.60	1,118
1-Penten-3-ol	15.67	1,146
trans-2-Hexenal	16.79	1,175
cis-3-Hexenal	17.42	1,191
1-Pentanol	19.11	1,236
6-Methyl-5-hepten-2-one	21.90	1,312
cis-3-Hexen-1-ol	23.33	1,353
2-Isobutylthiazole	24.14	1,376
Nonanal	24.54	1,387
trans-2-Octenal	25.77	1,424
6-Methyl-5-hepten-2-ol	26.65	1,450
trans,trans-2,4-Heptadienal	26.94	1,459
Linalool	29.40	1,535
Methyl Salicylate	35.65	1,744
cis-Geranylacetone	38.53	1,834*
Benzyl Alcohol	39.01	1,846*
β-Ionone	40.02	1,873*

2.10 Quantitative Analysis of D-Glucose and D-Fructose

The concentration of D-glucose and D-fructose was determined using a commercially available assay kit (K-FRGLQR, Megazyme, Ireland) and absorbance measurements using a Spark 10M complete with lid lifter and two, external reagent pumps (TECAN Trading AG, Switzerland). Enzymatic reactions were carried out in UV-Star 96-well plates (Greiner Bio One, Stonehouse, UK) by following the procedure described by the kit manufacturer. Duplicate, 6-point calibration curves were prepared between 0.6-9.0 µg of each sugar per well on each experimental plate. Aliquots (1 µL) of the general purpose extraction were pipetted into the remaining wells, each sample analysed in duplicate. Reagent 1 (40 µL) was added to each well, followed by deionised water as a balance to make up the volume of each well to 203 µL. A magnetic patch was stuck to the lid and the plate was placed inside the Spark 10M plate reader ready for analysis. The plate reader methodology consisted of an initial pre-heating time to allow the incubator to reach 37 °C, followed by 20 s of orbital shaking at speed setting 1.5. The plate was then transferred to the incubation compartment for 5 min, before a baseline absorbance reading (A_1) (340 nm) was taken.

Immediately after 20 µL of Reagent 2 was added to each well by injector 1 and the plate was shaken and transferred for incubation (as above). After 20 mins the plate was removed and a further absorbance reading (A_2) was taken at 340 nm, this measured the absorbance change between A_1 and A_2 following the enzymatic conversion of all D-glucose and ATP to glucose-6-phosphate and ADP through the action of hexokinase. G-6-P and $NADP^+$ was then converted to gluconate-6-phosphate, NADPH and H^+ by glucose-6-phosphate dehydrogenase. The increase in absorbance is due to NADPH formation, which is stoichiometric with D-glucose. Following this, 20 µL of Reagent 3 was deposited in each well by injector 2 and the plate was shaken and incubated as above. After 20 mins of incubation a final absorbance reading (A_3) at 340 nm was taken. The final absorbance reading identifies the difference in absorption between A_2 and A_3, caused by the conversion of D-fructose and ATP to fructose-6-phosphate and ADP through the action of hexokinase. F-6-P is then converted to G-6-P through the action of phosphoglucose isomerase, which then reacts with $NADP^+$ to form further NADPH; this reaction is stoichiometric with fructose concentration. The amount (µg) of D-glucose per well was calculated using the formula $A_2 - (A_1 * 203/223)$. Additionally, the amount (µg) of D-fructose per well was calculated using the formula $A_3 - (A_2 * 223/243)$.

2.11 Quantitative Analysis of L-Malic Acid

The concentration of L-malic acid was determined using a commercially available assay kit (K-LMALQR, Megazyme, Ireland) and absorbance measurements using a Spark 10M complete with lid lifter and two, external reagent pumps (TECAN Trading AG, Switzerland). Enzymatic reactions were carried out in UV-Star 96-well plates (Greiner Bio One, Stonehouse, UK) by following the procedure described by the kit manufacturer. Duplicate, 7-point calibration curves were prepared between 0.6-13.8 µg of L-malic acid per well on each experimental plate. Aliquots (1 µL) of the general purpose extraction were pipetted into the remaining wells, each sample analysed in duplicate. Solution 1 (50 µL), Solution 2 (20 µL) and Suspension 3 (2 µL) were added to each well, followed by deionised water as a balance to make up the volume of each well to 272 µL. A magnetic patch was stuck to the lid and the plate was placed inside the Spark 10M plate reader ready for analysis. The plate reader methodology consisted of an initial pre-heating time to allow the incubator to reach 37 °C, followed by 20 s of orbital shaking at speed setting 1.5. The plate was then transferred to the incubation compartment for 3 min, before a baseline absorbance reading (A_1) (340 nm) was taken. Immediately after 20 µL of Reagent 2 was added to each well by injector 1 and the plate was shaken and transferred for incubation (as above). After 20 mins the plate was removed and a further absorbance reading (A_2) was taken at 340 nm, this measured the absorbance change between A_1 and A_2 following the enzymatic conversion of all L-malic acid and NAD^+ to oxaloacetate and NADP through the action of L-malate dehydrogenase. Oxaloacetate and L-glutamate was then converted to 2-oxaloglutarate and L-

aspartate by glutamate-oxaloacetate transaminase. The increase in absorbance is due to NADP formation, which is stoichiometric with L-malic acid. However, the conversion of L-malic acid to oxaloacetate is reversible and the reaction equilibrium causes the reformation of L-malate. Therefore, oxaloacetate is immediately converted to 2-oxalglutarate to prevent the breakdown of the NADP. The amount (µg) of L-malic acid per well was calculated using the formula A_2 - (A_1 * 203/223).

2.12 Quantitative Analysis of L-Citric Acid

The concentration of L-citric acid was determined using a commercially available assay kit (K-CITR, Megazyme, Ireland) and absorbance measurements using a Spark 10M complete with lid lifter and two, external reagent pumps (TECAN Trading AG, Switzerland). Enzymatic reactions were carried out in UV-Star 96-well plates (Greiner Bio One, Stonehouse, UK) by following the procedure described by the kit manufacturer. Duplicate, 7-point calibration curves were prepared between 0.1-10.0 µg of L-citric acid per well on each experimental plate. Aliquots (10 µL) of a $^1/_{10}$ dilution of the general purpose extraction were pipetted into the remaining wells, each sample analysed in duplicate. Solution 1 (50 µL), Solution 2 (20 µL) and Suspension 3 (2 µL) were added to each well, followed by deionised water as a balance to make up the volume of each well to 272 µL. A magnetic patch was stuck to the lid and the plate was placed inside the Spark 10M plate reader ready for analysis. The plate reader methodology consisted of an initial pre-heating time to allow the incubator to reach 25 °C, followed by 20 s of orbital shaking at speed setting 1.5. The plate was then transferred to the incubation compartment for 3 min, before a baseline absorbance reading (A_1) (340 nm) was taken. Immediately after 20 µL of Reagent 2 was added to each well by injector 1 and the plate was shaken and transferred for incubation (as above). Solution 4 was made up with 10 times more deionised water than recommended in the kit so as to be useable in the injector system. After 20 mins the plate was removed and a further absorbance reading (A_2) was taken at 340 nm, this measured the absorbance change between A_1 and A_2 following the enzymatic conversion of all L-citric acid to oxaloacetate and acetate through the action of citrate lyase. Oxaloacetate, alongside NADH and H^+ was then converted to L-malate and NAD^+ through the action of L-malate dehydrogenase. The decrease in absorbance is due to the conversion of NADP to NAD^+, which is stoichiometric with L-citric acid acid. However, endogenous oxaloacetate decarboxylase present in the sample may convert some oxaloacetate to pyruvate, preventing quantitation. Therefore, any pyruvate is immediately converted to D-lactate by D-lactate dehydrogenase, a stoichiometric process that also degrades NADP to NAD^+. The amount (µg) of L-citric acid per well was calculated using the formulas $\Delta A = A_2 - (A_1 * 272/292)$ and $g/L_{sample} = \Delta A_{sample} / \Delta A_{standard} \times g/L_{standard} \times F$.

2.13 Metabolomics Extraction and Methodologies

2.13.1 Sample Extraction and Preparation

The extraction methodology of De Vos *et al.* was used for the metabolomic profiling in Chapters 7 and 8. Tomatoes were grown, shipped, stored and homogenised as previously described above. Centrifuge tubes, 50 mL, containing frozen tomato homogenate were suspended in liquid nitrogen during sampling. A subsample, 500 mg ±25 mg, was transferred to precooled, 2 mL centrifuge tubes. Ice cold, 100%, LC-MS grade methanol containing 0.125% formic acid, 1.5 mL, was pipetted into the tube and the contents shaken and then vortexed for 10 s. Tubes were placed in floating racks in a Sonorex RK 510 H sonicating bath (Bandelin, Berlin) and sonicated at nominal 160 W and 35 Hz in an ice slurry for 20 mins. Tubes were dried and then centrifuged for 3 min at 12,500 rpm in a Harrier 18/80 refrigerated centrifuge (MSE, London, UK) centrifuge until the insoluble material formed a firm pellet. An aliquot of the supernatant, 400 µL, was pipetted into a maximum recovery vial and frozen at -80 °C overnight. Following thawing of the samples precipitation of a creamy, yellow precipitate had formed on the base of the vial, thought to be lipids. Once at room temperature, a 250 µL aliquot was removed and placed into a fresh maximum recovery vial and stored at -80 °C until required for analysis.

2.13.2 Chromatographic Separation of Extracted Metabolites

The chemical analysis were performed on a Dionex UltiMate 3000 UHPLC system (Dionex, Sunnyvale, CA) connected to a Thermoscientific Q-Exactive mass spectrometer system (Thermo, Loughborough, UK). Chromatographic separations was achieved using a Waters T3 high strength silica (HSS) C18 UHPLC column (150 x 1.8 mm, 1.7 µm) (Waters, Elstree, UK) with a flow rate of 0.4 mL/min operating at 45 °C and a 5 µL injection volume. Samples were held at 4 °C within the autosampler module prior and following injections. A binary solvent system was used for the LC mobile phase and consisted of Buffer A (Ultrapure water 18.2 Ω collected from a Millipore Deionizer (Milli-Q Integral 3), ≤ 2 ppb TOC and 0.1% formic acid) and Buffer B, which consisted of LC-MS Optima grade acetonitrile with 0.1% formic acid. The LC profile was as follows: 0 min (5% B) hold for 1 min and then a linear gradient to 100% B at 12 mins, hold for further 2 min (wash period) and return starting conditions at 14 min with a column stabilization time of another 4 mins. The total run time per analytical run is approximately 20 min when including needle wash cycles.

2.13.3 Mass Spectrometer Settings

The heated electrospray ionization (HESI) introduction source, the capillary temperature and voltage were maintained at 325 °C and 3.8 KV (Positive mode)/ 3.5 KV (Negative mode) respectively. (N_2) sheath flow was set to 45, an auxiliary flow was set to 15 and a sweep flow of

5 was applied (all arbitrary units). The radio frequency of the S-lens was set to 50. For MS1 profiling the mass spectrometer was operating at 17.5K mass resolution with a scan rate of 13.2 scan/s^{-1} with automatic gain control (AGC) at 1^{e6} and a maximum injection time of 100 ms. For MS2 profiling the mass spectrometer was operating at 35K mass resolution with a scan rate of 8 scan/s^{-1} with automatic gain control (AGC) set to 5^{e5} and a maximum injection time of 50 ms. The mass ranges were set to 100-950 for positive ionisation and 115-950 for negative respectively. Positive and Negative polarity data sets were acquired independently using the identical cinematographic profile as describe above.

2.13.4 Data Alignment and Processing

For the remaining sections of this chapter and the Chapters 7 and 8, 'mass spectral feature' or MSF is used as a term to describe a signal or peak of interest in the collected data, prior to confirmation and identification. Once a compound has been identified this moniker is dropped in favour of the identified name or 'compound(s)' when discussing identified features collectively.

Post data acquisition processing and alignment was performed using Thermo Scientific Compound Discoverer 2.0 software suite (Thermo Fisher Scientific, Loughborough, UK). The data files were organised into respective polarities and further grouped by cultivar and additional truss positions. Pooled QC samples and sample blanks were also included and grouped accordingly in order to assess and evaluate system stability and tracked potential carry over effect throughout the entire batch analysis. Chromatographic alignment window was set to 0.15 min/9 second, with mass tolerance of 5 ppm using an adaptive curve algorithm. The minimum peak intensity was set to 1,000,000 counts with minimum signal/noise threshold of 3/1 with protonated adducts (M+H) preferred, the gap filling protocol was also turned on and missing values were replaced based on a predefined experimental class design. Positive and Negative polarity data sets were processed independently.

The resulting peak table was sequentially filtered to include pertinent, stable peaks and to eliminate those peaks that did not remain stable throughout the analysis. Of the 5,284 mass spectral features that were successfully detected by the MS and aligned in the previous processing step many were not stable in the samples and caused by fluctuations in the MS, contamination or a product of the system itself, henceforth referred to as 'artefact peaks'. Therefore, true, metabolite signals had to be separated from those that were considered as artefact peaks. The global experimental pools, created by combining 25 µL of each experimental sample into single vials, were used to confirm system stability and remove features that showed fluctuations in signal throughout the analyses. Firstly, sum signal intensities of the eleven global pool samples were calculated, showing a 13.7% relative standard deviation with all 5,284 peaks included. There was an obvious outlier in the global QC pools, the final sample hadn't injected correctly and presented

with $1.51E^{-09}$, far lower than all other pools which were ~ $2.49E^{-09}$. Removal of the final pool reduced the RSD of sum signal intensity in the QC pools to 6.1% as well as preventing the artificially low value dramatically increasing the RSD on a per feature basis, and was therefore deemed justified. The stability of each mass spectral signal was then assessed by determining the mean, standard deviation and relative standard deviations on a per peak basis. Initially, relative standard deviation of the peaks ranged from 1.4- 295.5%. As the global pool should have been representative of the full complement of metabolites present in each of the samples present in this current study, an elective threshold value of 15.0% RSD, inclusive of values up to 15.049%, was used to screen out features that showed instability or irreproducibility across the dataset, resulting in the retention of 1,329 features. These features were the focus for the remaining sections of this chapter. Features were organised by retention time and then coded as Mxxxx, where xxxx represents 0001 to 1329, the same code was applied to each feature for all three cultivars and those codes are retained throughout the remainder of the chapter. This initial check monitored the system stability and the reproducibility of the dataset across the experiment, however, this experimental design also had an 18-class structure, whereby one class (one cultivar, one ripening stage) may present markedly higher concentrations of a compound than the remaining classes. Therefore, the remaining 1,329 compounds were checked for intensity and abundance in each ripening stage of each cultivar. A peak area threshold of 100,000 aU as well as a 20% RSD per class was applied to each of the remaining mass spectral features to determine their presence and stability in each experimental class. Those features that met these criteria in one or more class were included, giving 2,783 entries which pertained to 451 unique mass spectra features of interest which were stable and at concentrations greater than 100,000 in at least one experimental class. The original dataset was then filtered to only include these 451 mass spectral features along with accurate mass, M+H and retention time. Individual cultivars were separated into independent worksheets and blank samples and global CQ pool samples were removed from each. Cultivar and positional pools were retained as representative samples of the respective groups. Data was uploaded to MetaboAnalyst 4.0 for multivariate statistical analysis and correlation.

2.13.5 Using MetaboAnalyst 4.0 for Visualisation of Relationships between Ripening Stages and Identifying Potential Biomarkers for Further Analysis

The ‘statistics’ node of MetaboAnalyst was used to process the filtered peak table for each of the analysed cultivars. Peak tables were uploaded as ‘peak intensity values’, if present, missing values were estimated using the k-nearest neighbour algorithm (KNN). The data was filtered using non-parametric standard deviation and was log transformed (generalised log transformation, g^{log}) and auto-scaled (mean-centred and divided by the standard deviation of each variable). Following normalisation and the removal of a single outlier in the Juanita dataset the data followed the standard bell curve format for each of the three cultivars. The outlying sample was an extremely anomalous value which dramatically distorted the rest of the dataset and overpowered any of the

true relationships present in the data as can be seen in **Figure 2.1**. Upon close inspection of the peak intensities in the outlying sample it became obvious that it was a 'dummy' injection, much more comparable to a blank sample than any other tomato extraction, therefore its removal was deemed to be justified to preserve the valuable relationships present in the samples. The improvement in the PCA plot indicates the validity in this choice, immediately resulting in the expected horseshoe shaped sample distribution across the first two principal components.

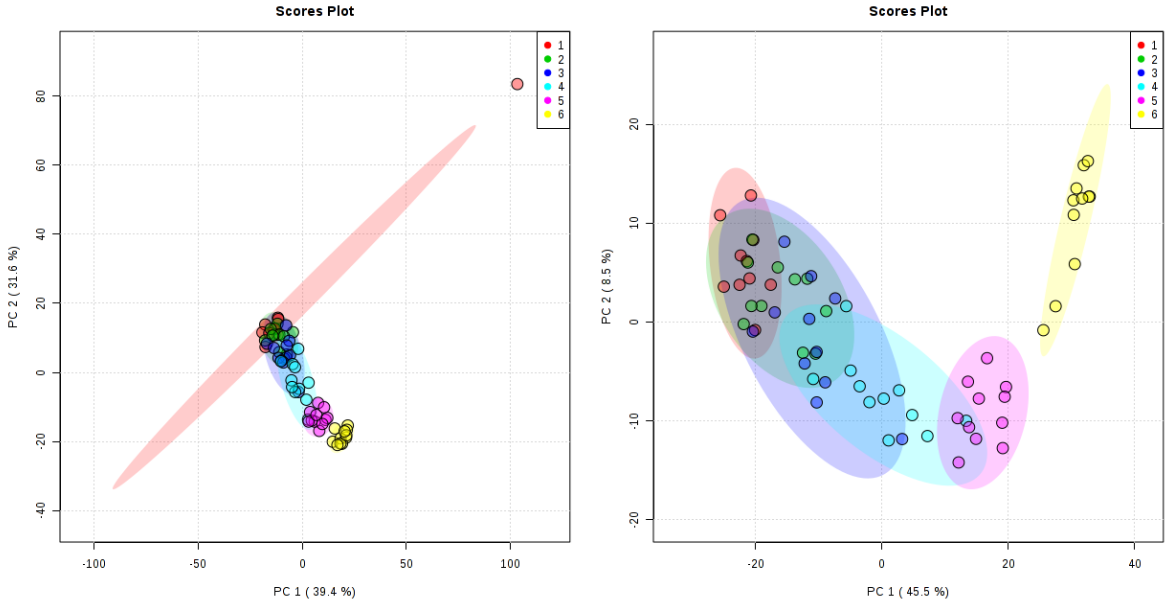


Figure 2.1 – Change in 2D-PCA plot for Juanita following outlier removal. Groups in order of descending ripeness Red Ripe, Red, Light Red, Orange, Breaker/Turning, Mature Green. Corresponding background colours indicate 95% confidence intervals of each sample cluster.

The distribution of samples in the principal components plots was very comparable between the analysed cultivars as shown in **Figure 2.2**.

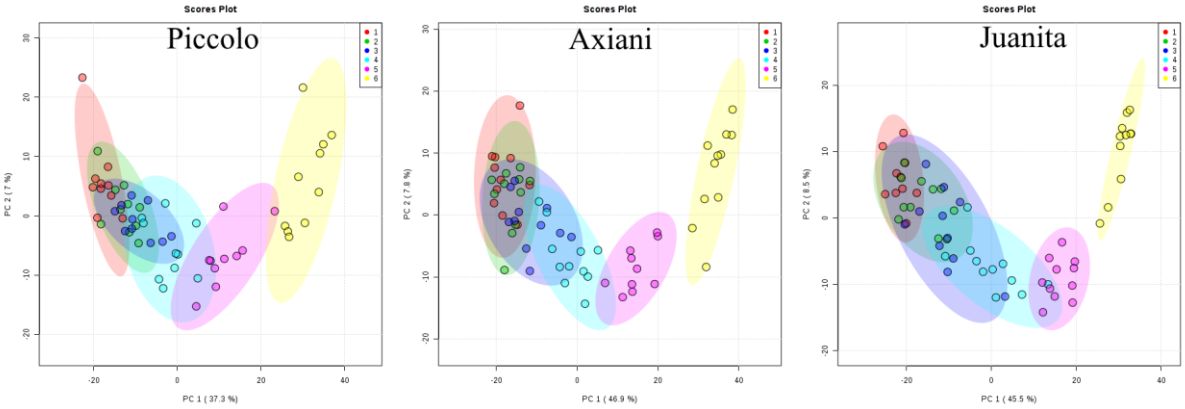


Figure 2.2 – Two dimensional PCA plots composed of the 1,320 mass spectral features of interest in ripening Piccolo (left), Axiani (centre) and Juanita (right). Groups in order of descending ripeness Red Ripe, Red, Light Red, Orange, Breaker/Turning, Mature Green. Corresponding background colours indicate 95% confidence intervals of each sample cluster.

It was immediately obvious that the more under-ripe fruits in the mature green and breaker categories were drastically different to the other populations of samples, with a visible merging of the 3 ripest groups and a noticeable overlap in the turning samples with the more ripe fruits. This is likely due to the somewhat subjective categorisation of samples into their respective experimental groups. The transition throughout ripening is a continuous process and therefore it is expected that perfect categorisation would be difficult to achieve.

Principal components was utilised as a purely visual assessment of the relationships present in the sampled populations. Following confirmation of the expected sample distribution the relationships were further assessed using partial least squared-descriptive analysis (PLS-DA) to determine which of the mass spectral features were explained the largest proportion of the variance between sample groupings. The PLS-DA function of MetaboAnalyst utilises the heirarchical experimental design, in this case, decreasing fruit ripness from groups 1-6, and maximises the degree of co-varience observable between the sample groups and data matrix, represented by *y* and *x* respectively. Partial least squared (PLS) regression is then performed on the data matrix and ranked class order. In this case, the categorical class order is following the progression of fruit ripening and associated biochemical changes, therefore PLS will involve constructing linear models that best explain the variation in intensities of the measured compounds accoring to class structure. One of the key benefits to PLS regression modelling in chemometrics and analytical environments is due to its ability to deal with large, complex datasets consisting of noisy and colinear data including missing values (Akarachantachote *et al.*, 2014, Farrés *et al.*, 2015). Moreover, multivariate techniques such as PLS and PCA are capable of elucidating relationships in datasets where the number of independent variables far outwieighs the number of individual samples (Andersen and Bro, 2010). PLS models rank variables using two metrics, variable importance in projection (VIP) and absolute regression coefficients for each correlated variable, both representing the influence that variable has over the relationship in the data. Feature selection can be based on either VIP or regression coefficient score, as both have benefits when trying to elucidate the relationships between features and the experimental design. The VIP may provide the best discrimination versus extremely high numbers of variables as seen in complex datasets such as total ion current mass spectrometry peak tables (Farrés *et al.*, 2015). Variables with higher VIP scores are strongly correlated to the class structure, either positively or negatively, and therefore are powerful drivers in the relationship seen in both PCA and PLS-DA plots. The threshold used as a cut-off to separate useful independent variables (mass spectral features, in this case), is undefined and subject to change based on the objectives of the study. Some studies extract all variables with VIP scores greater than a predetermined value, commonly this is 1 or 2 (Pérez-Enciso and Tenenhaus, 2003, Cho *et al.*, 2002). Other studies have utisied the top *x* variables, ranked by VIP score (Ji *et al.*, 2011, Akarachantachote *et al.*, 2014). Both of these techniques are valid and take the most important findings of the PLS model onward for further

investigation. For the purposes of this study, all features with a VIP score greater than 1 was used to determine those features that warrented further investigation and tentative identification through MS/MS analysis.

2.13.6 Potential Biomarker Isolation and Fragmentation by MS/MS

Those features shown to be important to the experimental design through PLS-DA previously, were selected as targets for MS/MS analysis. Pool samples for each of the positions per cultivar were rerun in MS1 mode to confirm that the chromatography, mass calibration and system stability was comparable to that of the original MS1 experiment. Accurate mass of the M+H adducts, using the accurate mass of a proton to 5 d.p., was used to locate the correct peak in the chromatograms of each of the pool samples. Retention time shifts of ±12 s were considered acceptable. The original accurate mass of the target compound, as determined through original MS1 profiling, was compared to that of the selected peak using both detected masses and the mass error. The mass error was determined by calculating the mass difference between the exact mass (original observed mass) and accurate mass (mass of target peak). The mass difference was then divided by the exact mass and multiplied by $10e^{-6}$ to represent it as ppm. Mass errors lower than 10ppm paired with reproducible retention times were deemed to be sufficient to confirm that the peak was that of the original analyte. For each compound, the sample which presented the largest peak area in the 'cleanest' section of the chromatogram was selected for injection for each target. Multiple targets were isolated per MS2 run, between 3 and 30 in the 11.5 min of analytical space in each chromatogram. A 6 s window between peaks was employed to ensure that there was no overlap in isolated compounds. Initial MS2 isolation was conducted at the lowest fragmentation energy level, 10 NCE (normalised collision energy), to preserve the molecular ion of the compound. Even at this lowest energy level, the variability in fragmentation between target analytes was extreme, with some compounds exhibiting no fragmentation and other where the molecular ion was ~10% that of certain fragment masses. The mass error of the isolated mass versus target exact mass was determined as a method of isolation confirmation, with the same 10 ppm tolerance employed in an earlier step. Subsequent fragmentation at increasing energy levels did not perform this check as the molecular ion was often lost completely and isolation at 10 NCE implied that further isolation and fragmentation at higher energy levels using identical method and sample would be equally successful. Isolation and fragmentation of each target compound was performed at 10, 30, 50 and 70 NCE, although higher energy levels for some compounds were not used in data processing as they had fragmented too much or were low abundance.

2.13.7 Tentative *in silico* Biomarker Identification though Isotopic Abundance Patterns and Fragmentation Trees with SIRIUS

Due to the limitations of the LC-MS system coupled with the older version of Xcalibur, certain routes of identification and characterisation of target compounds were not viable. The inability to specify electron volts (eV) as the collision energy used for analyte fragmentation resulted in spectra that, for the most part, would not be comparable to identical compounds analysed by other laboratories and made available online. Normalised collision energy (NCE) is an energy conversion for small molecule analysis, which takes into account the increased ability of larger molecules to absorb energy when compared to those with lower molecular weights. Imparting 20 eV of energy into a compound with a low molecular weight would, in general, result in greater fragmentation than a larger molecule with more bonds able to absorb the energy and retain its structure. This is a general rule, with some bonds types significantly more resistant to breakage through ionisation than others . However, NCE is an ionisation setting exclusive to ThermoScientific instruments and therefore, spectra produced using these energy settings are less prolific than those produced using the more common eV setting. Standard practice when attempting to elucidate the identity of compounds using LC-MS/MS approaches is comparison of fragmentation patterns between the unknown analyte and spectra from various, open-access mass spectral databases (Halket *et al.*, 2005, Kind and Fiehn, 2010). The object of this is to find directly comparable, experimentally derived, mass spectra from standard compounds analysed in isolation by various metabolomic laboratories and research groups worldwide. Getting near perfect matches in spectra comparison is highly desirable, but imperfect matches can still provide information on the chemical structure of the analyte, which may narrow down its true identity (Kind and Fiehn, 2010). Under optimum circumstances, pure, analytical grade standards of these compounds would then be analysed in parallel with the samples to confirm that the system and conditions utilised also produce near identical spectra following sequential fragmentation. Unlike ionisation using eV, NCE is a far less popular technique, this results in much narrower options for comparisons. The only online, open access database that utilises NCE levels and has a large number of available spectra is MZCloud (https://www.mzcloud.org/). However, MZCloud, at the time of writing only contains 8,237 compounds, as opposed to the 114,100 offered by the Human Metabolome Database (HMDB, http://www.hmdb.ca) or the 100,000 analytical standards with MS2 scans available on METLIN (metlin.scripps.edu) (Guijas *et al.*, 2018, Wishart *et al.*, 2007). As the fragmentation of targets in this study was achieved through NCE, the resulting spectra are not directly comparable to those found in either HMDB or METLIN, amongst other, popular databases. Comparisons to the fragmentation patterns available on MZCloud, is possible, but due to the low number of compounds present in the database, many targets with high VIP scores had no reference spectra available. Therefore, the use of alternative identification techniques was required in this case.

SIRIUS, Sum formula Identification by Ranking Isotope patterns Using mass Spectrometry, is a mass spectral processing technique developed at Friedrich-Schiller-Universität, Germany. SIRIUS is based on two, core concepts to enable structure elucidation and confirmation, namely natural isotopic abundance ratios and fragmentation tree computation and comparison (Böcker *et al.*, 2009). The former, was previously suggested by Kind and Fiehn and utilised by Iijima *et al.* prior to its incorporation into the SIRIUS workflow (Iijima *et al.*, 2008, Kind and Fiehn, 2006). As described by Böcker *et al*., the initial, monoisotopic sum formula of the analyte is determined using the natural distribution of the most common elements present in biomolecules as shown in **Table 2.5**.

Table 2.5 – Natural distribution of the isotopes of the most common elements present in biomolecules, for the purposes of isotopic abundance ratios and pattern analysis in mass spectrometry (adapted from Böcker *et al.*, 2009).

Element	Isotope	Mass	Mass difference	Abundance (%)
Hydrogen	^{1}H	1.007825		99.985
	^{2}H	2.014102	+1.006277	0.015
Carbon	^{12}C	12.0		98.890
	^{13}C	13.003355	+1.003355	1.110
Nitrogen	^{14}N	14.003074		99.634
	^{15}N	15.000109	+0.997035	0.366
Oxygen	^{16}O	15.994915		99.762
	^{17}O	16.999132	+1.004217	0.038
	^{18}O	17.999161	+2.004246	0.200
Phosphorus	^{31}P	30.973762		100
Sulphur	^{32}S	31.972071		95.020
	^{33}S	32.971459	+0.999388	0.750
	^{34}S	33.967867	+1.995796	4.210
	^{36}S	35.967081	+3.995010	0.020

Simulated isotopic abundance distributions are the created for the potential compounds and ranked based on similarity to the input spectra (Böcker *et al.*, 2009). Due to the extreme bias in the proportion of the most abundant isotope of each element, the isotopic abundance pattern appears as an exponential decrease from the molecular ion peak of the compound. Depending on the elemental composition of the compound, the ratio of isotopes and abundance of the analyte, some isotopic abundance peaks may not be visible, hence the elimination of mass spectral features that presented with a mean peak area less than 100,000 aU in this dataset.

3 Consumer Preference, Engagement and Opinion of Fresh Tomato Quality

3.1 Chapter Abstract

Consumer understanding of tomato flavour and drivers of preference were determined by market research and untrained sensory analysis. The market research proportion of the chapter found that, although there has been significant research into factors which affect the quality of tomatoes, consumers are largely unaware of actions which would be within their own control. For example, ~75% of respondents still store their tomatoes under refrigerated conditions following sale, which has been demonstrated by numerous studies to negatively affect the flavour profile. This was independent of the class of fruit purchased, inferring that consumers that place higher value on the tomatoes they consume are as likely to 'mistreat' them as those who spend less on them. However, storage practices were influenced by the self-proclaimed level of knowledge of tomato flavour, specified by each panellist, with just ~20% of those respondents believing themselves to have limited or average levels of knowledge of tomato flavour storing fruits at room temperature as opposed to 40% and 60% for informed and specialised participants, respectively. In both the online survey and sensory analysis, low importance was placed on attributes known to be vital to good tomato flavour, namely 'green/grassy', 'mouldy/musty' and 'earthy', indicating a possible difficulty in isolating and/or recognising these more complex flavours, or a potential influence through negative connotations of the names of these descriptors. Negative association in terms of the terminology used to indicate the acidity of fruits was also apparent, with 'tangy' and 'acidic' prioritised over 'sour', perhaps due to the values attributed to these terms by consumers. Penalty analysis was used to determine which attributes of the 5 cultivars analysed by sensory analysis influenced the overall liking. It was found that fruit texture, in terms of firmness and juiciness, was more negatively associated than fruit colouration, even though many consumers also gave low appearance ratings to the 'tangerine' and 'yellow' tomato cultivars. The replacement of Oranjestar with Royal Star as the primary commercial 'tangerine' cultivar by the growers seems to have been driven primarily by increased disease resistance and reliability, rather than by improved organoleptic metrics, which has led to a significant drop in acceptability and other measured attributes compared to the other cultivars.

3.2 Introduction

Ultimately, consumer preference and satisfaction are the most important aspects in the sale/purchase of all fresh produce. Dissatisfaction with tomato quality, although acknowledged for decades, has not reduced tomato sales in the UK. On the contrary, tomato sales and annual consumption by capita appear to be steadily increasing. This indicates that although consumer satisfaction with tomato quality, particularly with regards to flavour, aroma and taste, has diminished since the 1960-70's, tomatoes are deeply embedded in our cuisine and the food of many cultures around the world. This rising demand per capita has resulted in many strategies to increase fruit yield, reduce losses due to mechanical or biological damage and to produce more uniform, easy to handle and transportable fruit. This was addressed by commercial breeders through successive cross breeding and hybridisation of cultivars. This resulted in higher yielding, resistant and uniform fruit production, ideal for harvest, shipping and increased shelf-life, all of which increase commercial productivity and profits. However, prioritisation of hybrids with these traits simultaneously resulted in a loss of genetic diversity and a suppression of genes responsible for many organoleptic attributes (Tieman *et al.*, 2017, Jones and Scott, 1983, Causse *et al.*, 2004, Klee and Giovannoni, 2011). In addition, many of the industry practices have been shown to adversely affect the overall fruit quality. This includes fruit maturity at harvest, handling practices and post-harvest treatments (Beckles, 2012, Kader *et al.*, 1978, Kader *et al.*, 1977, Maul *et al.*, 2000, Passam *et al.*, 2007, Stern *et al.*, 1994, Gómez *et al.*, 2009, de León-Sánchez *et al.*, 2009).

It is widely acknowledged that the losses in organoleptic traits of fresh tomato have had an impact on consumer acceptance of the crop. However, the strategies employed to fully characterise this shift in acceptance are principally focused on profiling and quantifying flavour, with subsequent correlation to quantitative analytical data (Baldwin *et al.*, 1998, Cortina *et al.*, 2018, Baldwin *et al.*, 2008, Tandon *et al.*, 2003). This allows for the important characterisation of the dynamic flavour of tomatoes between cultivars, growth conditions and ripening patterns. To achieve this level of detail trained sensory analysts are required as they are able to distinguish between individual aromas, flavours and tastes and score the perceived intensity of each. However, this approach, whilst it is able to characterise both the intensity and complexity of the organoleptic traits of tomatoes, fails to determine whether this increases overall liking and acceptability of the crop by consumers. Liking is equally complex, but less defined. There are many drivers that can determine the liking of a product by consumers as a population and as individuals; making the overall 'profile' of a product subjective to each consumer. Previous studies have utilised both descriptive and preference-based profiling to fully elucidate the intricate relationship between chemical composition, flavour intensity and consumer liking in fresh tomatoes (Auerswald *et al.*, 1999a, Johansson *et al.*, 1999, Auerswald *et al.*, 1999b, Causse *et al.*, 2003). This approach allows for parallels to be drawn between flavour intensity and profiles and the influence this has on

everyday consumers. The understanding, engagement and desires of consumers are powerful driving forces behind all fresh produce sales, including tomatoes. By coupling sensory analysis and market research a broader picture of consumer understanding and engagement can be determined. Moreover, specific traits and attributes may be seen as more or less important dependent on the context in which the question is posed. For example, sensory analysis presents participants with the material under scrutiny and asks them to determine how much certain attributes meet their desires and influence their liking of the product. Online surveys ask similar questions, but without the prompts provided by the presence of the object under scrutiny. These contrasting approaches may identify attributes that are undervalued by consumers outside the context of tomato consumption and reveal some of the less recognised relationships that drive consumer preference in fresh tomatoes.

This chapter will focus on market research, consumer opinion and sensory preference testing, in an attempt to determine public opinion of fresh tomatoes and identify those areas responsible for the greatest levels of dissatisfaction among consumers. Moreover, the level of understanding of the more intricate traits of tomato flavour of the sampled population will be discerned, highlighting areas that consumers struggle to recognise both when reflecting on and when sampling tomatoes.

3.3 Methods and Materials

Fruits for this study were sourced from Thanet Earth Marketing (Kent, UK) and stored as described in the main M&M section of this thesis in Chapter 2.

The questionnaire for both sensory analysis sessions can be seen in **Appendix 3**. Due to the nature of panellist recruitment, the questionnaire aimed to gather preference and liking data on those attributes which were considered accessible to the wider public. Therefore, important quality parameters such as specific flavours/aromas (green/fruity/earthy etc.) and tastes (sweet/sour/bitter etc.) were omitted for the sake of reducing confusion and drop-out rate. Sensory analysis was conducted in the lobby of the Health and Life Science building at Northumbria University and relied on footfall and recruitment posters and emails to attract participants.

All statistical analyses in this chapter were conducted using SPSS version 24. The individual tests applied to the datasets under scrutiny in the chapter are fully explained below.

For the analysis of the sensory, data both Kolmogorov-Smirnov (K-S) and Shapiro-Wilk (S-W) tests were employed to determine if the distribution of the data followed a Gaussian curve and could be considered normally distributed. For the individual sensory analyses periods in 2017 and

2018, data was analysed independent of the second session, using a Friedman's Test to determine the scoring of the measured attributes on a per panellist basis. Where the Friedman's test showed significance between the measured groups, pairwise comparisons were conducted between the three measured populations per question. Due to the increase in likelihood of type 1 errors (rejecting a true null hypothesis) during multiple comparisons, a Bonferroni correction was applied, thereby multiplying the original significance by the number of comparisons and represented as the adjusted significance. To complement the Friedman's Test, medians were calculated for the data to identify central ranks of data as significance as calculated by Friedman's Test is independent of the mean and based on sample ranks.

The combined sensory dataset was then analysed, independent of the panellists, looking at aggregated scores to understand the general liking for each of the cultivars. Normality was assessed using K-S and S-W tests and confirmed that the datasets were not normally distributed. Therefore, a Kruskal-Wallis-H test was chosen to investigate the difference in scores per cultivar.

Potential annual, seasonal and environmental effects on liking of the Piccolo fruits was analysed by sequential Mann-Whitney-U tests.

3.4 Results and Discussion

3.4.1 Market Research Survey

The current purchasing habits of consumers is an important guide to understanding their opinions and how much value they place on tomatoes as a commodity in daily life. Increased spending on tomatoes, particularly the purchase of more premium fruits, may indicate a greater interest in and understanding and valuing of tomato flavour. Some of the demographic questions included in the survey were designed to produce data that can be used to identify trends and relationships in purchasing habits as well as level of understanding with regards to tomato flavour and quality.

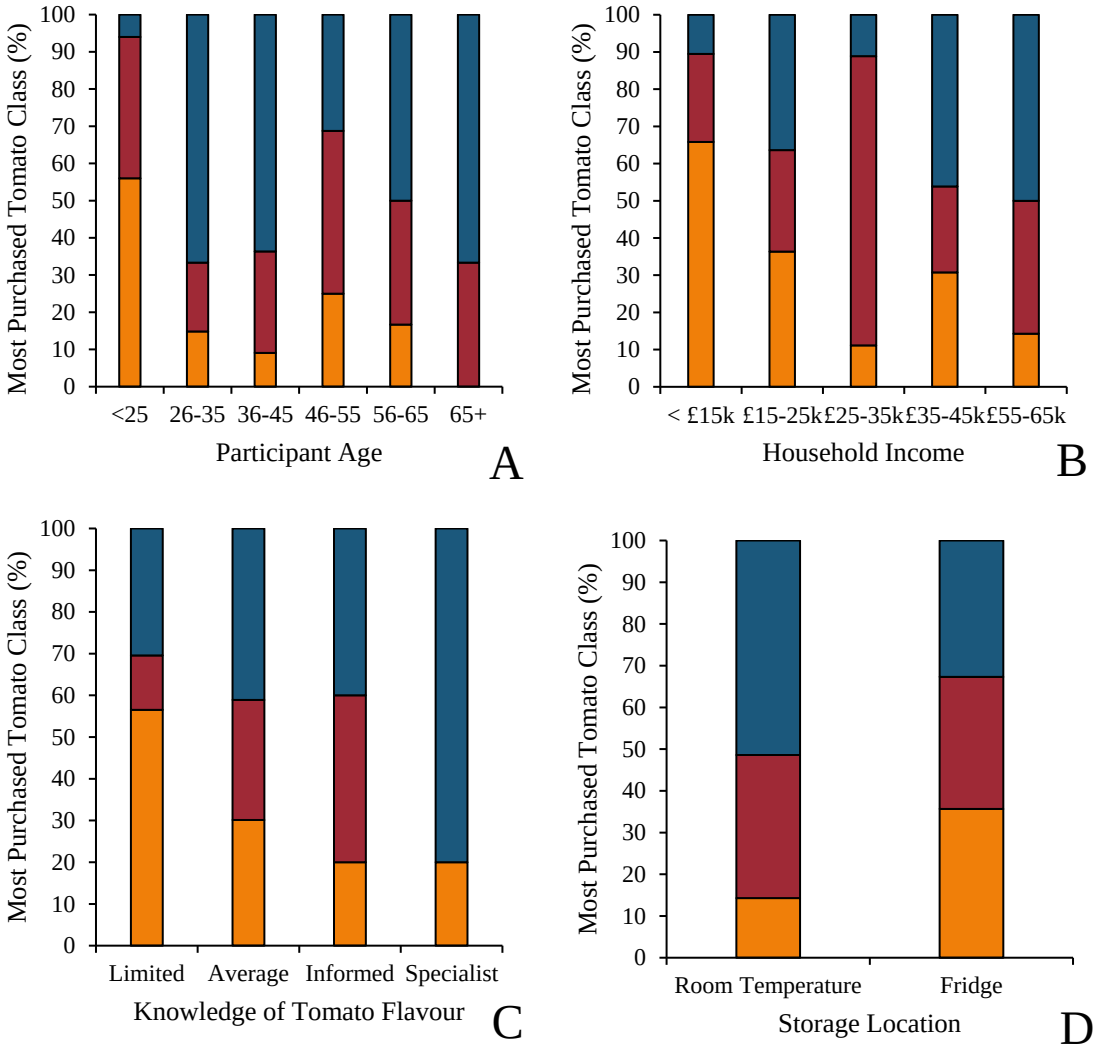


Figure 3.1 - Purchasing habits of consumers separated into three classes of tomato fruit by: **A –** Panellist age group. **B** – Personal income of panellists. **C –** Knowledge of tomato flavour. **D –** Tomato storage practices. ■ - Premium Class £6-7/Kg ("Finest", "Taste the Difference" etc.), ■ - Mid-range Class ~£4/Kg (Supermarket own brand), ■ - Lower Class £2-3/Kg ("Value", "Basics" etc.). Responses from 136 participants.

Figure 3.1A-D shows the distribution of the purchasing habits of consumers with regard to fruit class. Premium fruits often cost more than 2-3 times the price of value fruits, which is reflected in the quality of the fruit and the flavour and taste that they provide. This may indicated that consumers who willingly pay the 'premium' for the first class of fruit are likely to be more invested in the overall experience of eating the tomatoes, than those that pay half that amount for basics tomatoes. Alternatively, lower income households may be relegated to purchasing lower quality fruits due to the lower proportion of income assigned to grocery shopping. **Figure 3.1A** reveals that lower-class fruit are preferred by younger participants, with only 6% of under 25 year olds preferentially purchasing premium class fruits. The opposite appears true of those participants over the age of 65, where low grade tomatoes are never purchased in this sample population and the ratio of premium to mid-range fruit purchasing is 2:1. This relationship can also be explained by approximate disposable income, which is illustrated in **Figure 3.1B**, which

sorts purchasing behaviour by approximate household income. A similar trend is seen as was seen in age ranges, the lower earning brackets are far more likely to purchase lower class fruits than they are to buy either mid-range or premium class fruits. This is driven by 81% of the under £15k group being less than 25 years old, suggesting that the relationship may be less dependent on age and more on disposable income. **Figure 3.1C** aims to understand if there is a relationship between the self-specified level of understanding and knowledge of tomato flavour and quality and the purchasing habits of individuals. For the most part it seems that the more people believe they know about fresh tomato flavour, the more likely they are to buy higher cost, premium fruits. **Figure 3.1D** separates responses based on the home storage practices of purchased tomatoes. The majority of the 136 people surveyed, 101 people, store tomatoes in the fridge, which has been previously demonstrated to arrest sugar accumulation, inhibit volatile formation and increase the acidity of fruits stored between 10-13 °C, even before visible signs of chilling injury (CI) (Stern *et al.*, 1994, Maul *et al.*, 2000, Kader *et al.*, 1978). Common home refrigeration temperatures are between 1-4 °C, sufficiently cool to cause perceivable quantitative shifts in organoleptic quality of the fruits and trigger the manifestation of CI symptoms. The symptoms of chilling injury include surface/skin 'pitting', mottling or uneven colouration/formation, uneven ripening and cell wall loosening, loss of flavour and taste and increased susceptibility to biological spoilage (Maul *et al.*, 2000, Lyons, 1973).

Lower class fruits often consist of the 'off cuttings' of premium trusses when harvested, with the fruits not at suitable ripening stages removed from the truss and held back to continue the ripening process. This is advantageous for the grower, as it reduces wastage and allows better selection of premium fruits, however it results in lower class fruits consisting of tomatoes that have been removed from the plant earlier than is desirable, which has been shown to negatively affect the final quality of the crop (Auerswald *et al.*, 1999a, Baldwin *et al.*, 2000, Carrari and Fernie, 2006, Deltsidis *et al.*, 2018). The reliance of a large proportion of the sampled population on lower class fruits may also be partly responsible for the attributes determined as needing improvement. Only 16.9% of respondents thought that tomato flavour required no improvement, in contrast to 43.4% that requested significant improvements be made in this area. This opinion may be influenced by the lower class of fruit potentially presenting with reduced organoleptic profile, rather than those of premium fruits which would have been removed from the plant at the optimum ripeness.

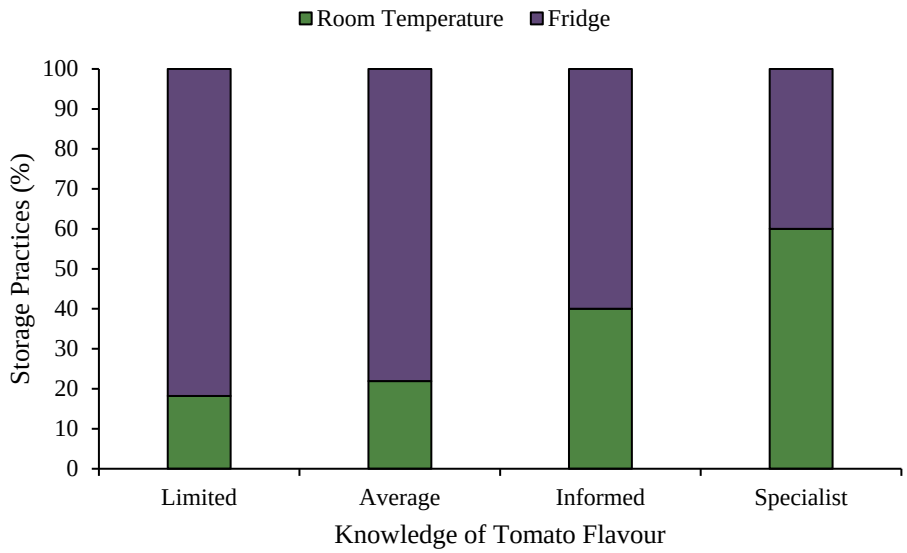


Figure 3.2 – Storage practices of respondents categorised by their self-specified levels of knowledge of tomato flavour.

Therefore, as the negative effects of chilled storage are demonstrable, individuals who refrigerate their fruit may also notice significantly worse sensorial experience when eating tomatoes at home. Potentially, this may devalue the product in their eyes and reduce the purchasing of premium class fruits, which is shown in **Figure 3.1D**. Those respondents who store tomatoes at room temperature, buy premium fruits more than 3 times as frequently as lower-class fruit. This is further corroborated by **Figure 3.2**, above, which shows that the more knowledgeable respondents are about tomato flavour and quality, the less likely they are to improperly store their tomatoes in the fridge. However, even in the 'specialist' category, which was largely composed of sensory analysts, 40% (2 of 5) still stored their tomatoes in the fridge. This raises the concern that the current and ongoing dissatisfaction with tomato flavour could be, in part, due to loss of genetic diversity, but further exacerbated through improper treatment of fruits following purchase. This argument gains traction when considering that the first instances of complaints over decreased quality coincided with the availability and widespread use of the home refrigerator in the UK in the 1960-70's. If this is the case, a supermarket led advertising campaign may help to sway consumers into storing tomatoes at room temperature, allowing for flavour development, rather than suppression within the home.

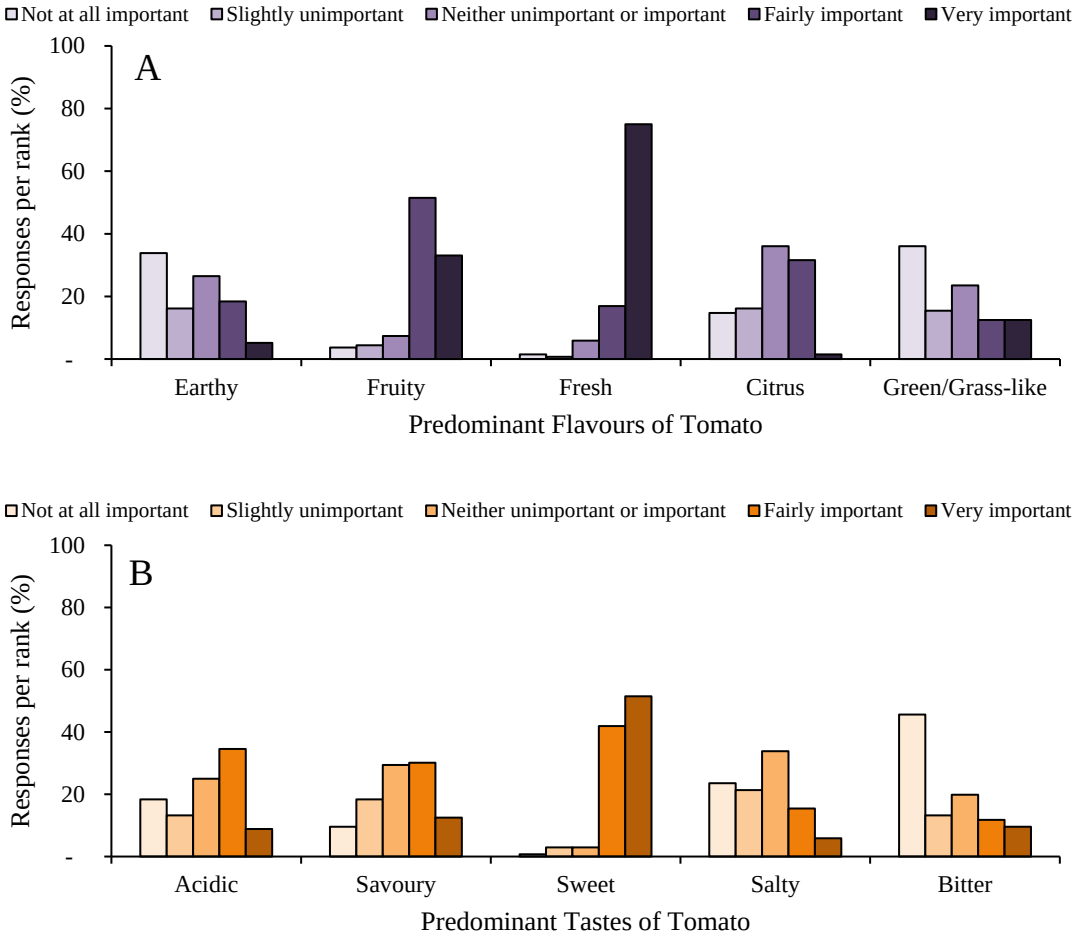


Figure 3.3 – Importance of various attributes relevant to **A** tomato flavour, **B** tomato taste. Colour gradient depicts "not important" (palest) to "very important" (darkest). Flavour and taste attributes were compared in separate sets (n=136).

To understand the requirements and motivations of consumers in relation to tomato quality, it is important to determine their level of understanding of the key organoleptic elements at play. Respondents' opinions on five flavour and five taste sensations of varying significance to fresh tomato quality are displayed in **Figure 3.3**. By determining the relative importance placed in each of the attributes, rather than their intensity, the data can be used to deduce the understanding of the complexity of fresh tomato flavour of the sampled participants. Fresh and fruity flavours were the most valued, with 75% and 33% of participants respectively specifying they were very important to tomato flavour. Both green/grassy and earthy flavours were thought to be largely unimportant by most participants, which is at odds with the accepted importance of both attributes within the academic community.

The 'mean response score' (MRS) was calculated for each of the attributes in question. Each of the scale values was weighted based on its impact, with "not important at all", "slightly unimportant", "neither unimportant or important", "fairly important" and "very important"

represented by -2, -1, 0, +1 and +2 respectively. This weighting system was used, as the closer to -2 and +2, the more negatively or positively important that value was considered to be. Attributes with means that are close to zero are not thought to affect the flavour or taste of tomatoes. Larger error bars are indicative of disagreement between responses over the importance of the attribute in question. The population of specialist responses was much smaller than other groups and therefore display higher error rates. As shown by **Figure 3.4** below, the four attributes that are consistently believed to impact the flavour and taste of fresh tomatoes are "fruity", "fresh", "savoury" and "sweet".

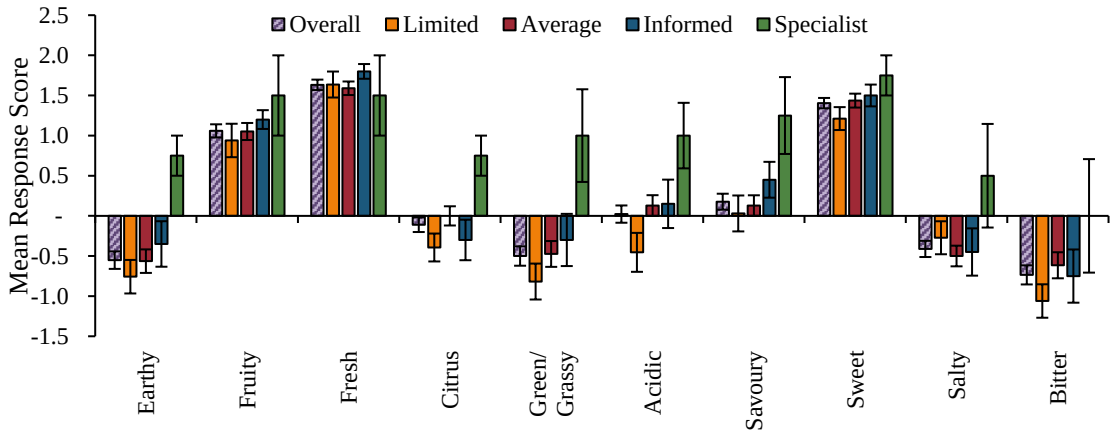


Figure 3.4 – The mean response score (MRS) of the importance of various flavour, aroma and taste attributes of fresh tomato. The "Overall" sample group is the mean response from all respondents, whereas the other groups represent the degree of knowledge of tomato flavour given by each participant. Positive values represent high importance of that attribute in relation to flavour or taste. Negative values represent negative importance associated with the attribute. Values close to zero suggest that value does not have a significant role in flavour/taste. Error bars represent standard error of the mean (Overall n=136, Limited n=33, Average n=78, Informed n=20, Specialist n=5).

When these values are further explored there is a general trend of increasing importance of "fruity", "savoury" and "sweet" with increased prior knowledge of tomato flavour, which is expected as they are all important and easily identifiable during tomato consumption. The most obvious change is in the importance level of "earthy", "citrus", "green/grassy" and "salty", whereby respondents who had classified themselves as "specialist" in terms of their knowledge of tomato flavour, specified these attributes as important to the flavour of fresh tomato, when other groups did not. In general, the other groups rated each of the aforementioned attributes as either neutral or unimportant to tomato flavour, which is contrary to the findings of many published studies (Tieman *et al.*, 2012, Petro-Turza, 1986, Hongsoongnern and Chambers IV, 2008, Buttery, 1993, Baldwin *et al.*, 2000). This indicates that, without the opportunity to taste tomatoes, when trying to recall or measure an attribute, regular consumers may not be able to isolate specific parameters that are less well-known and commonly associated with tomato. The best examples of this are green/grassy and earthy, which are flavour defining sensations in tomato.

The importance of green/grassy notes formed through lipid oxidation has been previously reported to influence consumer liking and preference in tomatoes (Tieman *et al.*, 2012, Krumbein and Auerswald, 1998, Baldwin *et al.*, 1998). However, consumers may find it difficult to imagine green/grassiness in the context of tomatoes, thereby lowering its perceived importance. Mischaracterisation of green/grassy aromas by panellists was also apparent when asked to specify which flavours and tastes are impactful in overall tomato flavour, where only 45 of the 136 respondents believed that fresh tomato flavour contains green/grassy notes as shown in **Figure 3.5**.

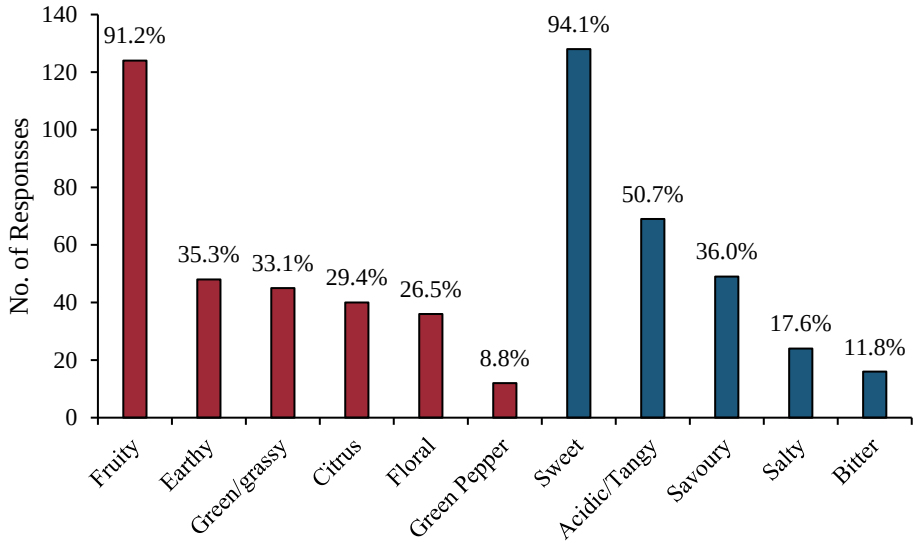


Figure 3.5 -The frequency of use of specific flavour, aroma and taste descriptors in the context of fresh tomato by 136 respondents. Respondents were allowed to select as many or as few as they thought were applicable to fresh tomato. Red bars represent aroma/flavour descriptors, blue bars represent taste descriptors. Data call outs represent the percentage of the 136 participants that believe that descriptor applies to tomatoes.

Fruity and sweet descriptors are the most frequently used with 91.1 and 94.1% usage between respondents respectively. The remaining aroma descriptors are associated with tomato far less frequently, with only between 25-35% of people believing earthy, green grassy, citrus and floral are applicable to tomatoes. In terms of taste, sweetness is the most common descriptor, followed by acidity/tanginess, which are two of the driving factors in consumer preference of tomatoes (Auerswald *et al.*, 1999b, Jones and Scott, 1983, Stevens *et al.*, 1979). The lower frequency of usage of acidity/tanginess could be due to negative connotations associated with acidity in many foods or the intensity of acidity in relation to sweetness, making some participants overlook the more background acidity in favour of the more dominant sweetness. It has been previously demonstrated that strong sweetness and moderate acidity is most acceptable to consumers (Jones and Scott, 1983, Stevens *et al.*, 1979).

The firmness of the fruit flesh and juiciness of the fruit were the major textural parameters that consumers looked for in tomatoes. For juiciness, 41.9% and 50.0% of participants stated that it was 'fairly' or 'very' important, respectively, in influencing their choice of and purchasing habits for tomatoes (data not shown). Similarly, 30.1% and 59.6% of respondents claimed that the firmness of flesh was respectively fairly important or very important in their choice of fruit. The thickness of skin was considered to be less impactful to the majority of respondents, with only a cumulative 57.0% of respondents selecting fairly important (40.7%) and very important (16.3%). The only textural parameter that was not considered influential in consumer choice by the majority of participants was the number of seeds, with a total of 41.8% believing it to be unimportant when choosing tomatoes and a further 39.6% claiming it didn't affect their choice in either a positive or negative way. As expected, although many textural parameters are important in consumer acceptability, juiciness and fruit firmness more significantly influence overall acceptability than number of seeds or thickness of skin, both of which would need significant deviations from normal to yield a reduction in overall liking.

3.4.2 Consumer Preference Testing through Sensory Analysis

To complement the open access survey format of data collection, a set of two consumer preference sensory analysis sessions was conducted in May 2017 and a combined set of sensory analysis in January 2018 and March 2018. One cultivar was analysed on both sessions, Piccolo, alongside two cultivars which differed between sampling periods, Sweetelle and Oranjestar for the 2017 session and Summer Sun and Royal Star for the 2018 session, were used for the analyses. Samples have been assigned codes for the purposes of data analysis, as well as randomly generated numerical tags for the sensory sessions, as shown in **Table 3.1**. The tomatoes used were a selection of small-fruited cultivars including one standard red cherry, Piccolo, two tangerine cherry cultivars, Oranjestar and Royal Star, one yellow cherry, Summer Sun and a baby plum type, Sweetelle. The tangerine tomatoes were sourced from the same breeder, Majestar (Sakata Vegetables Europe, Boston, UK), with Royal Star superseding Oranjestar in commercial growing in 2018, due to the increased disease resistance and fruit yield provided by its hybridisation.

Table 3.1 – Coding system used for both sensory analysis and data processing and analysis.

Cultivar	Sampling Year	Cultivar Code	Code	Numerical Tag
Oranjestar	2017	ORA	ORA_'17	808
Piccolo		PIC	PIC_'17	316
Sweetelle		SWE	SWE_'17	291
Royal Star	2018	ROY	ROY_'18	164
Piccolo		PIC	PIC_'18	728
Summer Sun		SUM	SUM_'18	493

The responses received from the two separate periods of sensory analysis, May 2017 and January and March 2018, were initially analysed independently to better understand the likings and preferences on a per panellist basis. Questions had been structured in two ways; firstly, a 10-point hedonic scale was used to assess the overall liking of general quality parameters. A 10-point scale was deliberately chosen to eliminate the middle point present in the more common 9-point version, forcing participants off the non-committal middle ground. Secondly, more targeted questions focused on the colour, shininess, firmness and juiciness used a "just about right" (JAR) scale, whereby 3 represented the JAR point, 2 and 1 represented increasing lack of the attribute and 4 and 5 representing increasingly too much of the attribute. Conversion of JAR data for graphical representation was carried out by subtracting the JAR point (3) from the provided value, converting the data to -2 to 2, which represented the dissatisfaction with negative values indicating too little, positive values too much and zero values representing content/ "just about right" in that attribute.

As seen in **Table 3.2**, all cultivars were generally well liked in the 2017 sensory sessions, with median values for appearance, flavour, texture and acceptability scoring above 8 on the hedonic scale. However, once responses were ranked, the Friedman test showed significant differences in liking across the population regarding a number of attributes. The overall appearance of Oranjestar was rated as significantly lower than both Sweetelle and Piccolo, likely due to its characteristic colouration. Oranjestar is a non-standard ripening mutant, resulting in a deep orange colour at full ripeness, often referred to as a tangerine tomato. This is due to the inability to isomerise tetra-*cis*-lycopene to all-*trans*-lycopene, preventing red colouration (Vogel *et al.*, 2010). Both the overall appearance and the JAR assessment of colour value, have Oranjestar scoring lower than the other cultivars, indicating that the public may not be as engaged with non-standard colouration in tomatoes as they are with classically red coloured fruits. This agrees with the findings of the 2018 sensory sessions, where both orange and yellow fruits scored significantly lower than red fruits in both attributes.

Table 3.2 – Preference and acceptability data from the 2017 and 2018 sensory analysis sessions, as analysed by Friedman's test with Post-Hoc analysis by pairwise comparisons. Where pairwise comparisons are displayed the Friedman's test was significant ($p \leq 0.05$); non-significant Friedman's did not warrant Post-Hoc investigations and are marked as such. Adjusted significance for pairwise comparisons is displayed as calculated by the Bonferroni correction for multiple comparisons. Group Medians displayed to highlight the areas of difference per attribute. Questions have been separated into their two types, hedonic preference and JAR scales. (2017 n=101, 2018 n=111)

		Group Medians			Pairwise Adjusted Significance		
		2017 Study					
	Attributes	ORA	SWE	PIC	ORA vs SWE	ORA vs PIC	SWE vs PIC
Hedonic Preference	Overall Appearance	8	9	9	<.0005	<.0005	1.000
	Overall Flavour	8	9	9	.202	.005	.579
	Overall Texture	8	8	8	Friedman NS (p=0.198)		
	Overall Acceptability	8	8	9	.088	.001	.448
"Just about right"	Colour	2	3	3	<.0005	<.0005	.448
	Shininess	3	3	3	Friedman NS (p=0.380)		
	Flesh Firmness	3	3	3	.041	.037	1.000
	Juiciness	3	3	3	.159	.015	1.000
		2018 Study					
	Attributes	ROY	SUM	PIC	SUM vs ROY	SUM vs PIC	ROY vs PIC
Hedonic Preference	Overall Appearance	7	7	9	.447	<.0005	<.0005
	Overall Flavour	6	8	8	<.0005	1.000	<.0005
	Overall Texture	7	8	8	<.0005	1.000	<.0005
	Overall Acceptability	6	8	9	<.0005	.344	<.0005
"Just about right"	Colour	2	2	3	.016	<.0005	<.0005
	Shininess	3	3	3	1.000	.226	.447
	Flesh Firmness	3	3	3	<.0005	.895	<.0005
	Juiciness	3	3	3	Friedman's Test NS (p=0.927)		

The data collected in the market research section of this chapter further supports this hypothesis, indicating that orange and yellow fruits have only been purchased by 41.1% and 35.2% of respondents respectively. The widespread availability of non-standard colouration in tomato fruits is a relatively recent trend, only becoming prevalent in the UK in the last 5 years. Additionally, orange and yellow fruits are often confined to the more premium brackets, which may exclude them from large parts of the population who do not typically purchase premium fruit, 62.5% of respondents of the online survey. The texture of the fruits analysed in 2017 was considered by panellists to be equally well-liked. However, results from the 2018 period indicated increased dissatisfaction with the texture of Royal Star when compared to Summer Sun and Piccolo, which was not apparent in 2017 with Oranjestar. This was supported by the JAR scale responses of fruit

firmness which showed that the tangerine tomatoes, Oranjestar and Royal Star, were considered to be significantly poorer than the other cultivars in both 2017 and 2018. This difference is not apparent when comparing median values across cultivars. However, it is clearer when the mean responses are compared, indicating that both tangerine tomatoes tend to be considered slightly too soft, whereas, Piccolo from both years, Summer Sun and Sweetelle were all rated as being on the too firm side of the JAR point. The impact on overall texture liking is depicted in **Figure 3.6A-B**. The replacement of Oranjestar with Royal Star in the commercial tomato growing environment may be a perfect representation of growers and breeders prioritising improved efficiency and commercialisation over organoleptic quality of the fruits. Royal Star received significantly lower mean scores in the four overall liking questions than any of the other analysed cultivars, a strong indicator that it would be less acceptable to purchasing consumers. Additionally, a similar trend was seen in negatively associated traits in the descriptor selection and 'just about right' scale questions, as further explored below. Based on these findings, the economic benefits of a more standardised and resistant crop with a higher fruit yield may be outweighed by the notable loss of quality and consumer acceptance.

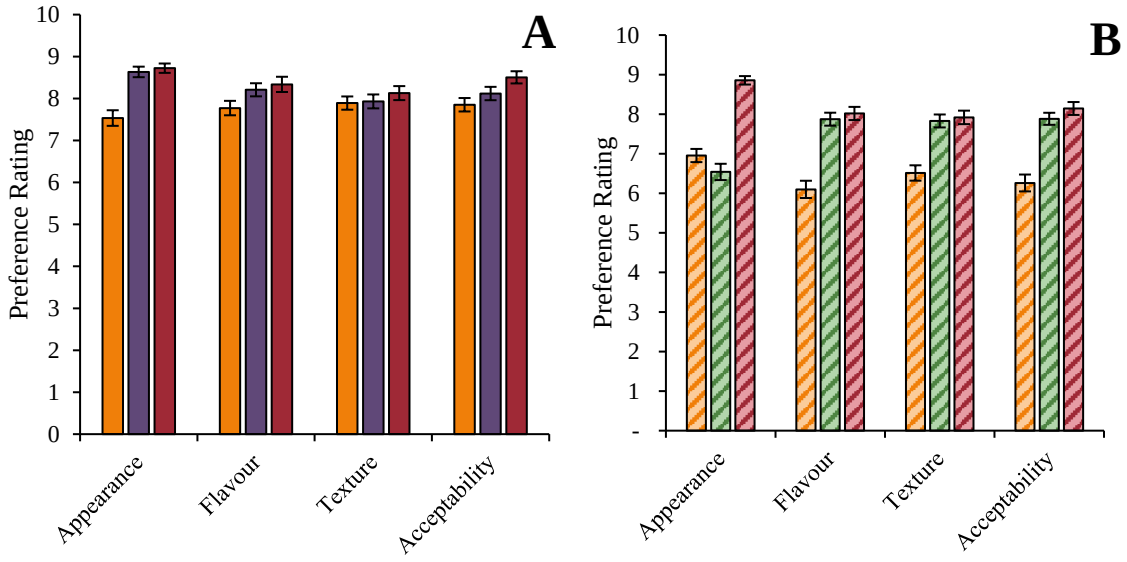


Figure 3.6A-B – Sensory preference data of four cultivars gathered over two sensory sessions in 2017 and 2018. **A)** Mean hedonic preference rating of general quality parameters of fresh tomato gathered in 2017 (n=101). **B)** Mean hedonic preference rating of general quality parameters of fresh tomato gathered in 2018 (n=111). Error bars for both figures represent SEM. Solid bars = 2017, striped bars = 2018, ■ Oranjestar '17, ■ Sweetelle '17, ■ Piccolo '17, ■ Oranjestar '18, ■ Summer Sun '18, ■ Piccolo '18.

Participants in the study were completely untrained in sensory analysis; it is likely that only a small proportion of them were familiar with the requirements of the process. Due to this, it was assumed that complex terminology, associated with and characteristic of, tomato flavour may be misunderstood or inaccessible to some participants, increasing the frequency of inaccurate responses or mischaracterisation of attributes. Therefore, the 2017 survey utilised open and

simplistic descriptors alongside more complex terminology to accord with the range of understanding that may have been present in panellists. The frequency of descriptor usage for each cultivar can be seen in **Figure 3.7**.

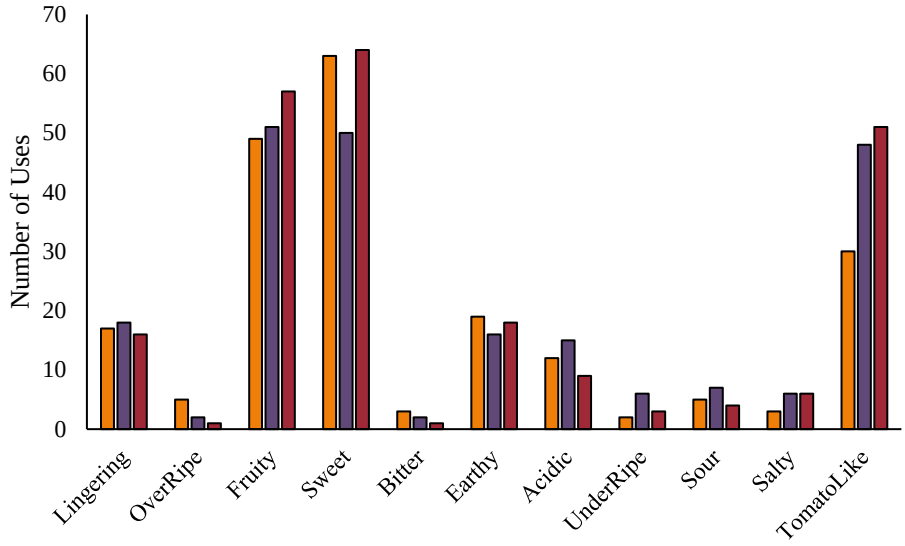


Figure 3.7 – Frequency of usage of the descriptors provided in the questionnaires. Participants were encouraged to select as many values as they thought applied to the fruit in question. Sample population is 101 individuals that provided a total of 208 responses for Oranjestar (■), 221 for Sweetelle (■) and 230 for Piccolo (■).

Although descriptors can provide informative profiles of the tested materials, they are often misused or not well understood by participants who do not have prior experience in trying to verbalise their sensorial experiences. This is particularly apparent with complex flavours and aromas or those with negative connotations, like green/grassy, mouldy, earthy and sourness. Without prior training or understanding of sensory analysis, it is often difficult for panellists to differentiate specific flavours from the whole experience, leading to artificially lower usage of specific aroma and flavour descriptors than would be expected in the matrix in question. This is particularly apparent in fresh tomato where many of the individual flavour characteristics are both difficult to understand and are portrayed as negative through naming. Sourness/acidity is one of the most important gustatory aspects of tomato flavour, acting as a counterpoint to the high levels of sweetness in the fruit. However, sourness can be interpreted as undesirable as it is the most dominant taste in under-ripe fruit, particularly by participants less well versed in tomato flavour. Therefore, it was observed that less participants selected sourness as being present in the sampled fruits than would be expected in a panel well-versed in fresh tomato flavours. Moreover, this is even more prevalent in aroma description, where green/grassiness and mouldy aromas, by definition, are not appetising flavours, but both play important roles in overall tomato flavour. Tomato-like was added into the descriptors as an aggregate description of these more confusing flavours (green, grassy, mouldy etc.). Unfortunately, eliminating the more complex descriptors,

in favour of more simplistic phrases that would be well-understood by the untrained panellists, caused an unanticipated issue. The majority of participants (85%) used a combination of the descriptors sweet, fruity and tomato-like all of which are too simplistic and obvious to be particularly valuable in terms of understanding the consumers' sensorial experiences of each cultivar. This is compounded by the fact that 'tomato-like' was deliberately left undefined and open for interpretation by the panellists. Additionally, panellists offered an average of 2.2±1.1 flavour descriptors and only 1.7±0.7 appearance descriptors (data not shown) across all cultivars sampled in 2017. With this average, it can be deduced that panellists are likely to use between 2-3 flavour descriptors and of those, 85% of people would use one or more of sweet, fruity and tomato-like. Providing obvious descriptors, alongside more complex ones, enabled the selection of descriptors that were definitely applicable to tomatoes and, possibly considered 'correct answers' in panellists' eyes. Additionally, the open-ended nature of the question allowed any number of descriptors to be selected but, as previously stated, many participants selected the easy options, possibly reducing the degree to which they thought about tomato flavour and engaged with the question. In the 2018 iteration of the descriptor selection question, sweetness was removed and 'tomato-like' was separated into its component flavours in an attempt to remove the 'easy choices' and force consumers to select more valuable descriptors, the results of which can be seen in **Figure 3.8**. Fruity was maintained, due to its importance in describing the impact of carotenoid derived volatiles on tomato flavour.

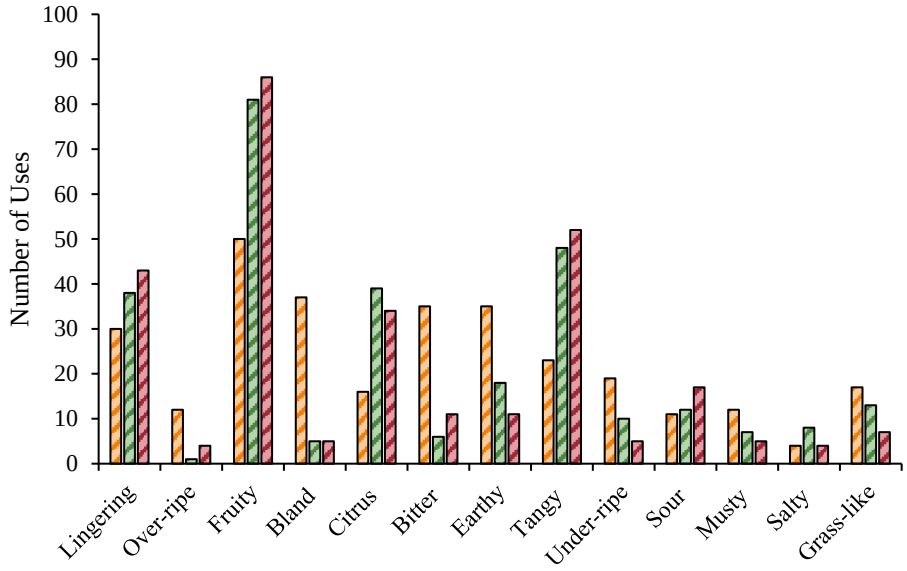


Figure 3.8 - Frequency of usage of descriptors from the adapted descriptor list of 2017 provided in the 2018 questionnaires. Participants were encouraged to select as many values as they thought applied to the fruit in question. Sample population is 111 individuals that provided a total of 301 responses for Royal Star (▢), 286 for Summer Sun (▢) and 284 for Piccolo (▢).

Adaptation of the list of descriptors presented to participants achieved the expected result. Removal of simplistic descriptors forced panellists to engage with the analysis in a more in-depth way, yielding an increase in the mean descriptors selected per panellist, 2.7±1.1 up from 2.2±1.1, and a more comprehensive profile from the new descriptor list. As with the hedonic and JAR tests, the tangerine tomato Royal Star received a higher frequency of negatively associated attributes than either of the other cultivars in 2018. Particularly notable is the rise in frequency of blandness and bitterness in the Royal Star fruits. Blandness is a good indication of dissatisfaction in the flavour profile or intensity of flavour present in the fruits, particularly of low levels of sugars and acids. The general dissatisfaction with Royal Star can be largely explained by the increased instance of 'over-ripe' votes and concomitant decreased acidity and fruitiness. This would likely yield a blander, less engaging overall flavour, which is reflected in the data. Interestingly, although Piccolo and Summer Sun are extremely different cultivars, the frequency of descriptor usage was comparable in each case.

Penalty analysis was performed on the JAR and hedonic overall acceptance responses for both the 2017 (**Figure 3.9**) and 2018 (**Figure 3.10**) sensory analysis results. Penalty analysis calculates the impact of attributes that are not 'just about right', in the opinion of a proportion of the participants, based on the frequency of votes higher and lower than the JAR point. The JAR point is a 3 on the 1-5 scale used in this study. The mean overall acceptance score given by each panellist that votes a 3 in each attribute is then compared to the mean of all those that vote lower in that attribute and all those that vote higher. Weightings for values on the extreme of the scale (1 or 5) are not applied and aggregate groups for 1 and 2 votes (low group) and 4 and 5 votes (high group) are calculated. The low group mean is subtracted from the JAR mean acceptability. This is repeated for the high group and then for each other attribute in question. The value received indicates the difference in overall acceptability of the product between respondents that believed it was 'just about right' and those that were dissatisfied with the attribute. Larger values indicate significantly lower overall acceptability in low or high groups. These effect intensity values are then plotted against the frequency by which the dissatisfaction occurs in the sampled population. Attributes that occur in more than 20% of the responses and which also result in an average decrease in acceptability of greater than one point are considered to impact the overall liking of the product significantly, often referred to as the 'critical corner'.

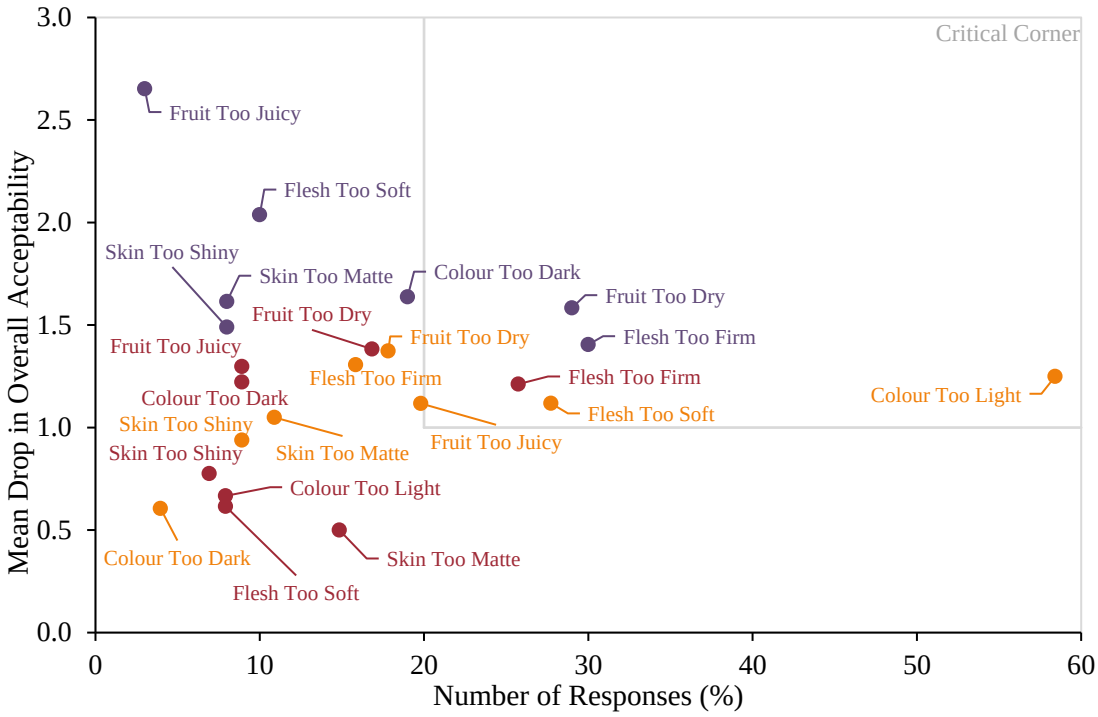


Figure 3.9 - Penalty analysis of JAR mean scores vs frequency of complaint about each measured attribute in the 2017 sensory analysis study. Attributes that score in the 'Critical Corner' (≤25% responses, ≤ 1 drop in acceptability) can be considered to influence overall acceptance scores for that cultivar. ● = Oranjestar'17, ● = Sweetelle'17, ● = Piccolo'17. (n=101)

As can be seen in **Figure 3.9**, there was at least one JAR attribute that resulted in a greater than one-point drop in overall acceptance for each of the cultivars analysed in 2017. Piccolo only consistently lost overall acceptability scores when panellists identified that the fruit flesh was firmer than they would prefer. This attribute was identified by 25.7% of participants and caused an average drop of 1.21 in overall acceptability in this group, when compared to those that did not identify an issue with the firmness. Sweetelle was identified as having too firm flesh and being too dry by 29.7% and 28.7% of participants respectively. Panellists that identified that the fruit were too dry showed a larger mean drop in overall acceptance, 1.58, than those that considered fruit to be too firm, 1.40. It is worth noting that ~46% of participants that noted Sweetelle was too dry also thought the fruit was too firm, so a cumulative action between the parameters is likely to have affected the overall acceptance of the cultivar and the individual effects may be less potent. Oranjestar was also deemed to have two characteristics that impacted the perception of the cultivar. It is not unexpected that the colour of the tomato was considered to be too light by 58.4% of participants, resulting in a 1.25 mean point drop in acceptability. In addition, the fruit of Oranjestar were generally considered to be too soft by 27.7% of the sampled population, causing a decrease of 1.12 points in average overall acceptability. It is worth noting that there were several attributes that caused a significant point drop in panellists that recognised them, but that the frequency of the complaint was insufficient to affect the overall perception of the cultivars. This seems particularly prevalent in Sweetelle, where complaints were few for most of the measured

attributes, but did significantly sway the overall acceptance of the cultivar in these groups. This could be indicative of poor homogeneity of crop quality, with a small proportion of fruits in the same harvest being of lower quality than average. Participants who selected one of these tomatoes would rate the whole cultivar lower due to the experience.

Figure 3.10 performs the same penalty analysis on the second round of sensory testing data, conducted in 2018. The findings of this set of data collection markedly differ from those in the 2017 period. The majority of JAR influenced dissatisfaction was focused on Royal Star, with three attributes well into the 'critical corner' of the penalty analysis plot. Royal Star fruits were simultaneously considered to be both too dry and too juicy by 32.4% and 33.3% of panellists, which resulted in a mean drop of liking of 1.93 and 2.32 respectively. The contrasting experience of these groups of panellists is difficult to explain. The respondents are almost perfectly split into thirds over this issue, with each third thinking the fruit were too dry, 'just about right' or too juicy. As with Sweetelle above, this could be indicative of crop variability and a reduction in predictability of fruit characteristics across a harvest. The indecisiveness around this characteristic could suggest another textural parameter causing dissatisfaction, but one that it is not adequately covered in the answers available on the questionnaire. Unfortunately, this cumulative dissatisfaction in the fruit texture seems to have had a significant impact in the overall liking of the cultivar. In addition, Royal Star fruits were also thought to be too soft by 44.1% of panellists, resulting in a mean drop in overall acceptability of 1.88 points. As above, this fuels the argument that the texture is a strong driver in cultivar dissatisfaction for both tangerine tomatoes, Royal Star and Oranjestar.

Figure 3.10 - Penalty analysis of JAR mean scores vs frequency of complaint about each measured attribute in the 2018 sensory analysis study. Attributes that score in the 'Critical Corner' (≤25% responses, ≤ 1 drop in acceptability) can be considered to influence overall acceptance scores for that cultivar. ● = Royal Star'18, ● = Summer Sun'18, ● = Piccolo'18. (n=111)

The only attribute that was thought to significantly impact the acceptability of Piccolo was too firm fruit, which echoes the findings of the 2017 period. In this case, only 23.4% of participants complained that the fruit were too firm for their liking, resulting in a drop of 1.79 points in overall acceptability. The fact that this attribute has been identified as unsatisfactory over two years of sampling, two crop harvests and two independent panels of sensory analysis, is a strong indicator that Piccolo is too firm for a significant proportion of the sampled population. If the percentage of complaints were extrapolated to the wider population, it may indicate that Piccolo falls short in the public perception, at present, and efforts to slightly soften the texture may yield a more widely accepted fruit. Summer Sun was the only cultivar over the two periods which had no JAR parameters in the 'critical corner'. This indicates that, although some attributes were identified as unsatisfactory to consumers, the overall acceptability was not significantly swayed by any of these parameters and overall liking of the cultivar was determined by unexplored drivers.

3.4.3 Annual of Preference for Piccolo Fruits

Piccolo was used as a 'control cultivar' for the two sensory sampling periods in order to gain further insights and understanding of the markedly different consumer preferences demonstrated between the two sampling periods and separate sensory panels. As the panellists that took part in both batches of sensory analysis were recruited independently for each study and were not required to participate in both, samples were not analysed pairwise. The distribution of responses was compared and shown to be comparable, allowing the use of a Mann-Whitney-U test, the results of which are presented in **Table 3.3**.

Table 3.3 – The overall ratings for specific attributes of fruit quality, as assessed by hedonic scale, on Piccolo fruits across two separate sensory analysis sampling periods. Significance determined by Mann-Whitney-U test. (n=101 for PIC'17, n=111 for PIC'18)

		Quality Parameter			
		Appearance	Flavour	Texture	Acceptability
PIC_'17	Median	9	9	8	9
PIC_'18		9	8	8	9
PIC_'17	Mean Ranks	102.65	113.70	110.37	112.63
PIC_'18		110.00	99.95	102.98	100.92
Piccolo	U	5,994.5	4,878.0	5,214.5	4,986.0
	z	0.911	-1.669	-0.895	
	Asymp. Sig.	.362	.095	.371	.153

*Significance presented at the *p≤0.05 or **p≤0.01 level*

Piccolo was homogeneous across the two sampling periods, with non-significant changes in the responses provided by panellists; although there was a non-significant decrease in the mean scores for overall flavour, texture and acceptability.

In a recent paper by Causse *et al.* 19 commercial tomato cultivars were analysed by expert sensory assessors in France, Italy and The Netherlands to determine geographical and cultural shifts in preference and liking as well as to identify those attributes and traits that are common to consumer acceptance. The authors confirmed that sugars, acids and flavour intensity were some of the key drivers for consumer acceptability and that this relationship was common across each of the countries involved. However, they also noted that a certain degree of diversity in flavour profile and fruit texture is required to please all European demographics, as there was less agreement in these parameters between the panels (Causse *et al.*, 2010).

3.5 Conclusions

The aim of this chapter was two-fold, firstly to understand the drivers, engagement, purchasing and handling practices of consumers in relation to fresh tomatoes. Secondly, to better understand the drivers and misconceptions of consumer preference of fresh tomatoes in regular, everyday consumers, both during sampling of tomatoes through sensory analysis and reflectively during the online survey. Many prior studies have aimed to use sensory analysis as a quantitative tool for characterisation of flavour in tomato fruits, which is extremely valuable, in tandem with quantitative chemometric analysis of the matrix. However, preference testing on a large scale can reveal the perceived desirable and negative attributes of products as well as elucidating the level of understanding and engagement presented by the panellists. Many attributes, previously shown to be key to tomato flavour, are either not detected, are poorly understood or are given low importance by untrained consumers, suggesting that, although they are important to overall tomato flavour, consolidated attempts to improve these values may not be fully appreciated by the everyday consumer, at least not initially. To reiterate previous claims, sweetness, fruitiness and, to a lesser extent, sourness, appear to be attributes that consumers can identify, relate to and which drive their overall liking of cherry tomato fruits. The fact that fruitiness is both understood and is a strong driver in overall liking, indicates that a focus on increased carotenoid derived volatiles and high sweetness may be the most promising direction for smaller fresh tomatoes, such as cherry and tangerine types. Non-standard ripening tomatoes, such as tangerine or yellow types seem to be less well-liked than their red counterparts. However, much of the dissatisfaction surrounding these fruits comes from their unusual appearance, with more comparable liking scores following consumption. As long as these types of tomato retain similar organoleptic quality, unlike Royal Star, to the standard red fruits they will be accepted by the public. More time and exposure to these fruits should yield higher appearance scores as the public accepts that the colouration is not indicative of unripeness or poor fruit quality.

In addition, the market research survey revealed a number of issues both with understanding and handling of tomatoes in the home. It has been well documented that tomatoes, like many other fruits, are significantly impacted by low temperature storage (Hobson, 1987, Maul *et al.*, 2000). However, all the findings of these researchers have yet to be effectively instilled in the general population. Over three quarters of respondents still store tomatoes in a refrigerated environment, severely hampering flavour and quality development in the days following purchase and prior to consumption. Household refrigerators commonly operate at 1-4 °C, below the temperature previously demonstrated to suppress respiration, metabolism and flavour formation in tomato fruits. This indicates that only those involved in the growth or sale of tomato fruits are aware of proper, household storage practices. There is an opportunity to educate the public on this

issue and it would be beneficial on the part of the retailer to lead efforts to communicate this to consumers.

3.6 Future Work

This work demonstrates that there are several attributes that are important to tomato flavour, but which are both misunderstood and undervalued by the general population. This lack of understanding of terms such as 'green/grassy', 'earthy', 'musty/mouldy' etc. hampers detailed description of tomato flavour by untrained participants, both in sensory analysis and market research survey sections of this chapter. Consumers are still unaware of the best storage practices of tomato fruits in the home, probably leading to suppression of organoleptic quality during the period between purchase and consumption. Previous publications have confirmed post-harvest treatments below 15 °C are detrimental to the formation of sugars, accumulation of acids and modifies the volatile profile, however, the information has not percolated from the scientific community to the consumer it would seem (de León-Sánchez *et al.*, 2009, Maul *et al.*, 2000, Ponce-Valadez *et al.*, 2016).

A valuable avenue for utilising this data more effectively, would be to further adapt and validate the widely accepted 'penalty analysis' method, commonly used on 'just about right' scales in sensory analysis to use binary descriptor data, which is often difficult to interpret. This would require an expansive dataset from untrained panellists, preferably using both open choice descriptor maps and forced-choice binary descriptor questions, in order to understand the impact of directly querying the attribute, as opposed to allowing participants to select all those that apply.

4 Chemical Differentiation of Ten Commercial Tomato Cultivars

4.1 Chapter Abstract

Tomatoes (*Solanum Lycopersicum cv.*) from 10 commercial, UK cultivars were grown in glasshouses, at Thanet Earth, Kent, throughout 2015. Flavour and taste-active components, along with some volatile precursors, were quantified by GC-TOF-MS for volatiles, GC-MS for amino acids, HPLC-UV for nucleotides and enzymatic assays from sugars and organic acids. Additionally Total Soluble Solids (TSS), acidity (pH) and external colour were elucidated by %Brix, pH meter and DigiEye digital imaging, respectively. Furthermore, complementary sensory analysis on 68 organoleptic attributes was conducted by MMR Research Worldwide using a trained panel for each of the cultivars and compared to the analytically derived chemical composition. The analysed cultivars presented, individual, distinctive profiles based on both the quantification of quality defining components and through sensory profiling. The distribution of various flavour or taste-active compounds enabled cultivars to be easily distinguished. Discriminant classification of the cultivars was possible using just the volatile profile, with 91.7% correct classification rate of 252 samples. The misclassified samples were mainly a single salad-type cultivar that was misclassified into similar cultivars. The mean intensity scores for sweetness, sourness and umami were strongly correlated with the analytically calculated means for the same cultivars with linear regression models for mean sweetness, umami and sourness explaining 93.7%, 82.8% and 80.3% of the variability between sensorial and analytical datasets, respectively. Correlation of flavour attributes to volatiles was not possible due to the complexity of olfaction and sample handling procedures. A distinct difference between two harvest seasons of 'Elegance' tomatoes was observed in both volatiles and taste active components. Although this difference was significant for the analytical data, little change was observed by the sensory panel, particularly based on the significant shift in volatile profile. The shift in flavour and taste active components between seasons was attributed to the artificial lighting and heating used for winter fruits, particularly high pressure sodium (HPS) lamps.

4.2 Introduction

For many years, tomato quality and flavour has been the subject of much contention. There has been ongoing public debate relating to the decline in flavour of commercially grown tomatoes, in the media, commercial sector and scientific community. Many studies have attempted to identify the root cause of the loss in tomato flavour, with some attributing it to poor post-harvest practices by the industry and retailers (Boukobza and Taylor, 2002, Kader *et al.*, 1978), some noting the shift in vital flavour components such as sugars, acids and volatiles (Malundo *et al.*, 1995, Stevens *et al.*, 1979) and others examining the newly constructed tomato genome for clues as to the aforementioned changes (Baldwin *et al.*, 2000, Klee and Giovannoni, 2011, Tieman *et al.*, 2006b). However, most authors agree that the partial loss of important flavour characteristics and intensity has been exacerbated by the need for effective commercialisation; suitability for transport, uniform size/shape/colour, increased fruit yield and disease and pest resistance.

Since tomatoes were cultivated as a commercial food crop, growers have selectively bred and crossbred to form new species with a range of desirable characteristics. Many cultivars that are within the same class of tomatoes display different flavour profiles and chemical composition (Baldwin *et al.*, 1998, Krumbein and Auerswald, 1998, Hernández Suárez *et al.*, 2008b). As tomatoes now account for a significant proportion of many diets worldwide, and have been incorporated into the cuisines of numerous cultures, improvement in their quality is highly sought. It is widely accepted that the organoleptic qualities and profiles of tomato vary between cultivars. This is desirable, as cultivars are often created to provide a different sensorial experience for the consumer. Tomatoes possess a highly varied flavour profile, mainly due to the complex interactions and compositional differences of taste and/or flavour active compounds such as sugars, organic acids, volatiles, amino acids and nucleotides (Buttery, 1993, Buttery *et al.*, 1987, Petro-Turza, 1986). Primary flavour components include glucose, fructose and, to a lesser extent, sucrose; citric and malic acids; over 400 volatiles, with between 10 to 30 believed to directly influence flavour; glutamic and aspartic acids alongside the 5'monophosphate nucleotides (Malundo *et al.*, 1995, Oruna-Concha *et al.*, 2007, Petro-Turza, 1986). The volatile profile of tomato fruits is extensive, but many of these are present below the odour detection threshold (ODT) and therefore are unlikely to influence the perceived flavour. In 1987, Buttery, Teranishi and Ling found that only (*Z*)-3-hexenal, 3-methylbutanal, β-ionone, 1-penten-3-one, hexanal, (*Z*)-3-hexanol, (*E*)-2-hexenal, 3-methylbutanol, 2-isobutylthiazole, 6-methyl-5-hepten-2-one and methyl salicylate were present at concentrations in excess of their ODT. Contrary to this, Tandon *et al.* assessed the detectability of the same volatiles, using abundance data from tomatoes from Buttery, Teranishi and Ling and found that only (*Z*)-3-hexenal, hexanal, 1-penten-3-one and 3-methylbutanal were detectable in spiked deodorised tomato homogenate (Buttery *et al.*, 1987, Tandon *et al.*, 2000). Tandon *et al*. also noted that the matrix of evaluation had a

significant impact both on the detectability and odour profile of the compounds thought to influence the flavour of tomato, with deodorised tomato homogenate increasing the threshold of detection substantially over aqueous solutions of each volatile. Additionally, the volatiles identified as being flavour contributors by Buttery, Teranishi and Ling in 1987 were determined based on the odour thresholds presented by Buttery *et al*. in an earlier paper (Buttery *et al.*, 1970, Buttery *et al.*, 1987). However, these odour thresholds were elucidated using aqueous solutions of the volatiles, which Tandon *et al.* demonstrated provided artificially low ODT when compared to a deodorised tomato homogenate matrix. Therefore, of the eleven volatiles that were present in fresh tomato at amounts that exceeded the odour thresholds defined by Buttery *et al.*, as few as three could be present in sufficient quantities to be perceived. In a study by Ruiz *et al.* significant differences were found in 5 closely related tomato cultivars when comparing the concentrations of 5 key tomato volatiles and sugar, organic acid and L*a*b* colour levels (Ruiz *et al.*, 2005). Additionally, Baldwin *et al.* and Krumbien, Peters and Brückner found that the volatile profile was significantly different between a number of tomato cultivars (Baldwin *et al.*, 1991a, Krumbein *et al.*, 2004, Baldwin *et al.*, 2000). However, other authors have found that the concentration of volatile components is unrelated to the perceived flavour of tomatoes. Buttery *et al.* was not able to significantly differentiate tomato cultivars based on the concentrations of volatiles, specifically in regards to C_5-C_{13} volatiles; however, limited statistical analysis was performed on the dataset (Buttery *et al.*, 1988). Notable differences in the flavour volatiles that are at/or above their respective odour detection thresholds are indicative of a shift in the perceivable tomato flavour between cultivars. Complementing the analytical flavour data with comprehensive organoleptic analysis is likely to allow for correlation or regression analysis in identifing relationships between the different analytical and sensorial profiles of cultivars.

The combination of chemometric and sensory profiling of fresh produce is one of the most useful tools to convert analytical, quantitative data into a format that is more comparable to consumer liking and acceptance. Isolated concentration measurements of taste and flavour active compounds in foodstuffs are difficult to relate to how consumers perceive the food, without sensory analysis to complement the data. The ability to differentiate cultivars on both a chemical and sensorial level is highly valuable to the tomato growing industry. The ability to rapidly evaluate cultivars for organoleptic quality using simplistic measurements would be a great asset in ruling out underperforming cultivars and redirecting growers toward higher quality produce. A threefold correlation of sensory analysis, detailed analytical data and simplistic on-the-spot measurements will allow growers to quickly evaluate their crop in the greenhouse. However, the relationships between chemical constituents may be different between cultivars and, therefore, it is vital to understand the relationships between chemical composition and the inter-cultivar variability.

4.3 Methods and Materials

All methods and materials used in this chapter have been previously described in Chapter 2 of this thesis.

For the purposes of this work and to facilitate discussion, ELE and EL2 are treated and referred to as separate cultivars. In practice, they are the same cultivar grown during different seasons, which will also be explored.

4.4 Results and Discussion

4.4.1 Total Soluble Solids (TSS) and Locular pH

Total Soluble Solids (TSS) and pH are commonly used methods of rapidly assessing the quality of plant crops, including tomatoes (Balibrea *et al.*, 2006, Beckles, 2012). TSS is a refractive measure of soluble dry matter, this includes sugars and acids, which contribute approximately 70-80% of the dry matter, but is also influenced by other soluble compounds such as amino acids, vitamins, minerals (Beckles, 2012). Due to this, TSS, although commonly represented as 'sugar content', often is misrepresentative of the sugar and, therefore, sweetness of fruits. This often occurs due to highly varied acid content, either due to cultivation or post-harvest practices, or specific cultivar traits. As organic acid content accounts for a significant proportion of TSS it can dramatically change the %Brix value, and therefore misrepresent the true sugar content. It is widely accepted that larger fruited cultivars have proportionally lower TSS content than smaller tomato types. This relationship is easily observable in **Figure 4.1**, where the fruit weight and %Brix values of each of the fruits used in this chapter was compared. The observed relationship is non-linear, but this is primarily due to the use of multiple high sugar/acid cherry tomato cultivars in this study. The four cherry and two plum tomato cultivars had very similar fruit weights, ranging from 11.05-19.76 g for the Piccolo and Sunstream respectively. Unsurprisingly, Elegance fruits recorded the lowest TSS, but the average fruit weight of Elegance fruits across the two harvests was approximately 55% larger than those of the next largest cultivar, Temptation.

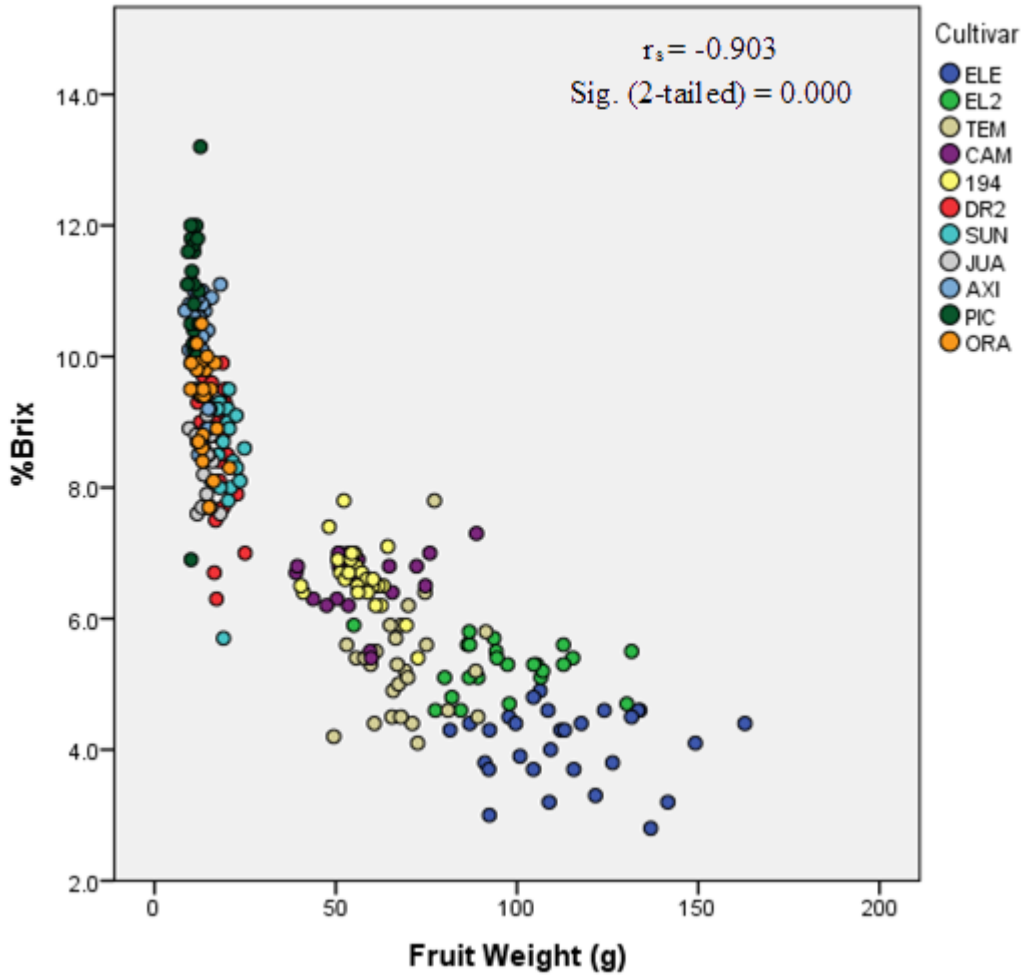


Figure 4.1 – Strong, non-linear, inverse correlation (r_s = -0.903, p<0.0005) between the weight of fruit and total soluble solid (TSS), represented as %Brix. Correlation assessed using Spearman's rank-order correlation (n=274).

The relationship between soluble solid content and locular pH was unpredictable, as seen in **Figure 4.2**. This highlights the limitations of TSS and %Brix measurements for accurate prediction of sugar content, as the acidity, amongst other dry matter contributors such as amino acid content, can be highly variable. These soluble components may therefore significantly alter the detected TSS content of fruits, incorrectly assuming lower or higher sugars than are truly present. It should be noted that the pH measurements are solely based on the locular cavity pH and not fruit homogenate. Moreover, as identified in Chapter 6, the locule fluid contains significantly higher proportions of acidic components than the surrounding flesh, therefore decreasing the pH relative to the pH of a homogenised fruit.

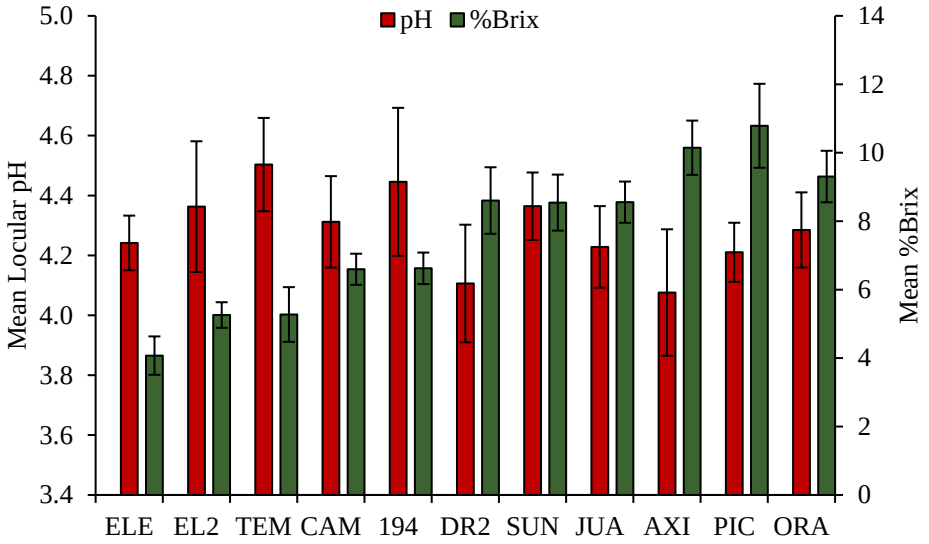


Figure 4.2 – The mean locular pH and %brix of fruits of the studied cultivars. Error bars represent standard deviation (n= ELE=29, EL2=24, TEM=27, CAM=24, 194=26, DR2=24, SUN=21, JUA=25,AXI=28, PIC=26, ORA=22).

In general, cherry tomatoes presented with lower pH and higher TSS content than the larger fruited cultivars, which agrees with previous findings. The highest TSS content was observed in Piccolo fruits, followed by Axiani and Oranjestar, whereas the lowest soluble solids were present in both harvests of Elegance tomatoes and the Temptation crop. The most acidic locular contents were found in Axiani and DR2 fruits, with the least acidic tomatoes being P194 and Temptation fruits.

4.4.2 Composition and Ratio of Sugars and Acids and the Respective Gustatory Impact of Each

One of the most important characteristic organoleptic properties of fresh tomato is the intensity and balance between the sweetness, provided by sugars, and acidity, contributed primarily by organic acids, although many other acidic components are also present in tomato (Bauchet *et al.*, 2017, Gautier *et al.*, 2008, Hernández Suárez *et al.*, 2008a, Jones and Scott, 1983, Malundo *et al.*, 1995). The sweet/sour balance in tomatoes, coupled with the savouriness provided by umami compounds and certain volatiles promotes their inclusion in many savoury dishes and cuisines.

The content of sugar and acids, their ratios and respective gustatory intensities in fruits of the studied cultivars can be seen in **Table 4.1**.

Table 4.1 – The sugar and organic acid content of the 11 cultivars studied along with ratios and their contributions to the perceived taste of fresh tomatoes of these cultivars. Data is presented as mean ± standard deviation. Sweetness and sourness conversion based on the values presented by McLaughlin and Margolskee. Significance was determined be Kruskall-Wallis-H test and subsequent Post-Hoc analysis by pairwise comparisons. Significance was accepted at the $p<0.05$ level, following Bonferroni correction for multiple comparisons. Pairwise significance is indicated by the same alphabetic character per column, a to z plus α (McLaughlin and Margolskee, 1994).

Cultivar	Harvest	n	Glucose (mg/g FW)	Fructose (mg/g FW)	Total Sugars (mg/g FW)	G/F Ratio	Total Sweetness (mg Sucrose/ g FW)	Citric acid (mg/g FW)	Malic acid (mg/g FW)	Total Acids (mg/g FW)	C/M Ratio	Total Sourness (mg Citric acid/ g FW)	Sugar/Acid Ratio
ELE	Winter	33	11.9 ± 2.5 a,b,c,d,e,f	12.4 ± 2.5 a,b,c,d,e,f	24.3 ± 4.1 a,b,c,d,e,f	1 ± 0.2	25.7 ± 4.5 a,b,c,d,e,f	3.3 ± 1.1 a,b,c,d,e	0.5 ± 0.1 a,b,c,d,e,f,g	3.8 ± 1.1 a,b,c,d	6.9 ± 2.6 a,b,c,d,e,f	4 ± 1.1 a,b,c,d	6.9 ± 2.6 a,b,c
EL2	Summer	26	12.7 ± 2 g,h,i,j,k,l	13.3 ± 2.6 g,h,i,j,k,l	26 ± 3.6 g,h,i,j,k,l	1 ± 0.2	27.5 ± 4.1 g,h,i,j,k,l	2.9 ± 0.7 f,g,h,i,j	0.4 ± 0.1 a,h	3.3 ± 0.7 e,f,g,h,i	7.9 ± 1.8 g,h,i,j,k,l	3.4 ± 0.7 e,f,g,h,i	8.4 ± 2.8
TEM	Winter	28	14.5 ± 3.3 m,n,o,p,q	15.7 ± 3.7 m,n,o,p,q,r	30.2 ± 6.3 m,n,o,p,q,r	0.9 ± 0.2	32.1 ± 6.9 m,n,o,p,q,r	4 ± 0.7 k,l,m	0.4 ± 0 i	4.4 ± 0.7 j,k,l	9.6 ± 2.2 m,n,o,p	4.5 ± 0.7 j,k,l	7.1 ± 2 d,e
CAM	Summer	25	14.8 ± 3 r,s,t,u	17 ± 2.6 s,t,u	31.8 ± 4.5 s,t,u	0.9 ± 0.2	34.1 ± 4.7 s,t,u	3.1 ± 0.7 n,o,p,q,r	0.3 ± 0.1 b,h,i,j,k,l,m	3.4 ± 0.7 m,n,o,p,q	11.4 ± 2.7 a,g,q,r	3.5 ± 0.7 m,n,o,p,q	9.5 ± 2.3 a,d,f,g,h,i
194	Summer	28	13.4 ± 2.9 v,w,x,y,z,α	16.8 ± 2.3 v,w,x	30.2 ± 4.1 v,w,x,y,z,α	0.8 ± 0.2	32.9 ± 4.2 v,w,x,y,z	3 ± 0.7 s,t,u,v,w	0.4 ± 0.1 c,o	3.4 ± 0.7 r,s,t,u,v	8.6 ± 2.1 s,t,u,v,w	3.5 ± 0.7 r,s,t,u,v	9.3 ± 2.5 b,j,k,l
DR2	Summer	27	19.9 ± 4.9 a,g,m,r,v	23.2 ± 5.1 a,g,m,s,v	43.1 ± 9.4 a,g,m,s,v	0.9 ± 0.2	46.4 ± 10 a,g,m,s,v	3.6 ± 0.7 x,y,z	0.4 ± 0.1 d,j	4 ± 0.7 x,y,z	9.4 ± 1.6 x,y,z,α	4.1 ± 0.7 w,x,y	10.8 ± 2 c,e,m,n,o,p
SUN	Winter	23	19.4 ± 2.6 b,h,n,s,w	21 ± 3.5 b,h,n	40.4 ± 5.1 b,h,n,w	0.9 ± 0.2	43 ± 5.9 b,h,n,w	4.6 ± 0.9 a,f,n,s,α	0.4 ± 0.1 e	5 ± 0.9 e,m,r,α	13.2 ± 3.9 b,h,s	5.1 ± 0.9 e,m,r,z	8.3 ± 1.8 q,r
JUA	Summer	27	18.2 ± 4.6 c,i,x	20.4 ± 3.4 c,i,o	38.5 ± 6.7 c,i,o,x	0.9 ± 0.2	41.2 ± 6.7 c,i,o	5.3 ± 1.6 b,g,o,t	0.4 ± 0.1 f	5.7 ± 1.6 a,f,n,s	15.1 ± 5.7 c,i,m,t,x	5.8 ± 1.6 a,f,n,s	7.4 ± 3 f,m
AXI	Summer	28	22.1 ± 4.4 d,j,o,t,y	25 ± 4.3 d,j,p,t,w	47.2 ± 7.6 d,j,p,t,y	0.9 ± 0.1	50.5 ± 8.1 d,j,p,t,x	7.4 ± 1.3 c,h,k,p,u,x,α	0.4 ± 0.1 k	7.8 ± 1.3 b,f,j,o,t,x	18.8 ± 5.1 d,j,n,q,u,y	7.9 ± 1.3 b,g,j,o,t,w	6.2 ± 1.4 g,j,n,q
PIC	Winter	27	22.8 ± 4.9 e,k,p,u,z	24.9 ± 4.4 e,k,q,u,x	47.7 ± 9 e,k,q,u,z	0.9 ± 0.1	50.8 ± 9.3 e,k,q,u,y	7.2 ± 1.5 d,i,l,q,v,y	0.4 ± 0.1 l	7.6 ± 1.6 c,g,k,p,u,y	17.2 ± 4.3 e,k,o,r,v,z	7.8 ± 1.6 c,h,k,p,u,x	6.5 ± 1.8 h,k,o
ORA	Winter	27	19.2 ± 3.9 f,l,q,α	20.9 ± 3.2 f,l,r	40.1 ± 6.6 f,l,r,α	0.9 ± 0.1	42.7 ± 6.7 f,l,r,z	6.6 ± 1.7 e,j,m,r,w,z	0.4 ± 0.1 g,m	7 ± 1.8 d,h,l,q,v,z	17.9 ± 6.5 f,l,p,w,α	7.1 ± 1.8 d,i,l,q,v,y,z	6.3 ± 2.6 i,l,p,r

ELE/EL2 – Elegance, TEM – Temptation, CAM – Campari, 194 – P194, DR2 – DR28090TC, SUN – Sunstream, JUA – Juanita, AXI – Axiani, PIC – Piccolo, ORA - Oranjestar

One of the main drivers in both consumer acceptance of tomatoes and viability of new cultivars in the commercial environment is their sugar/acid content and balance. One of the prime examples of this is Piccolo, which has been commercially sold for 15-20 years, largely due to its high sugar and acid content amongst other parameters. In contrast, many other tomato cultivars are replaced every couple of years, as the industry aims to improve and innovate their product. As expected, Piccolo tomatoes present with the strongest sweetness and second highest sourness, very comparable to Axiani. Axiani has slightly lower sugars, but greater acids, slightly lowering the sugar/acid ratio and creating a marginally more tart taste, which is popular in cherry tomatoes. Oranjestar and Juanita tomatoes presented approximately 20% less sweetness than the aforementioned cherry types. Although similar in sweetness, Juanita and Oranjestar were very different in sourness, with Oranjestar just ~10% less sour than Piccolo, unlike the ~35% decrease seen in Juanita. This may be beneficial in Oranjestar tomatoes which are classically more 'fruity' where both sweetness and acidity are essential. Sunstream and DR2 were also noticeably different for the plum tomatoes. Both cultivars present similar sweetness, but DR2 contained 20% less total acids, resulting in a comparable decrease in sourness. The salad types can be loosely categorised on fruit size, as previously examined in **Figure 4.1**. Campari, Temptation and P194 are medium sized salad tomatoes and are relatively indistinguishable based on sugar and acid content and the resulting gustatory sensations. The primary difference is notable in Temptation fruits which generate ~30% more sourness than the other salad types, although this may also be an effect of season as TEM is the only winter crop in this category. The two harvests of Elegance fruits are marginally different. Between the two harvests the summer crop, EL2, presented with slightly greater sweetness, but lower sourness, which is reflected in their total sugar/acid ratio.

The rate at which tomatoes amass sugars, and the proportion of each sugar in 'Table Ripe' fruit is highly variable, with commonly reported glucose/fructose ratios ranging from 0.7-1.4 (Beckles, 2012, Luengwilai and Beckles, 2010, Luengwilai *et al.*, 2010, Rosales *et al.*, 2011, Vermeir *et al.*, 2009, Vogel *et al.*, 2010). The glucose/fructose ratios observed in this study are relatively predictable, slightly higher fructose accumulation in smaller fruited cultivars leads to a slight decrease in ratio and boost in sweetness. The reasons for variable ratios of glucose and fructose have not yet been determined. Both glucose and fructose are primarily liberated from the catabolism of sucrose and starch at the onset of ripening, with one molecule of sucrose liberating one molecule each of both glucose and fructose . The metabolism of sucrose and generation of starch has been shown to be primarily catalysed by sucrose synthase, with acid invertase and ADP-glucose pyrophosphorylase playing more minor roles (Schaffer and Petreikov, 1997, Wang *et al.*, 1993). The metabolism of the fructose derived from imported sucrose is catalysed by fructoskinase or hexokinase, as all fructose metabolic pathways require the phosphorylation prior to further metabolism (Kanayama *et al.*, 1998, Kortstee *et al.*, 2007). This conversion happens upon transient starch accumulation in maturing fruits and subsequent breakdown and generation

of hexoses. Imbalance in hexose ratios is relatively rare in fleshy fruits with sucrose as the primary imported carbohydrate. An exception is observed in apples, which generate higher proportions of fructose due to high proportions of imported sorbitol and conversion via sorbitol dehydrogenase (Li *et al.*, 2012). Genetic tools that will enable proportionally higher accumulation of one sugar over another may be a viable target for future breeding strategies i.e. favouring the preferential catabolism of glucose instead of fructose will increase the perceivable sweetness without actually affecting the total sugar content, thereby improving the sensorial properties without altering the caloric load significantly. Each molecule of glucose which is consumed in favour of fructose, essentially doubles the sweetness of sequestered sugars, due to the relative sweetness of 0.7 and 1.4 sucrose equivalents for glucose and fructose respectively. Therefore, lower glucose/fructose ratios are highly desirable in terms of the organoleptic properties of commercial tomato cultivation. The method by which this could be achieved is difficult to ascertain. Unfortunately, the initial step in the inclusion of fructose in many biochemical pathways is its conversion to fructose-6-phosphate (F6P), which is catalysed by hexokinases. In general, hexokinases are not substrate specific, phosphorylating a number of hexoses, whereas glucokinases are more specific to glucose (Causse *et al.*, 2004). Therefore, blocking or inhibiting this conversion may not be a viable target as it would disrupt the conversion of glucose and other hexoses, along with fructose, which would significantly hamper cellular function and many biochemical pathways. Downregulation of hexokinases and upregulation of glucokinases, which are much more substrate specific, primarily targeting glucose, may yield the desired accumulation of fructose. Modifying hexokinases in tomato fruits has been previously shown to be detrimental to plant health and growth, however most approaches aim to increase or supplement hexokinase content/activity (Dai *et al.*, 1999, Menu *et al.*, 2004, Roessner-Tunali *et al.*, 2003). A recent paper by Shammai *et al.* proposed an alternative method of increasing fructose accumulation in tomato fruits. By using introgression lines of commercial *Solanum lycopersicum* containing a Fgr^{H} allele from wild-type *Solanum habrochaites*, the authors were able to increase fructose accumulation by 60% with glucose content unaffected (Shammai *et al.*, 2018).

4.4.3 Concentration of Certain Amino Acids and Nucleotides and Umami Intensity

Umami is one of the five basic tastes, commonly considered to be synonymous with meaty/savoury attributes in many dishes and foodstuffs. Tomatoes are considered a respectable source of umami components, some α-amino acids and 5'-monophospahte nucleotides, which justifies their inclusion in savoury dishes (Ninomiya, 2002). Fresh tomatoes contains a relatively high concentration of glutamic acid and moderate aspartic acid (Ninomiya, 2002, Oruna-Concha *et al.*, 2007, Yamaguchi and Ninomiya, 2000). The umami sensation is solely provided by amino acids, in an additive relationship. However, the presence of certain monophosphates, including guanosine 5'-monophosphate (GMP) and adenosine 5'monophostphate (AMP), both present in fresh tomato, has been previously shown to enhance the intensity of the umami

sensation (Yamaguchi *et al.*, 1971). The total umami contribution of a foodstuff is determined by the presence of the aforementioned compounds, amongst others, and their impact relative to standard compounds. The standard used for the intensity provided by α-amino acids is monosodium glutamate (MSG). MSG was the first umami compound discovered, isolated and analysed, providing the highest intensity of umami taste of the α-amino acids (Yamaguchi *et al.*, 1971). Inosine 5'monophosphate is used as a benchmark for the enhancing properties of specific nucleotides. During the identification of umami as an individual taste sensation in the early 20th century, it was noted by multiple authors that IMP, commonly found in fish or fish based preparations, and GMP, which is abundant in dried mushrooms and other vegetables, enhanced the umami intensity (Yamaguchi, 1967, Yamaguchi and Ninomiya, 2000, Yamaguchi *et al.*, 1971). As the initial investigations into umami focused on its role as a chemical seasoning, IMP was used as a baseline due to its abundance and importance in fish sauces and dashi, kelp stock (Lindemann *et al.*, 2002, Yamaguchi and Ninomiya, 2000).

Table 4.2 – The relative contribution of several compounds to the overall perceived umami sensation in many foodstuffs. Relative comparisons made within classes of compounds, therefore amino acids are relative to MSG and 5'monophosphate nucleotides are relative to inosine 5'monophosphate (Mau, 2005, Yamaguchi *et al.*, 1971).

Compound	Relative Umami Concentration (RUC)
Glutamic acid	1.0
Aspartic acid	0.077

Compound	Relative Umami Concentration (RUC)
Inosine 5'monophosphate (IMP)	1.0
Guanosine 5'monophosphate (GMP)	2.3
Xanthosine 5'monophosphate (XMP)	0.61
Adenosine 5'monophosphate (AMP)	0.18

The initial findings of Yamaguchi *et al*. focused on the synergistic, but binary relationship between glutamate and a single purine ribonucleotide in solution. However, due to the additive nature of umami and using the original calculations put forward in the paper by Yamaguchi *et al.*, Mau was able to adapt the formula to account for multi-component mixtures like those found in most foods (Mau, 2005). Their equation was proposed as a method of converting the concentrations of the various umami components to a single, intensity rating referred to as Equivalent Umami Concentration (EUC) and represented as grams of MSG/100 g of material. The equation is as follows:

$$Y = \sum a_i b_i + 1218 \left(\sum a_i b_i\right) \left(\sum a_j b_j\right)$$

Whereby Y represents the EUC of the matrix in question. The concentration of the α-amino acids (g/100 g) is represented by a_i, and their intensity values, relative to MSG, used to modify their umami contribution is b_i. The concentration of umami enhancing, purine 5'monophosphate nucleotides (g/100 g) is represented by a_j, and their enhancing power, relative to IMP, is represented by b_j. The relative contributions of each of the components commonly found in foodstuffs can be seen in **Table 4.2,** for reference. By using this equation the EUC of foods can be determined relative to the amount of MSG alone required to emulate the same umami intensity. The umami intensity provided by the 11 crops of tomato fruits used in this study as well as the concentration of the related components in each can be seen in **Table 4.3**.

Table 4.3 – Concentration of umami components of 10 different cultivars and 2 harvest seasons of 'Elegance fruits. Data is presented as mean ± standard deviation. Significance was determined be Kruskall-Wallis-H test and subsequent Post-Hoc analysis by pairwise comparisons. Significance was accepted at the $p<0.05$ level, following Bonferroni correction for multiple comparisons. Pairwise significance is indicated by the same alphabetic character per column, a to z and α to γ.

Cultivar	Harvest	n	Concentration (µg/g FW)				EUC (g MSG/100g)
			Aspartic acid	Glutamic acid	GMP	AMP	
ELE	Winter	32	106 ± 26 a,b,c,d,e,f,g,h	1021 ± 249 a,b,c,d,e,f,g	15 ± 4 a,b,c,d	235 ± 53 a,b,c	1.1 ± 0.4 a,b,c,d,e,f,g
EL2	Summer	27	374 ± 85 a,i,j	1286 ± 292 h,i,j,k,l,m	17 ± 3 e,f,g,h	238 ± 43 d,e,f	1.4 ± 0.4 h,i,j,k,l,m
TEM	Winter	29	154 ± 36 i,k,l,m,n,o,p	1606 ± 291 a,x,y	18 ± 3 i,j,k	238 ± 39 g,h	1.8 ± 0.5 a,n,o,p
CAM	Summer	27	432 ± 117 b,k,q,r	1415 ± 353 n,o,p,q,r	18 ± 4 l,m,n	260 ± 48 i,j	1.7 ± 0.5 q,r,s,t
194	Summer	28	396 ± 125 c,l,s	1448 ± 479 s,t,u,v,w	17 ± 4 o,p,q	234 ± 43 k,l,m	1.6 ± 0.7 u,v,w,x,y
DR2	Summer	30	542 ± 153 d,m,t,u,v,w,x	2028 ± 473 b,h,n,s	20 ± 4 r,s	270 ± 50 n,o	2.6 ± 0.9 b,h,u,z,α
SUN	Winter	28	349 ± 52 e,n,t,y	2829 ± 375 c,i,t,x	25 ± 7 a,e,i,l,o,t	280 ± 71 p	4.1 ± 1.2 c,i,n,q,v
JUA	Summer	20	268 ± 116 f,q,u	2313 ± 892 d,j,o,u	19 ± 6 u,v	229 ± 75 q,r	2.7 ± 1.4 d,j,β,γ
AXI	Summer	20	309 ± 68 g,o,v	2359 ± 492 e,k,p,v	23 ± 7 b,f,w	287 ± 88 a,d,k,s	3.2 ± 1.1 e,k,r,w
PIC	Winter	20	346 ± 85 h,p,w	3152 ± 652 f,l,q,w,y	32 ± 10 c,g,j,m,p,r,u	362 ± 116 b,e,g,i,l,n,q	5.5 ± 1.3 f,l,o,s,x,z,β
ORA	Winter	20	209 ± 119 j,r,s,x,z	2185 ± 828 g,m,r	51 ± 10 d,h,k,n,q,s,t,v,w	554 ± 115 c,f,h,j,m,o,p,r,s	6 ± 2.3 g,m,p,t,y,α,γ

ELE/EL2 – Elegance, TEM – Temptation, CAM – Campari, 194 – P194, DR2 – DR28090TC, SUN – Sunstream, JUA – Juanita, AXI – Axiani, PIC – Piccolo, ORA - Oranjestar

It is immediately apparent that the larger fruited cultivars produced lower levels of most umami components on a weight for weight basis, than the smaller fruited cherry tomato cultivars. This agrees with the previous findings of Oruna-Concha *et al.* as well as the general trend of lower dry matter content in larger fruited cultivars (Oruna-Concha *et al.*, 2007). Piccolo had the highest concentration of glutamic acid as well as the second highest concentration of both umami nucleotides. Piccolo fruit also contained the most aspartic acid of the cherry type tomatoes, but significantly lower than that seen in DR2, P194 and Campari fruits. There is a general trend of increasing umami intensity with smaller fruited cultivars, but this is not absolute. For example, Sunstream presented with the third highest umami intensity, ahead of both Juanita and Axiani fruits, which were on average, 30% and 35% smaller than Sunstream fruits respectively. Interestingly the tangerine tomato, Oranjestar, presented the highest umami sensation (average EUC =6 g MSG/100g), which was not driven by the glutamic acid content, but by significantly higher GMP and AMP concentrations, which enhanced the taste intensity past that of Piccolo fruits. Oranjestar is typically considered to be quite a fruity and sweet cultivar, which is likely to be due to its inherent high sugar content and certain carotenoid derived volatiles, which add fruity/floral notes as seen in **Table 4.4**. Most importantly, based on the sensory profiling of the cultivars carried out by MMR Research Worldwide and presented in **Table 4.6**, Oranjestar scored second highest for umami intensity, behind Piccolo (MMR Research, 2018). The winter crop of Elegance, ELE, contributed the lowest umami sensation, the lowest concentration of both umami amino acids and produced the least GMP, which presents the most powerful enhancing capacity of the present nucleotides. There was also a distinct difference between the winter and summer crops of Elegance fruits, but this will be further explored in the chapter. The data presented in **Table 4.3** clearly shows that the cherry and plum tomatoes contribute significantly more intense umami than the salad, cocktail or beefsteak cultivars studied here. This relationship may not be true for all tomato cultivars available for purchase by consumers in the UK, but it seems likely that salad type fruit will provide less savoury tastes than other cultivars. This adds weight to the argument that the problems with tomato flavour, taste and quality are potentially not universal across all tomatoes, but more extreme in certain cultivars and tomato types, than in others.

The relationship between each of the umami components and the overall umami sensation is particularly interesting, as seen in **Figure 4.3A-D**. The least well correlated component is aspartic acid (r_s=0.321), due to a visible divide between the assessed cultivars. The large, fruited summer cultivars EL2, CAM and 194 as well as DR2 which was a summer, plum tomato, accumulate higher concentrations of aspartate, but this does not lead to the same increase in EUC as seen in the winter grown cultivars, or cherry tomatoes. The reason behind this increase in aspartic acid is not obvious, however its poor correlation to the total EUC of fruits can be attributed to its lower umami intensity. Aspartate provides just 7.7% of the umami sensation of glutamate, so it is possible that the accumulation of additional aspartate, although significant in itself, as seen in

Table 4.2, is not large enough to significantly drive the overall umami sensation. The generation of amino acids in ripening fruits is directly interlinked, with γ-aminobutyric acid (GABA) and glutamate playing key roles in the synthesis of other amino acids (Lam *et al.*, 1996, Umbarger, 1978). The deamination of glutamate to convert oxaloacetate, an intermediate in the citric acid cycle, to aspartate results in the production of aspartate and loss of glutamic acid in equal parts. The α-ketoglutarate formed from this reaction can be converted back to glutamic acid, however, it may be that larger fruited cultivars generate aspartate at a faster rate than they regenerate glutamate, leading to increased aspartate production, but suppressed glutamate accumulation, resulting in lower umami. The correlations in **Figure 4.3C-D** highlight that the significantly higher umami nucleotide content in Oranjestar fruits may be driving its higher umami sensation, not its glutamic acid content. In the case of both GMP (C) and AMP (D), Oranjestar fruits cluster separately, presenting significantly higher concentrations of both nucleotides than all other cultivars analysed. The same relationship is not apparent in the cultivars glutamate content, which, although high, is not significantly different to that of the other cherry tomatoes.

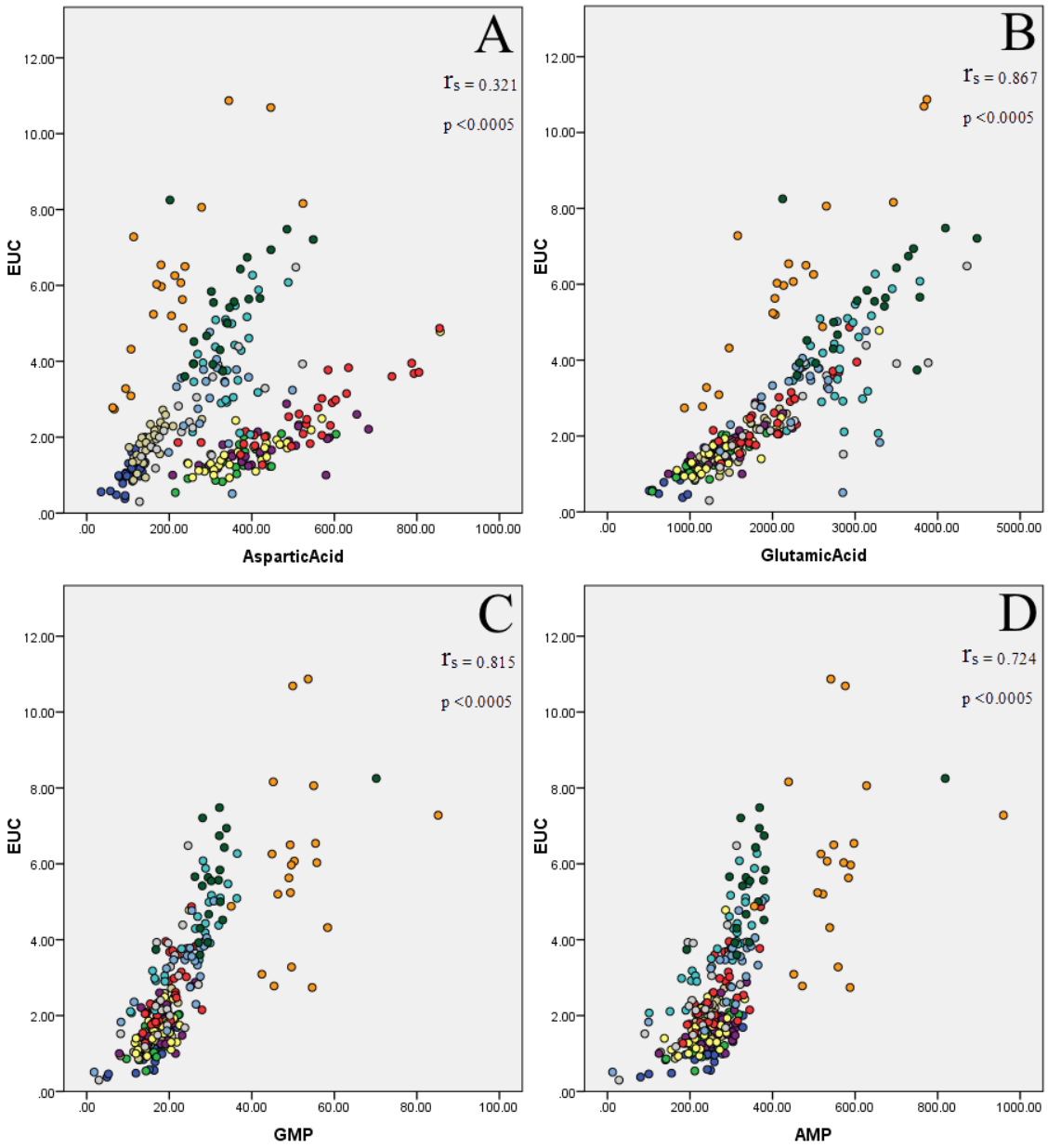


Figure 4.3A-D – Correlation between calculated Equivalent Umami Concentration (EUC) (g MSG/100 g) of each of the 11 cultivars and the concentration of each umami component. Correlations assessed using Spearman's Rank-Order correlation with a single, homogenous sample group (n=281). ● - ELE, ● - EL2, ● - TEM, ● - CAM, ● - 194, ● - DR2, ● - SUN, ● - JUA, ● - AXI, ● - PIC, ● - ORA.

The difference in nucleotide content is of particular interest as the only true outlying cultivar is the only non-standard ripening cultivar, a tangerine type cherry tomato. It is possible that one of the mutations that has led to the creation of tangerine tomatoes, which are characterised by their orange skin, has also affected the way fruits generate and store nucleotides. The apparent decrease in amino acids may indicate that the fruit are converting a greater proportion of GABA and glutamate to other products, including nucleotides. Althernatively, Chew *et al.* recently demonstrated that transgenic tomato cultivars overexpressing adenosine monophosphate (AMP) deaminase resulted in an increase of GMP concentration and simultaneous decrease in glutamate accumulation, which is very comparable to the findings here (Chew *et al.*, 2017). This finding

was accidental as the authors expected overexpression of AMP deaminase to convert AMP to IMP and, therefore, increase the umami sensation of the fruits. Potentially, a similar effect has occurred in the Oranjestar cultivar, whereby AMP deaminase or related enzymes is over expressed, leading to greater accumulation of these nucleotides.

4.4.4 Endogenous Volatile Profile and Discriminant Analysis

The endogenous volatiles of fresh tomato were quantified for each of the studied cultivars. As the samples were snap frozen in liquid nitrogen and homogenised at -195 °C and stored at -80 °C, there was little opportunity for the generation of volatile components through the action of enzymes. Therefore, many of the compounds that are commonly associated with fresh tomato aroma and flavour are underrepresented, as the sample handling practices inhibited their formation. Some compounds form endogenously, existing in fruit tissues naturally, without the requirement of tissue damage and disruption. These compounds are often used as methods of dissuading damage by insects or microorganisms, including moulds and bacteria (Hubert *et al.*, 2008, Matsui *et al.*, 2012). The generation of volatile compounds endogenously, has not been studied to the same level of scrutiny as the volatile complement formed in damaged or homogenised tissues. Most previous work macerates or homogenises tomatoes fresh, followed by incubation to enable enzymatic action and the production of volatiles (Baldwin *et al.*, 2008, Baldwin *et al.*, 1991b, Boukobza and Taylor, 2002, Buttery *et al.*, 1988, Krumbein and Auerswald, 1998, Ruiz *et al.*, 2005, Shen *et al.*, 2014, Simkin *et al.*, 2004, Song *et al.*, 1998, Tieman *et al.*, 2006a, Tikunov *et al.*, 2005). The preparation methodologies and sample handling used in these studies might represent the potential flavour and aroma of prepared tomatoes that have been cut or macerated prior to consumption, but potentially may overestimate the true concentration experienced by consumers due to the extended sample preparation time enabling greater opportunity for volatile formation. Many previous studies homogenise at room temperature for ~20-30 s, followed by holding for 2-5 min to allow for volatile formation (Baldwin *et al.*, 2008, Boukobza and Taylor, 2002, Buttery and Ling, 1993, Buttery *et al.*, 1987, Krumbein and Auerswald, 1998, Tieman *et al.*, 2006b, Tikunov *et al.*, 2005). In this present study, the endogenous volatiles, rather than those that form following maceration, were of primary interest since they are more representative of the metaboloic state of fruits as determined by cultivar specific genetic traits, ripening progression and environmental growth conditions. Metabolic arrest through sub-zero homogenisation and sample storage and followed by the addition of acidified, saturated sodium chloride solution inhibited the action of lipoxygenases, dramatically suppressing the rate of formation of lipid oxidation volatiles. Hence the profile of these cultivars is significantly different to those previously reported by other authors as apparent in **Table 4.4**. The main differences are accounted for by the C6 lipid oxidation products, being at significantly lower concentrations than those presented in the literature (Buttery *et al.*, 1970, Buttery *et al.*, 1987, Riley and Thompson, 1998, Shen *et al.*, 2014,

Tieman *et al.*, 2006b, Tikunov *et al.*, 2005). This is most apparent in hexanal and *cis*-3/*trans*-2-hexenal, which present with 6-10 times lower concentrations than those found in room temperature homogenisation and incubation practices. In contrast, the carotenoid derived volatile content of the fruits is much more comparable to that proposed by previous authors. The concentration of 6-methyl-5-hepten-2-one/-ol, β-ionone, linalool and *cis*-geranylacetone are all very comparable to previously reported values (Buttery *et al.*, 1987, Tieman *et al.*, 2006b, Buttery *et al.*, 1988, Lewinsohn *et al.*, 2005, Simkin *et al.*, 2004). Because these studies did not prevent enzymatic generation of volatiles to the same as was identified in this work, this suggests that these compounds exist at relatively stable levels in intact fruits, with little to no spike in production following tissue disruption.

The fact that the production of carotenoid derived volatiles does not seem to increase following tissue disruption indicates that the activity of carotenoid cleavage dehydrogenase activity is markedly increased following tissue damage or disruption. This suggests that the formation of carotenoid derived volatiles is relatively continuous following sufficient progression throughout ripening to generate the substrates. However, the carotenoids present in tomatoes are highly photosensitive and unstable, acting as powerful antioxidants and mitigating the effects of intercellular reactive oxygen species (ROS). The mechanisms behind the antioxidative function of carotenoids is two-fold. Firstly, carotenoids with high proportions of conjugated double bonds are able to absorb and mitigate the energy provided by singlet oxygen species ($^{-}O_2$), with the most potent carotenoid being lycopene, due to its open structure and high proportion of conjugated double bonds (Di Mascio *et al.*, 1989). Carotenoids are also particularly effective at capturing and neutralising peroxide radicals, commonly generated through lipid peroxidation, therefore protecting cellular lipids, including membrane bound lipoproteins from oxidative damage (Stahl and Sies, 2003). In doing so, the structure of the carotenoid is often changed, and its function reduced. It is possible that the carotenoid cleavage dehydrogenase enzymes commonly attributed to the formation of many of the carotenoid volatiles, preferentially catabolise the carotenoid fragments resulting from peroxide neutralisation (Stahl and Sies, 2003). As this process is relatively constant in living cells, due to the automatic and spontaneous generation of peroxides, this would potentially explain why these volatiles are relatively constant in tomato and unaffected by damage to the cells of the fruits.

Table 4.4 – Concentration of endogenous volatiles in fresh tomato fruits (ppb FW). Data is presented as mean ± SEM. Significance was determined be Kruskall-Wallis-H test and subsequent Post-Hoc analysis by pairwise comparisons. Significance was accepted at the $p<0.05$ level, following Bonferroni correction for multiple comparisons. Pairwise significance is indicated by the same alphabetic character per column, a to z then α to θ.

			Concentration (ppb FW)								
Cultivar	Harvest	n	Isovaleraldehyde	1-Penten-3-one	Hexanal	cis-3/ trans-2-Hexenal	6-Methyl-5-hepten-2-one	cis-3-Hexen-1-ol	2-Isobutylthiazole	Methyl Salicylate	β-Ionone
ELE	Winter	31	273 ± 5 a,b,c,d,e,f	77 ± 3 a,b,c,d	831 ± 18 a,b,c,d,e,f,g	860 ± 17 a,b,c,d,e,f	224 ± 6 a,b,c,d	6 ± 0.4 a,b,c,d	896 ± 34 a,b,c,d	18 ± 1 a,b,c,d	9 ± 0.6 a,b,c,d,e,f
EL2	Summer	26	141 ± 6 g,h,i,j	56 ± 3 e,f,g,h,i,j	475 ± 12 a,h,i,j	337 ± 9 a,g,h,i,j,k,l	177 ± 5 e,f,g	5 ± 1 e,f,g,h	628 ± 23 e,f,g,h	16 ± 1 e,f,g,h	8 ± 0.5 g,h,i,j,k,l
TEM	Winter	30	536 ± 13 g,k,l,m,n,o,p,q,r	129 ± 7 a,e,k,l,m	999 ± 25 h,k,l,m,n,o,p	1131 ± 30 g,m,n,o,p,q,r	291 ± 15 e,h,i,j,k,l	12 ± 2 a,e,i,j,k	1144 ± 50 e,i,j,k,l,m,n	11 ± 1 i,j,k	18 ± 1.4 a,g,m,n,o,p,q,r
CAM	Summer	31	141 ± 14 a,k,s,t,u	111 ± 10 f,n,o,p	389 ± 29 b,k,q,r,s	561 ± 29 b,m,s,t,u	180 ± 7 h,m,n,o	43 ± 11 b,f,l,m,n,o,p	860 ± 44 o,p,q,r	12 ± 0.5 l,m,n	8 ± 0.7 m,s,t,u,v,w
194	Summer	20	164 ± 88 l,v,w,x,y	121 ± 7 b,g,q,r,s	668 ± 16 i,q,t,u	487 ± 27 c,n,v,w,x	163 ± 5 a,i,p,q,r	11 ± 1 c,g,q,r,s	741 ± 63 i,s,t,u,v	17 ± 3	16 ± 1.5 x,y,z,α,β
DR2	Summer	26	47 ± 8 b,h,m,s,v,z	47 ± 5 k,n,q,t,u,v	501 ± 24 c,l,v	560 ± 16 d,o,y,z,α	196 ± 6 j,s,t,u,v	24 ± 4 d,h,t,u,v,w,x	636 ± 42 j,w,x,y	12 ± 0.4 o,p	3 ± 0.5 b,h,n,s,x,γ
SUN	Winter	33	87 ± 5 c,n,α	179 ± 8 c,h,o,t,w,x,y,z	829 ± 13 j,v,w,x,y,z	1299 ± 15 e,h,s,v,y,β,γ,δ	328 ± 5 b,f,m,p,s,w,x,y	8 ± 1 l,t	1134 ± 23 f,s,w,z,α,β,γ	15 ± 3 q,r,s	10 ± 0.3 δ,ε,ζ,η,θ
JUA	Summer	31	155 ± 14 o,z,β,γ,δ	114 ± 7 d,i,u,w,α,β	574 ± 21 d,m,r,w,α,β	1000 ± 15 i,t,w,z,ε,ζ	228 ± 7 q,w,z,α	8 ± 0.7 m,u,z	476 ± 19 a,k,o,z,δ	5 ± 0.2 a,e,i,l,o,q	3 ± 0.3 c,i,o,t,y
AXI	Summer	28	40 ± 4 d,h,p,t,w,β	77 ± 5 l,r,x,α	520 ± 12 e,n,x	787 ± 7 j,p,t,x,α,β	147 ± 3 c,k,t,x,z	7 ± 1 i,n,q,v	274 ± 15 b,g,l,p,t,α	9 ± 0.7 b,f	1 ± 0.4 d,j,p,u,z
PIC	Winter	27	48 ± 3 e,i,q,u,x,γ	75 ± 5 m,p,s,y,β	372 ± 24 f,o,t,y,α	670 ± 15 f,k,q,γ,ε	129 ± 4 d,l,n,u,y,α,β	6 ± 0.4 j,o,r,w	168 ± 15 c,h,m,q,u,x,β,δ	5 ± 0.7 c,g,j,m,p,r	2 ± 0.2 e,k,q,v,α,x
ORA	Winter	29	12 ± 1 f,j,r,u,y,α,δ	88 ± 4 j,v,z	398 ± 24 g,o,u,z,β	684 ± 11 l,r,δ,ζ	278 ± 13 g,o,r,v,z,β	5 ± 0.3 k,p,s,x,z	347 ± 31 d,n,r,v,γ	6 ± 1 d,h,k,n,s	4 ± 0.3 f,l,r,w,β,γ

ELE/EL2 – Elegance, TEM – Temptation, CAM – Campari, 194 – P194, DR2 – DR28090TC, SUN – Sunstream, JUA – Juanita, AXI – Axiani, PIC – Piccolo, ORA - Oranjestar

Table 4.4 – Continued.

Cultivar	Harvest	n	Concentration (ppb FW)								
			trans-2-Pentanal	1-Penten-3-ol	1-Pentanol	trans-2-Octenal	6-Methyl-5-hepten-2-ol	trans,trans-2,4-Heptadienal	Linalool	cis-Geranylacetone	Benzyl Alcohol
ELE	Winter	31	65 ± 2 a,b,c,d	37 ± 2 a,b,c,d,e,f,g,h,i	52 ± 4 a,b,c,d,e,f,g	22 ± 2 a,b,c,d	35 ± 3 a,b,c,d,e,f	75 ± 4 a,b,c,d	27 ± 1 a,b,c,d,e,f,g,h	203 ± 10 a,b,c,d,e,f	148 ± 15 a,b,c,d,e
EL2	Summer	26	58 ± 2 e,f,g,h,i	42 ± 2 j,k,l,m,n,o,p,q,r	59 ± 4 h,i,j,k	41 ± 4 a,e,f,g	13 ± 1 a,g,h,i,j,k	51 ± 2 e,f,g,h,i,j	12 ± 0.6 a,i,j,k	117 ± 5 a,g,h,i,j	66 ± 6 a,f,g
TEM	Winter	30	107 ± 6 a,e,j,k,l,m	82 ± 7 a,j,s	101 ± 9 a,h,l,m	35 ± 3 h,i,j,k	36 ± 2 g,l,m,n,o,p,q	110 ± 8 e,k,l,m,n,o	36 ± 4 i,l,m,n,o,p,q,r	204 ± 11 g,k,l,m,n,o	59 ± 5 b,h,i
CAM	Summer	31	107 ± 6 b,f,n,o,p,q	100 ± 4 b,k,t,u,v	82 ± 5 b,n	47 ± 5 b,l,m,n	20 ± 1 r,s,t,u	84 ± 5 p,q,r,s	19 ± 2 s,t,u,v,w	110 ± 5 b,k,p,q,r	88 ± 8 j,k,l,m
194	Summer	20	85 ± 3 g,r	68 ± 2 c,l,t,w	111 ± 6 c,i,o,p,q	98 ± 8 c,h,o,p,q,r	17 ± 1 l,v,w,x	97 ± 5 f,t,u,v,w	10 ± 1 b,l,x,y	167 ± 8 s,t,u	99 ± 12 n,o,p
DR2	Summer	26	63 ± 3 j,n,s,t	76 ± 4 d,m,x	97 ± 7 d,j,r,s	39 ± 4 h,s,t,u	11 ± 0.6 b,m,y,z	55 ± 3 k,x,y,z,α	9 ± 0.7 c,m,u,z,α	109 ± 7 c,l,v,w,x	190 ± 17 f,h,j,q,r,s
SUN	Winter	33	155 ± 4 c,h,k,o,r,s,u,v,w	153 ± 5 e,n,s,w,x,y,z,α,β	100 ± 4 e,k,t,u	45 ± 3 d,v,w,x,y	27 ± 1 n,y,α,β,γ,δ	119 ± 6 g,x,β,γ,δ,ε	9 ± 0.3 d,n,s,β,γ	152 ± 6 y,z,α,β	127 ± 5 g,i,t,u,v
JUA	Summer	31	77 ± 3 u,x	69 ± 3 f,o,u,y	79 ± 6 f,o,v	30 ± 3 o,v,z,α	5 ± 0.2 c,h,o,r,v,α	6 ± 0.8 a,g,l,p,t,y,β	1 ± 0.1 e,j,o,t,x,u,β,δ,ε	21 ± 4 d,h,m,p,s,v,y,γ	46 ± 5 c,k,n,q,t,w
AXI	Summer	28	67 ± 2 l,p,v,y	82 ± 2 g,p,z	80 ± 3 g,w,x	17 ± 1 e,i,l,p,s,w	3 ± 0.2 d,i,p,s,w,y,β	3 ± 0.2 b,h,m,q,u,z,γ	2 ± 0.1 f,k,p,u,y,z,γ,ζ,η	12 ± 3 e,i,n,q,t,w,z,δ	33 ± 2 d,l,o,r,u,x
PIC	Winter	27	73 ± 4 m,q,v,z	68 ± 4 h,q,v,α	55 ± 3 l,p,r,t,w	13 ± 1 f,j,m,q,t,x,z	33 ± 0.2 e,j,q,t,x,γ	5 ± 0.8 c,i,n,r,v,α,δ	9 ± 0.9 g,q,v,δ,ζ	66 ± 7 f,o,u,α,ε	112 ± 11 w,x,y
ORA	Winter	29	115 ± 4 d,i,t,x,y,z	72 ± 3 i,r,v,β	47 ± 2 m,n,q,s,u,v,x	15 ± 1 g,k,n,r,u,y,α	9 ± 1 f,q,u,δ	11 ± 1 d,j,o,s,w,ε	10 ± 1 h,r,ε,η	246 ± 12 j,r,x,β,γ,δ,ε	40 ± 3 e,m,p,s,v,y

ELE/EL2 – Elegance, TEM – Temptation, CAM – Campari, 194 – P194, DR2 – DR28090TC, SUN – Sunstream, JUA – Juanita, AXI – Axiani, PIC – Piccolo, ORA - Oranjestar

The only amino acid derived volatiles under investigation in this work were isovaleraldehyde (3-methylbutanal) and, potentially, 2-isobutylthiazole. As with the carotenoid derived volatiles, isovaleraldehyde is reported at very comparable concentrations as that in the literature. This further indicates that this compounds is likely to be endogenous, and produced relatively constantly in ripe fruit; either stored internally awaiting tissue damage, or slowly released as a method of attracting attack by herbivores. Based on these results, it is difficult to determine which method of accumulation is taking place, as this is single time-point measurement on frozen homogenate and understanding this process would require samples at various stages following tissue damage. It may be valuable to quantify the isovaleraldehyde exuded from whole, undamaged tomato fruits, to try to clarify its production and release from fruits. On the other hand, 2-isobutylthiazole is significantly more abundant than reported in previous works, by approximately 10 times (Buttery *et al.*, 1988, Kochevenko *et al.*, 2012, Mathieu *et al.*, 2009). This may be significant as higher concentrations of 2-isobutylthiazole have been strongly correlated with increased liking/acceptance of tomato fruits (Piombino *et al.*, 2013). Definitively identifying the root cause of this is challenging due to the uncertain biosynthetic pathway of 2-isobutylthiazole. The most likely pathway is through amino acid degradation, likely leucine or a derivative, coupled to cystamine, although this has not been confirmed (Fernandez *et al.*, 2002, Schutte and Teranishi, 1974). Schutte and Teranishi also noted that 2-isobutylthiazole was produced endogenously in whole, intact tomato fruits and was not reliant on tissue disruption or damage for its formation, which is an exceptional case for sulphur containing volatiles (Schutte and Teranishi, 1974). The authors further noted that the concentration of 2-isobutylthiazole was highly cultivar dependant. This is in strong agreement with the findings of this work, as the high concentration observed here is unlikely to have been generated following tissue disruption in this case. Additionally, there is an ~6.7 fold difference in the concentration of 2-isobutylthiazole between the cultivars, with Piccolo fruits presenting the lowest at 168 ppb and Sunstream the highest at 1,133 ppb. The reasons for the elevated concentrations of 2-isobutylthiazole in this study are unclear. As it is unaffected by tissue disruption, handling and differences in methodological approach may have resulted in a decrease of the endogenous levels as a proportion released on tissue damage may account for this discrepancy. However, the most plausible explanation might relate to the fact that all of the tomato cultivars were stored on the truss until sample preparation. Some preliminary investigations have shown that the truss itself contains a large quantity of the volatile (data not shown), indicating that the fruits may continue to import 2-isobutylthiazole or its precursor during on-truss storage.

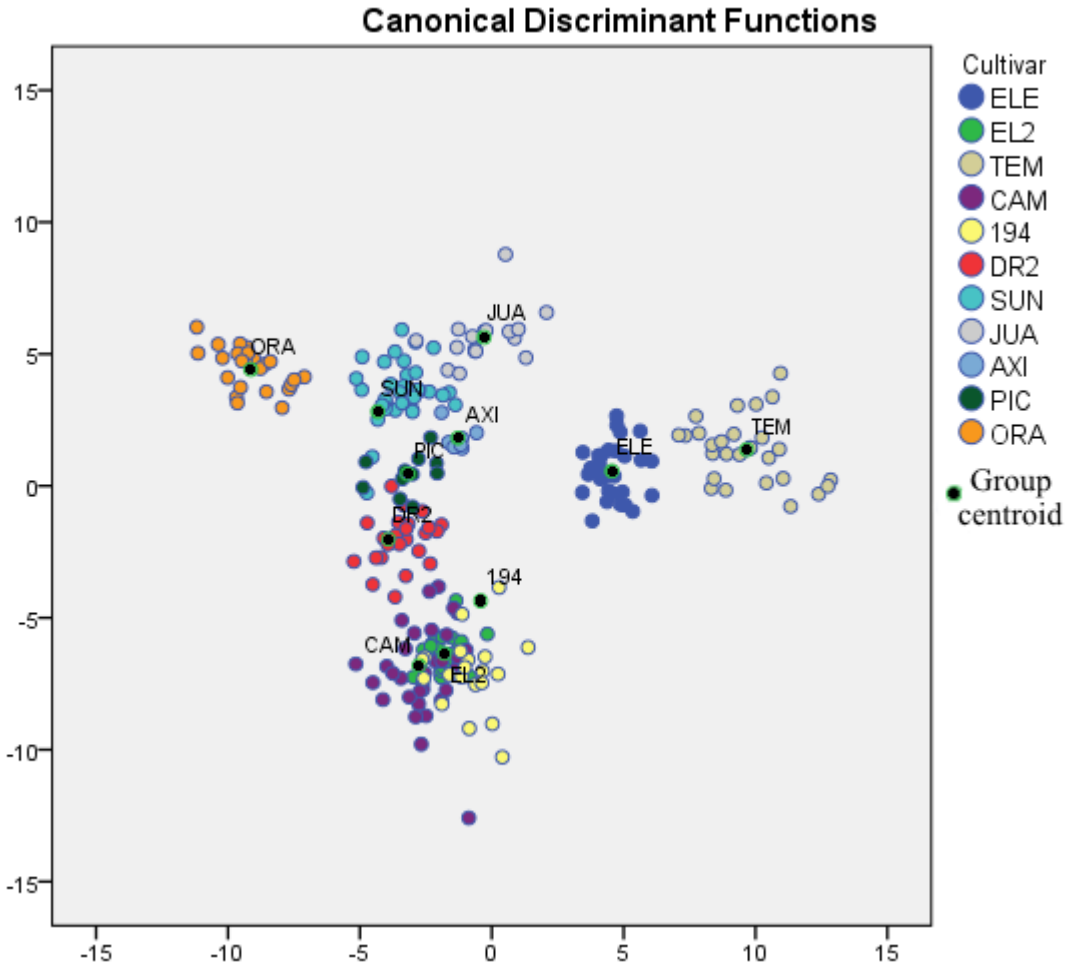


Figure 4.4 – Classification of samples based on detected volatile profile. A correct classification rate of 92.5% was produced by the initial model, dropping to 91.7% following cross validation (n=252).

As shown in **Figure 4.4**, the analysed cultivars can be correctly classified, for the most part, using their volatile profiles alone. Classification-based discriminant analysis of the volatile profiles was conducted with a total of 312 individual samples, split between 11 cultivar groups. Following construction of the model, 60 cases were removed due to containing trace levels (presented as missing values) of one or more discriminating volatiles. The final model, consisting of 252 samples, was able to correctly classify 92.5% of the samples, which dropped slightly following 'leave-one-out' based cross validation to 91.7%. Of the cultivars, a 100% classification rate was observed for Elegance, Temptation, Juanita, Axiani, Piccolo and Oranjestar fruits. One Elegance2, one Sunstream, three Campari and five DR2 samples were misclassified. In addition, 194 was so similar to both Elegance2 and Campari it was misclassified in 55% of its samples, primarily between these two cultivar groups. This methodology demonstrates both the power and usefulness of classification based analysis as well as its limitations. Between different cultivars classification analysis can be used to draw attention to group populations that are more similar than may seem apparent based on the numerical data alone. However, when trying to separate cultivars that present very comparable profiles it is difficult to find discriminating features.

4.4.5 Meta-Data Analysis with Complementary Sensory Data

All the sensory data presented in this subsection of the chapter has been collected by an industrial project partner, MMR Research Worldwide, and has only been included, following their approval, to allow for meta-data analysis between the chemical and sensorial datasets (MMR Research, 2018). The data was collected from a second batch of identical fruits of each cultivar that were picked on the same days and transported to our project partners.

Using chemical profiling to determine the quality of fresh produce is very common, due to its reliability and quantitative nature. One of the main benefits is the removal of consumers from the evaluation process, as the way in which people perceive and interpret the organoleptic sensations of foods is highly varied and specific to each individual. However, this also presents the problem of relating the chemical composition of a foodstuff back to how it will be perceived and liked by consumers. Trained sensory analysis and intensity testing is a commonly used method of comparing between the collected chemical profile to the end product that consumers will purchase (Auerswald *et al.*, 1999a, Auerswald *et al.*, 1999b, Causse *et al.*, 2002, Cortina *et al.*, 2018, Ponce-Valadez *et al.*, 2016). Complications arise when trying to understand the relative impact of volatile compounds, which provide highly varied and characteristic flavours and aromas to the perception of a foodstuff. Many volatiles present a character that can be classified into multiple aroma groups, therefore understanding the intensity of a single aspect of that sensation is extremely difficult. Additionally, the character of many volatiles is highly dependent on its abundance, testing environment and matrix of evaluation, which has been demonstrated multiple times in fresh tomato (Abegaz *et al.*, 2004, Bezman *et al.*, 2002, Tandon *et al.*, 2003, Tandon *et al.*, 2000, Tieman *et al.*, 2012). Tandon *et al.* used a regression model to determine the weightings of various volatiles of fresh tomato flavour to certain sensory descriptors. However, the authors struggled to find a correlation between 'green/grassy' and 'tomato-like' and the quantified volatiles, many of which were C6 aldehydes and alcohols known to specifically present these aromas/flavours (Tandon *et al.*, 2003). Abegaz *et al.* had similar objectives when trying to use regression modelling of volatile flavour and aroma compounds and gustatory intensity provided by non-volatile components to the sensorial profiles of fresh tomatoes. Relating the gustatory components to the sensory perception of tomato flavour was highly successful. However, the authors also noted that the flavour provided by the volatile complement was significantly less associated with the flavour and aroma as defined by sensory analysis (Abegaz *et al.*, 2004). In this study, the difficulty in correlating/relating volatile content to flavour and quality is further complicated by the different handling procedures for sensory and analytical samples for the fruits used, both post-harvest and during analysis. Trusses were picked and divided equally between project partners. The half that were used for chemical analysis were stored in unmonitored room temperature for the first day followed by storage at 15 °C for 24 hours prior to being directly snap frozen. The other half were stored in unmonitored room temperature for 48 hours and then

analysed by sensory analysis. The format for sensory analysis was to smell the whole tomato, cut and smell the tomato and then further cut and eat the tomato. During this time the enzymes present in the fruit would have had ample opportunity to generate additional volatile compounds, which would not have been possible in the samples taken for analytical determinations. This disparity in sample handling makes comparisons between volatile complement and sensory profile highly challenging.

Table 4.5 depicts the mean intensity scores for 6 flavour attributes of each of the studied cultivars. Depth of flavour aims to categorise each of the cultivars based on their overall flavour intensity, which will be a combined effect of the volatiles and gustatory sensations. As mentioned above, direct comparison between the instrumental analysis and these results would be likely to be inconclusive or misleading as the experimental design was not structured in a way as to enable close comparisons between these methodologies.

Table 4.5 – Intensity of different flavour attributes of fruits of the analysed cultivars as determined by a trained sensory panel and collected by MMR Research Worldwide (n=10). Values were taken from an unstructured, 100-point scale.

Cultivar	Type	Depth of flavour	Overall green	Green pepper	Earthy/ compost	Fruity	Savoury - overall
Elegance	Beefsteak	42	31	20	0.2	0.1	33
Elegance2	Beefsteak	41	32	19	0.1	0.0	30
Temptation	Salad	49	28	18	0.3	0.1	34
Campari	Salad	48	28	14	0.8	0.8	28
P194	Salad	46	28	12	0.3	0.6	30
DR28090TC	Plum	62	32	2	0.6	3.0	40
Sunstream	Plum	54	24	11	2.1	0.4	36
Juanita	Cherry	53	28	10	0.6	1.1	32
Axiani	Cherry	62	24	8	0.0	14	33
Piccolo	Cherry	62	13	9	1.3	1.9	52
Oranjestar	Tangerine Cherry	55	12	8	0.4	21	38

Correlation of the gustatory sensations of fresh tomato to the compounds responsible has been previously relatively successful, however as explained above, there are a number of limitations that make direct comparisons between the datasets challenging. Compounds responsible for gustatory senses are typically more stable following fruit damage than volatiles, mitigating some of the discrepancies in sample handling. The mean gustatory scores of the analysed cultivars can be seen in **Table 4.6**.

Table 4.6 - Intensity of different taste attributes of fruits of the analysed cultivars as determined by a trained sensory panel and collected by MMR Research Worldwide (n=10). Values were taken from an unstructured, 10-point scale.

Cultivar	Type	Salt	Sweet	Acid	Bitter	Umami
Elegance	Beefsteak	0.39	21.21	26.05	0.74	0.05
Elegance2	Beefsteak	1.67	20.19	25.89	0.69	1.86
Temptation	Salad	1.09	26.63	28.3	0.74	10.14
Campari	Salad	4.04	26.85	28.01	1.11	8.08
P194	Salad	4.51	25.79	27.5	1.06	10.06
DR28090TC	Plum	9.01	37.18	29.9	1.63	20.66
Sunstream	Plum	2.63	29.98	30.01	1.33	23.2
Juanita	Cherry	6.37	31.87	31.86	1.61	16.78
Axiani	Cherry	8.92	37.24	35.56	1.85	17.71
Piccolo	Cherry	3.76	40.22	32.71	1.06	42.75
Oranjestar	Tangerine Cherry	3.01	34.95	31.49	1.44	28.57

Of the primary senses, saltiness and bitterness were not assessed analytically as they are the least impactful gustatory senses in fresh tomato, as can be seen by their omission in several sensorial models, therefore those attributes will not be compared (Cortina *et al.*, 2018, Tandon *et al.*, 2003). Gustation and its impact on sensory perception is easier to compare than olfaction, due to the ability to convert the sum of components into a single numerical value, as seen below. Graphical representation of the sensory profiling showed similar relationships when compared to analytically derived data as shown in **Figure 4.5A-C**. This prompted further exploration using linear regression to determine the closeness of fit of the two datasets. The strongest relationship between the means of the perceived sensation versus the calculated intensity is observable in the sweetness of the cultivars. Following linear regression it can be determined that there is a strong relationship between the sensory profile based intensity data and the calculated sweetness of the same cultivars, $F(1,9) = 149.5$, $p<0.005$ and adjusted R^2 values indicating 93.7% coverage of the population. There is a slightly higher variance in the relationships of acidity and umami, however, strong linearity was also observed. Umami presented the second best prediction, $F(1,9) = 49.2$, $p<0.005$, with 82.8% of the variance explained by the adjusted R^2. Analytically versus sensorially detected sourness was also linear, $F(1,9) = 41.6$, $p<0.005$ and with a total explained variance of 80.3% as determined by the adjusted R^2. Part of the reason for the obtained relationships in perceived and calculated acidity is due to the narrow range of values obtained through sensory profiling, with the difference between the lowest (25. 9, EL2) and highest value (35.6, AXI) being proportionally smaller than the difference between the highest and lowest calculated acidity.

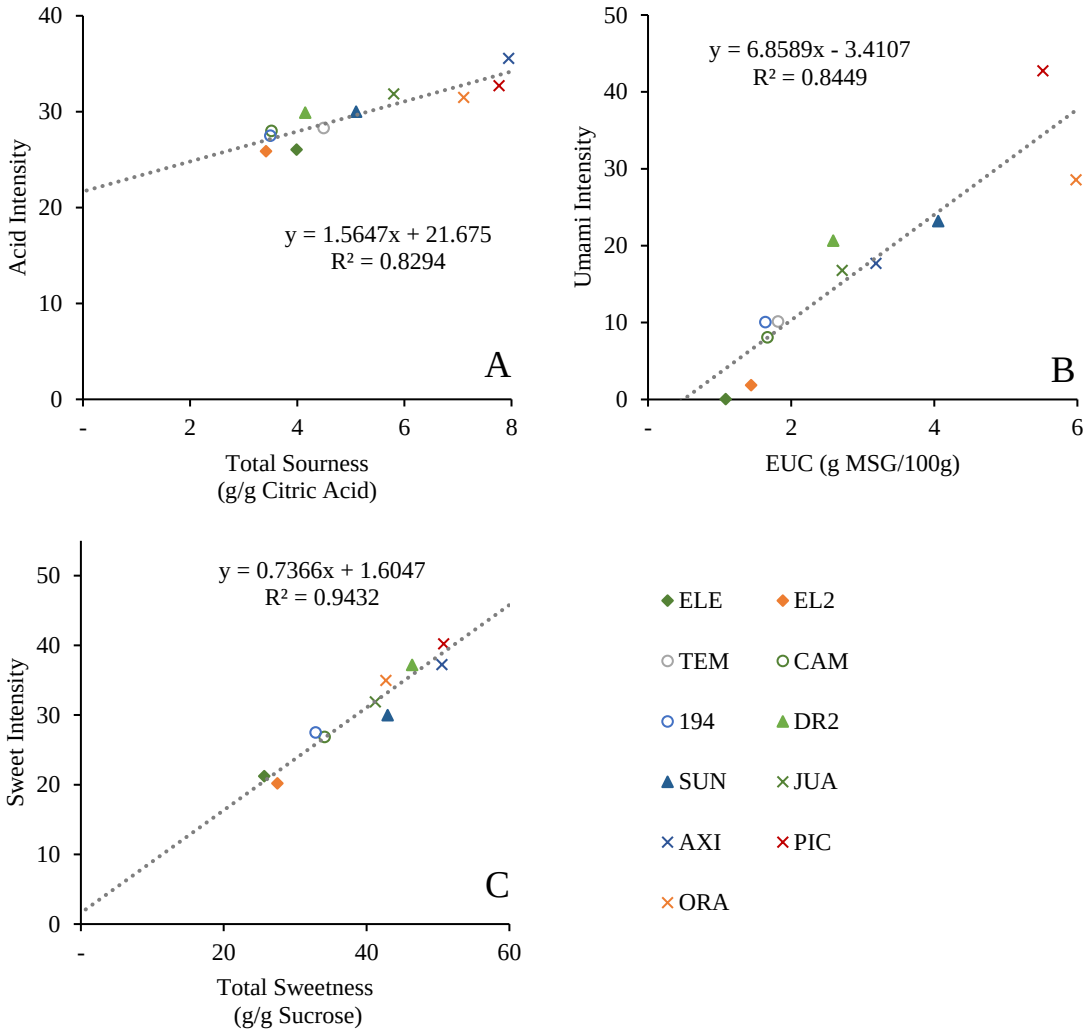


Figure 4.5A-C – Mean perceived gustatory sensation (as seen in **Table 4.6**) versus mean calculated gustatory sensation per cultivar. Trendline indicates the relatedness of sensory and analytical determinations of each gustatory sense. (n=10 for mean intensity ratings, n=33-ELE, 26-EL2, 28-TEM, 25-CAM, 28-194, 27-DR2, 23-SUN, 27-JUA, 28-AXI, 27-PIC, 27-ORA for sweetness and sourness means, n=32-ELE, 27-EL2, 29-TEM, 27-CAM, 28-194, 30-DR2, 28-SUN, 20-JUA, 20-AXI, 20-PIC, 20-ORA for equivalent umami concentration mean).

The relationship between the sensorially perceived and calculated umami intensity is strong when Piccolo and Oranjestar cultivars are omitted as there is a disagreement between the umami values for both of these cultivars, as assessed by each methodology. The calculated umami content of Oranjestar is significantly higher than anticipated when compared to the sensory values. It is worth noting that this was primarily driven by higher monophosphate nucleotides and not the umami amino acid content of the fruits. Calculated EUC for Piccolo may be underreported, as there was some degree of overload of the glutamic acid peak. Potentially, the relationship may be improved following clarification of this result, which would move Piccolo higher in terms of expected umami intensity, which would agree with the sensory findings. Alternatively, matrix interactions due to the distinct nature and physiological differences between these cultivars may be responsible for such a discrepancy.

4.4.6 Effect of Growth and Harvest Season on the Chemical Profile of 'Elegance' Tomatoes

There was a significant difference between the two harvests of Elegance fruits, both in terms of their chemical composition and detected sensorial profile, as assessed by MMR Research Worldwide, and previously shown in **Table 4.5** and **Table 4.6**. The summer harvest of Elegance, EL2, generally produced lower volatiles than the winter crop, as presented in **Figure 4.6A-B**, below. This phenomenon was not confined to a single class of volatiles, with those derived from lipid oxidation, carotenoids and amino acids displaying a similar trend. This decrease in volatile production in the summer crop, if mimicked following tissue disruption and the associated, rapid synthesis of addition volatiles, would lead to a less flavourful crop grown in summer months. It is difficult to suggest the reason for this, as there are many biochemical processes responsible for generating the precursor compounds as well as the volatiles themselves. It has been previously demonstrated that artificial lighting used in glasshouses can significantly affect overall crop quality and growth in many plant species including tomato (Gómez *et al.*, 2013, Liu *et al.*, 2009, Lu *et al.*, 2012, Ménard *et al.*, 2005, Olle and Viršile, 2013). The usage of artificial lighting has been previously shown to increase the production of certain volatiles, including in glasshouse tomato production (Tieman *et al.*, 2012, Colquhoun *et al.*, 2013). Current research efforts are exploring the replacement of high pressure sodium (HPS) lamps with more efficient LEDs which are tuneable to the desired wavelengths. Although suited to and commonly used in commercial growing, HPS lamps are known to poorly represent the blue and far-red wavelengths of natural light (Ménard *et al.*, 2005). Moreover, use of artificial light has been shown to increase fruit weight, number and leaf and stem length (Gómez *et al.*, 2013, Ménard *et al.*, 2005). It is possible that supplemental HPS lamp lighting employed in winter is partially responsible for the change in volatile composition between the analysed harvests of Elegance.

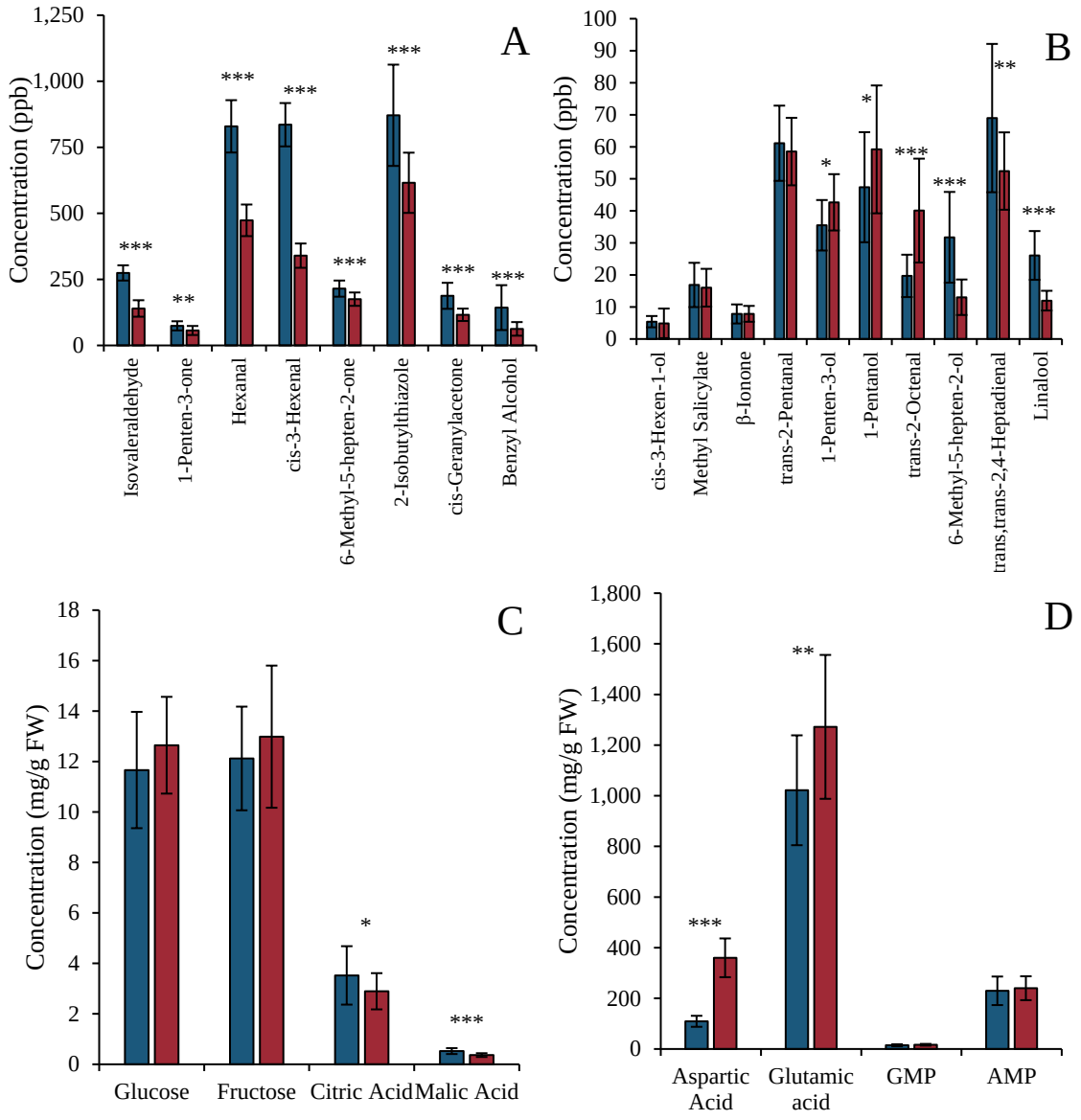


Figure 4.6 – Differences in the chemical composition of the two harvests of Elegance fruits, ■ - ELE (Winter), ■ - EL2 (Summer). Data presented is mean ± standard deviation. Significance determined by Mann-Whitney-U test. Significance denoted by * - p≤0.05, ** - p≤0.01 and *** - p≤0.001 (n=21 for ELE, n=22 for EL2).

Elegance tomatoes from the winter harvest accumulated significantly higher organic acid content than those of the summer harvest. This was significant enough to yield nearly a 20% increase in detectable acidity. The reason for greater organic acid accumulation is not apparent, but as discussed previously, is likely to result from the combination of artificial lighting, heating and environment used to enable year-round crop growth. There was no significant difference between the sugar content of the two harvests, although the two crops gave very different total soluble solids content as assessed using %Brix. Values of 4.0 ±0.6 and 5.3 ±0.4 %Brix were found for the winter (ELE) and summer (EL2) crops respectively, an increase of 32%. This further highlights the unreliability of %Brix as a measure of sugars in fresh tomato as there are too many other soluble components which can interfere with the reading. Potentially, the amino acids

content may have been partly responsible for the elevated TSS in EL2 fruits. The lowest values for both umami amino acids were seen in the winter crop of Elegance tomatoes, ELE. This resulted in ELE fruits providing 33% less umami than the summer crop of the same cultivar, EL2. Interestingly this may be due to one of two factors or a combination of both. Summer fruits are likely to be exposed to better environmental conditions for growth, including light quality and temperature, which will probably impact photosynthesis, generation of important assimilates and the total imported dry matter in developing fruits. In modern glasshouse grown tomatoes, like those used in this study, this is offset by lighting and heating supplementation of winter crops, in an effort to standardise the growth and quality of fruits produced. However, the average fruit size of ELE and EL2 was significantly different, 113.7 ± 12.8 g and 96.3 ± 12.2 g respectively. As the cultivar and growth location, handling and nutrition were all standardised between these harvests there is a strong likelihood that the artificial environment used in winter to mitigate poor growth conditions is not sufficient to establish a truly homogeneous crop all year round.

The combined difference in the profile of the summer versus winter harvest of 'Elegance' is a general increase in dry matter components and decrease in volatile production in the summer crop. The increase in dry matter seems to be largely driven by higher accumulation of amino acids and not sugars, which although slightly higher on average, were not significantly different between the seasons. The higher production of volatiles in the winter crop may be indicative of the artificial environment used to supplement the standard growth conditions following reduced solar radiation, heat and day/night cycles in winter. This reduction in total photosynthesis may explain the lower accumulation of amino acids and slightly elevated organic acid content in the winter crop.

4.5 Conclusions

The objectives of this chapter were two-fold. Firstly, the unique organoleptic character of each of the studied cultivars was determined through quantification of many of the compounds known to be important to the overall sensorial profile of tomatoes. Data on sugar, acid, amino acid, nucleotide and volatile composition of the cultivars revealed distinct differences allowing for accurate discrimination and classification based on chemometric profiles. In fact, the volatile complement in each of the cultivars was sufficient to allow for 91.7% correct classification across 252 individual samples. The sample handling procedures employed in this study allowed for the investigation of volatiles endogenously produced by in-tact fruit, with little to no enzymatic activity possible following tissue disruption. This suppressed the formation of lipid oxidation derived volatiles significantly, and also indicated a significant increase in 2-isobutylthiazole than reported in other studies. This was attributed to fruit-truss interactions, potentially importing 2-isobutylthiazole or its precursors during on-truss storage as the truss itself also presented high

concentrations of the volatile. Moreover, the unique nucleotide composition demonstrated by Oranjestar lead to significantly enhanced umami even with 33% less glutamate than its closes competitor, Piccolo. Understanding the genetic cause for this increase in nucleotide accumulation could be highly beneficial to future breeding programs, particularly if paired with even higher glutamate. Secondly, the work presented in this chapter shows that the combination of analytically derived data is strongly correlated the similar data derived from trained sensory profiling for certain attributes. As has been previously demonstrated by previous published works, the role of individual volatiles, their interaction with the matrix and other olfactory stimuli and their concentration dependant character makes correlation to sensory data challenging. However, the five basic tastes, particularly sweetness, sourness and umami, which are all essential to good tomato flavour, are highly correlated to their true values. This indicates that, renewed efforts to model these values based on comprehensive analytical and sensorial data may be highly advantageous to commercial tomato growers, particularly if establishing a trained sensory panel internally is possible.

4.6 Future Work

Repeat analysis of selected Piccolo fruits to ensure that the correct glutamic acid content is being reported would shed some light on the discrepancies between equivalent umami concentration (EUC) and the perceive umami content as identified by sensory analysis. If the glutamate content was underreported the umami based linear regression model would fit even better than at present, further solidifying this as a valuable route for commercial growers to take to estimate crop quality.

The abundance of 2-isobutylthaizole in these tomatoes is of great interest, particularly as liking has been correlated to the abundance of this volatile in fresh tomatoes before. We believe that there is a significant relationship between the truss and fruit during storage, either by continued import of compounds from the truss directly, or through surface-based adsorption of volatiles released by the truss on the skin of the fruits. The unconfirmed origin of 2-isobutylthiazole makes understanding this mechanism difficult, therefore further work may add significant value and understanding to fruit-truss interactions following removal from the plant.

5 Tissue Specific Localisation of Organoleptic Compounds in Three Commercial Tomato Cultivars

5.1 Chapter Abstract

Localisation of flavour and taste active compounds in different tissues of edible plants/fruits is of significance as it dictates the sensory experience during consumption. In the case of tomatoes, the presence of significant amounts of locule fluid, is important in taste/ flavour delivery and responsible for the initial high intensity sweet/sour taste. Other fruit compartments such as the flesh and seeds are also known to contain flavour and taste active components, which are important at different stages of mastication. To determine such potential differences in gustatory sensation, the distribution of taste and flavour-active coumpounds (sugars, organic acids, amino acids and volatiles) in the flesh, locule fluid and seeds of Genio (cherry/cocktail), Angelle (babyplum) and Valkiria (salad) were isolated and the relevant compounds extracted from the tissues. The locular fluid of each cultivar had comparable sugar concentration, but significantly higher citric acid content than in the flesh and seeds, 3.3, 3.5 and 4.0 times greater for Angelle, Genio and Valkiria fruits, respectively. The locular fluid also contained significantly higher concentrations of glutamate than the other tissues, although the same relationship was not apparent in aspartate. Moreover, to better understand the role of the locule fluid, sensory analysis was then used to determine the most well-liked composition, by modifying the acid content in simulated locule fluid samples as a method of varying the sugar/acid balance. Locule fluids containing high sugar and acids natively, like those of Angelle baby-plum tomatoes, were preferred when the acid content was lowered, whereas moderately high native sugar and acid, which emulated Genio cherry tomatoes, benefitted from increased acid content. Low sugar acid solutions, based on Valkiria salad tomatoes, were most preferred at native concentrations. This indicates that it is not just the ratio of sugar to acid that is important, but also in the combined intensity of both sweetness and sourness.

5.2 Introduction

When consuming whole, fresh tomatoes the sensation of the fruit bursting and the immediate sweet/sour tang of the juice and locule gel is very desirable. This sensation sets apart smaller fruits that can be consumed whole from larger tomatoes that are commonly cut before consumption, often resulting in the loss or dispersal of locular fluid. Such tomatoes also contain lower concentrations of both sugars and acids. This sensation is most important in cherry or baby plum tomatoes, as they are typically more reliant on sugar/acid content driving consumer preference, rather than impact of volatile compounds (Stevens *et al.*, 1979, Malundo *et al.*, 1995, Baldwin *et al.*, 2008). As both sugar and acid content, and the ratio between the two, are vital in consumer acceptance for fresh tomatoes, this initial reaction and taste is significant in determining the acceptability of cultivars (Salles *et al.*, 2003). Some cherry tomato cultivars are more popular than others in preference testing, which is largely due to sugar and acid content. Mencarelli and Saltveit noted that in ripening mature-green tomato slices, high sugar/acid ratio and content was correlated to consumer acceptance (Mencarelli and Saltveit, 1988). Schouten *et al*. previously demonstrated that the locular gel and fluid contains higher concentrations of citric and malic acid compared to pericarp tissues (Schouten *et al.*, 2016). Cherry tomatoes with higher sugar and acid content are preferred over those with less, indicating that the initial, intense burst of flavour is pivotal to consumer acceptance of a fruit, particularly those eaten whole.

Sugars are one of the most important classes of compounds in consumer acceptance of fruits. The perceived sweetness of ripe tomato fruits is primarily due to the high concentrations of both glucose and fructose, between 1.5-2.5% FW of each sugar. Tomatoes also contain a range of other compounds including sucrose, certain amino acids and volatile components that are perceived as sweet; however they are unlikely to significantly alter the overall perceived sweetness of the fruits. It has been previously shown that metabolite concentration can fluctuate, not only between fruits, cultivars and ripening stages, but also in the tissues of a single fruit (Gómez-Romero *et al.*, 2010).

Several organic acids are present in fresh tomato; however citric and malic acid are the most abundant and therefore the predominant drivers of fruit acidity. Citric acid is by far the most abundant organic acid, with malic acid present at only about 10% of this concentration. Other organic acids in tomato are present at even smaller concentrations, suggesting that citric and malic acid are primarily responsible for fruit acidity. Malic acid has been shown to be a precursor for the formation of glucose and fructose in ripe tomato fruits, decreasing during the ripening process, whilst directly boosting the sugar content of the fruits, hence causing an increase in the sugar/acid ratio (Petro-Turza, 1986).

The primary aim of the study was to ascertain if there was a preferred sugar/acid ratio in tomatoes through sensory preference testing. The matrix used was a simulated locule fluid containing fixed concentrations of certain food grade volatiles, 3 levels of hexose concentrations and 5 concentration of organic acids per sugar level. This allowed for the manipulation of the sugar/acid ratio of the juice, without a change in the sugar or volatile components, minimising the altered variables. The solutions were based on the concentration of sugars and organic acids of 3 tomato cultivars which acted as a baseline per tomato class (salad, plum and cocktail). Additionally, the perception of the odorants through olfaction has been previously demonstrated to be altered by the presence of compounds involved in taste (Stevenson *et al.*, 1999). The degree to which this occurs, over the range of sugar and acid contents studied, could indicate changes in tomato flavour between similar, native concentrations of the compounds in tomato. Finally, the distribution and localisation of hexoses and organic acids in the tomatoes was quantified in order to better understand the taste sensations observed when biting into a whole tomato. This was achieved by separating the locular fluid and gel from the pericarp and allowing the fluid to drain away from the gel and seeds. Once tissue types were isolated, the hexose and organic acid content of each was quantified in each tissue. The content of amino acids and potential for volatile formation for each tissue was also determined, in order to better understand the role each plays in the overall perceived flavour of fresh tomato.

5.3 Methods and Materials

5.3.1 Analytical Methodologies

Analytical methodologies employed in the collection of data for this chapter have been previously described in Chapter 2, Methods and Materials. The relevant methodologies include pH and °Brix measurement, determination of hexose and organic acid content using enzymatic assays and UV detection, free amino acid analysis following the EZ:FAAST methodology, nucleotide determination by HPLC-UV and determination of volatile composition through HS-SPME extraction and GC-TOF-MS analysis. Weights of sample used for the GC-TOF-MS analysis of volatiles were 250 ± 25 mg due to the limited weights of both fluid and seeds tissues, particularly in the smaller fruits. Additional methodologies unique to this chapter are detailed below.

5.3.2 Food Grade Compounds for Sensory Analysis

All compounds used for the preparation of ‘simulated locule fluid’ samples for sensory analysis were certified food grade/safe. Pure glucose and fructose (100%) were sourced from Bulk Powders (Sports Supplements Ltd., Colchester, UK). Citric acid (100%) was purchased from Bigger Jugs (Bigger Trading Ltd., Ilminster, UK) and malic acid (95%) from Meridianstar (Meridianstar, Walsall, UK). Volatile compounds used to simulate tomato flavour included

hexanal, 6-methyl -5-hepten-2-one, *cis*-3-hexen-1-ol, 2-isobutylthiazole and β-ionone were sourced from Sigma-Aldrich and were >98% purity (Sigma-Aldrich, Gillingham, UK). Additionally, cis-3-hexenal was sourced from Sigma-Aldrich and was 50% purity (Sigma-Aldrich, Gillingham, UK). Absolute, Food grade ethanol, 100% was sourced from Haymankimia (Haymankimia, Witham, UK). All deionised water used was purified using an in-house Milli-Q Integral 3 deioniser A10 TOC equipped with ProGuard TS2 and Quantum TEX cartridges and Millipak Express 40 filters (Merck-Millipore, Watford, UK).

5.3.3 Sensory Analysis Sample Preparation

Ethical approval for this project was received under ethics code RE18-01-131644.

The sensory analysis section of this chapter focused on determining consumer preference for sugar acid balance in the locule fluid of fresh tomatoes. Therefore, samples were formulated to emulate the locule fluid of commercial tomato based on the previously quantified native levels of sugars and acids in three tomato cultivars, Genio (cocktail), Angelle (baby plum) and Valkiria (salad).

Samples used for sensory analysis consisted of incremental concentrations of acids and set concentrations of sugars and volatiles. The inclusion of the volatile mix was to simulate the flavour of the locular fluid of tomatoes, add complexity to the organoleptic experience and give context to the perceived sugar and acid levels in the solutions.

The volatile spikes consisted of two solutions, the first contained 3.95 mg/mL hexanal, 2.68 mg/mL *cis*-3-hexenal and 7.48 mg/mL *cis*-3-hexen-1-ol and was solubilised in 100% food grade ethanol (Haymankimia, Witham, UK). An aliquot, 20 µL, of this solution was added to each sample solution used for sensory analysis. The second solution consisted of the remaining volatiles that were more soluble in aqueous solutions. Stock solutions for each volatile were made up in 50% ethanol. The concentrations of the stocks were as follows: 0.47 mg/mL 6-methyl-5-hepten-2-one, 0.17 mg/mL 2-isobutylthiazole and 0.23 mg/mL β-ionone. Aliquots, 1 mL, of these stocks were then combined and diluted in a 500 mL volumetric flask to create the second volatile mix. Each sensory sample was spiked with 1mL of the second volatile mix.

Separate solutions of sugars and acids were created for each type of tomato; salad, plum and cocktail. The concentration of each solution can be seen in **Table 5.1** Aliquots of each of the sugar and acid solutions (1 mL) plus 1.02 mL of the volatile mixture were combined prior to sensory analysis.

Table 5.1 - Composition of the solutions used for sensory analysis. Each sample contained 1 mL of sugar solution and 0.8, 0.9, 1.0, 1.1 or 1.2 mL of the corresponding acid solution. Additionally each solution contained 1 mL of volatile mix 2 and 20 µL of volatile mix 1.Water was used to make up the solutions to the final volume of 4.02 mL.

Cultivar	Glucose (mg/mL)	Fructose (mg/mL)	Citric Acid (mg/mL)	Malic Acid (mg/mL)
Valkiria	32.3	69.3	16.0	2.0
Angelle	67.6	88.1	40.4	2.2
Genio	59.1	125.5	29.9	0.2

The fifteen total sensory samples were split into three batches of 5, each batch representing one of the types of tomato. Of the 5 solutions, the acid content of the matrix was altered to cause a change in the sugar/acid ratio of the sample. Solutions were made up of 80%, 90%, 100%, 110% and 120% of the acid content shown in **Table 5.1** for each of the tomato types. The values of the acids shown in **Table 5.1** represents 100%.

Different sugar and acid solutions were added into sample according to type of tomato (salad, baby-plum and cocktail). Five samples with 80%, 90%, 100%, 110% and 120% of acid concentration were created for each different type of tomato juice as shown in **Table 5.2**. All samples contained 1 mL of sugar solution stock, 1 mL of volatile stock and 20 µL of C6 aldehydes stock. The volatile stocks were added to the samples 10 min prior to sensory analysis.

Table 5.2 Composition of the different simulated locule fluid samples used for sensory analysis.

Acid Composition (% of native)	Acid (mL)	Deionised Water (mL)	Sugar (mL)	Volatiles (mL)
120	1.2	0.8	1.0	1.02
110	1.1	0.9		
100	1.0	1.0		
90	0.9	1.1		
80	0.8	1.2		

5.3.4 Sensory Analysis

Sensory evaluation of the simulated locule fluid samples was carried out by 50 untrained panellists, 26 male and 24 female aged 20-39. The analysis took place in the sensory analysis suite at Northumbria University. The sensory analysis booths had controlled, diffuse red light, were held at 20-22 °C throughout the analysis and panellists were requested to refrain from

discussing with or distracting each other. Panellist were asked to rank 17 total attributes of the solution using a hedonic-scale from 1 (not similar/ extremely weak) to 10 (very similar/ extremely strong). A 10-point scale was used in favour of 9 as it forced responses of non-committal mid-ground. Panellists were asked to taste 5 different solutions in three separate sessions, with at least 45 minutes break between sessions to avoid sensory exhaustion.

Each sample was assigned with a randomised three-digit sample code. Each sample consisted of a mixture of each solution, with a total volume of 4.02 mL in a 25 mL transparent glass with mini cocktail straw to panellist in random order. Panellists were instructed to smell the aroma of the sample initially, then to taste the sample. The use of a straw was to allow for the possible aeration of the sample to improve the release of the volatiles from solution. Panellists were provided with sparkling water as a palate cleanser between samples.

5.4 Results and Discussion

5.4.1 Fruit Composition Relative to Each Tissue Type

From a consumer's point of view, there are three distinctive tissue types in whole tomato fruits during consumption; the seeds, flesh and juice. For the purposes of the work contained within this chapter, these have been categorised as follows. Henceforth, the 'fluid' refers to the liquid component, or locule fluid found within the locular cavity of whole fruits. The locule fluid was isolated from other tissues, with as little cross contamination as possible from the aqueous components that arise from cellular disruption and damage upon fruit dissection. The 'seeds' include the hard, fibrous seeds together with the surrounding, jelly-like parenchyma. Although these two tissues are clearly far from homogenous, it would be very difficult to separate the two during preparation and consumption. Therefore, they have been included together as a better representation of the tissue as it would be experienced by consumers. Finally, the flesh, now referred to as 'flesh', includes the entire pericarp and the columella. Again, the skin, or exocarp, is a distinctly different cellular morphology to that of the mesocarp, endocarp or columella, but in fresh tomatoes it is very unlikely to be removed prior to consumption, unlike in processing applications. Additionally, the exocarp is not likely to contain significant levels of either sugars or acids, and makes up a fraction of overall fruit weight, making an analysis on a per fruit basis very challenging. Due to these factors, the exocarp falls under the 'umbrella term' of flesh for the following analyses.

Initially, the weight contributions of each of the tissue types on a per cultivar basis were determined. This was due to the two-pronged approach to understanding how various tastes and flavours present in fresh tomato and whether specific tissues made had a more significant contribution to the various organoleptic experience during consumption. For this work, three

commercially available tomato cultivars were selected for analysis. A cocktail tomato cultivar, Genio, from Italy with an average fruit weight of 20.9g ± 1.5g. Baby plum tomatoes of cultivar Angelle from Morocco, with average fruit weights 9.1g ± 0.7g. The largest fruits were of the cultivar Valkiria, a salad tomato from Spain and with an average fruit weight of 78.7g ± 3.9g. The composition of the different cultivars can be seen in **Table 5.3**. Typically, cherry and cocktail tomatoes fill the role of smallest fresh eating tomatoes, however they are, on average, twice as large as the Angelle.

Table 5.3 - Morphological weight distribution of fruits of three tomato cultivars and the percentage of the overall fruit weight represented by the three distinct tissue categories previously described. Means represented alongside ± standard deviation of the twelve replicates per cultivar. Percentages are calculated from the means only. (n=12)

Cultivar	Type	Mean Weight (g)				Contribution to Total Fruit Weight (%)			
		n=12							
		Whole Fruit	Flesh	Seeds	Fluid	Flesh	Seeds	Fluid	Remainder
Genio	Cherry	20.9 ± 1.5	16.4 ± 1.3	0.9 ± 0.1	2.9 ± 0.6	78.4	4.1	13.8	3.7
Angelle	Baby-Plum	9.1 ± 0.7	7.0 ± 0.7	0.5 ± 0.1	0.9 ± 0.3	76.8	5.0	10.0	8.2
Valkiria	Salad	78.7 ± 3.9	61.1 ± 2.9	3.4 ± 0.6	11.2 ± 1.3	77.7	4.3	14.3	3.7

As shown in **Table 5.3,** even though there are vast differences in fruit weights between the cultivars, the proportional contribution of each of the three tissues is comparable, with the flesh tissue of Angelle only 1% lower than that of Valkiria, even though the fruits of Valkria are 8.6 times larger than those of the baby-plum. From this it can be deduced that the proportions of fruit weight attributed to each tissue type is likely to be predictable between cultivars, particularly the fruits of fresh eating cultivars. This trend may be less apparent when studying tomato cultivars developed for processing applications, rather than fresh eating, as they are often bred to produce higher levels of flesh than fresh eating types.

5.4.2 Distribution and Ratio of Sugars and Organic Acids in Different Tissues

Initial impressions with regards to many sensory experiences, but especially taste and flavour, are immensely important to consumer acceptance and repurchase. One of the most powerful sensations during fresh tomato consumption is the immediacy of the sweet and sour taste sensations upon biting into the fruit. This more apparent in smaller types of tomato such as cherry or baby plum, which are eaten whole, as the sensation is fully contained behind the skin and flesh and the tastants are not available for gustatory detection. In addition, due to the inverse relationship between fruit size and sugar and acid content, smaller fruited cultivars often trigger more powerful sweet/sour gustatory responses, due to the prevalence of sugar and acids. In larger

fruits this effect is less pronounced, firstly due to the typically lower sugar and acid content and secondly becasue larger fruits are often cut prior to consumption, allowing the cellular contents to mix with the locular fluid, diluting it, and increasing the chance that fluid will be lost prior to consumption. This often occurs during preparation of the fruits, as can be seen by the proportion of fruit weight that was lost when cutting these tomatoes before analysis, as shown in **Table 5.3**. All the material lost during the preparation of fruits was fluid or liquid released from cellular damage. If the majority of this lost fluid was locule fluid it result in reduced sugars and acids present for immediate tasting upon consumption, dramatically reducing the taste 'impact' of the fruit and reducing the sensory response. However, it is likely that some of the liquid released for cellular disruption during dissection of the fruits accounts for a proportion of this weight.

Table 5.4 – Concentration of the primary sugar and organic acids in three different tissues of three commercial tomato cultivars. Analyses were performed in triplicate on 6 fruits per cultivar/tissue. Significance is determined by Kruskal-Wallis H testing and pairwise comparisons according to Dunn's procedure with Bonferroni correction for multiple comparisons. Significantly different pairwise comparisons are represented by the same alphabetic character in a column and tissues were only compared intracultivar, not intercultivar. Significance is accepted at the $p<0.05$ level.

Cultivar	Tissue	Glucose (mg/Kg FW)	Fructose (mg/Kg FW)	Total Sugars (mg/Kg FW)	Citric Acid (mg/Kg FW)	Malic Acid (µg/Kg FW)	Total Acids (mg/Kg FW)	Sugar/Acid Ratio
Genio (n=6)	Fluid	8.7^{a}	16.8^{a}	25.5^{a}	9.7^{a}	233.0^{a}	9.9^{a}	2.6^{a}
	Seeds	8.9^{a}	14.1^{a}	23.1^{b}	5.4^{ab}	196.6^{a}	5.6^{b}	4.2^{b}
	Flesh	12.6^{b}	15.4^{a}	28.0^{c}	2.7^{b}	216.1^{a}	2.9^{a}	9.6^{a}
Angelle (n=6)	Fluid	14.5^{d}	20.3^{d}	34.7^{d}	13.0^{de}	191.1^{d}	13.2^{de}	2.6^{d}
	Seeds	11.8^{d}	15.9^{e}	27.7^{e}	4.2^{d}	262.9^{d}	4.4^{d}	6.2^{e}
	Flesh	18.7^{e}	19.2^{f}	37.9^{e}	3.9^{e}	215.8^{e}	4.1^{e}	9.2^{d}
Valkiria (n=6)	Fluid	8.1^{g}	14.2^{g}	22.3^{g}	5.2^{g}	488.3^{g}	5.6^{g}	4.0^{g}
	Seeds	7.6^{g}	11.7^{g}	19.3^{g}	1.8^{h}	456.0^{h}	2.3^{h}	8.4^{h}
	Flesh	10.4^{h}	12.6^{h}	23.0^{h}	1.3^{g}	569.9^{i}	1.9^{g}	12.4^{g}

As shown in **Table 5.4**, in general the level of sugars across different tissues is not significantly different when assessed using pairwise comparisons as a Post-Hoc analysis. However, the test statistic for the Kruskal-Wallis H test indicates that glucose is significantly different in Genio, Angelle and Valkiria fruits, $p=0.03$, 0.04 and 0.10 respectively. The same effect is also apparent in the fructose concentration for Angelle and Valkiria ($p=0.39$ and $p=0.024$), but not for Genio, where the concentration of fructose in each tissue is more homogenous. When looking at total sugars, calculated as the sum of glucose and fructose concentrations in each tissue, Angelle has significantly different concentrations between the seeds and flesh, whereas the fluid of Valkiria fruit contained on average 1.6 mg/g more than that of the seeds, which was significant. There appears to be a more significant deviation in citric acid content of each of the tissue types across

all three cultivars. Genio, Angelle and Valkiria fruits all showed significantly more citric acid in the fluid than in the flesh, approximately 3.5 times as much in the fluid than in the flesh. In terms of taste, the difference in citric acid distribution may explain the immediacy of the desirable sourness upon initial consumption of tomato fruits. The high proportion of citric acid in the fluid of the fruits would ensure that, following piercing the locular cavity during mastication, the locular fluid would be released and could immediately migrate into taste pores for detection and stimulation. Unlike the locular fluid, the citric acid in the flesh is probably intracellular and would, therefore, be released during continued mastication. The differences in this experience are probably paramount to how fresh tomato taste is perceived, with a strong immediate 'tang' provided by locular fluid shortly after tissue disruption, followed by a prolonged and less intense release of citric acid and other taste active metabolites during mastication. This argument is further supported by the difference in sugar/acid ratio between each of the tissues. The fluid of each of the cultivars has much more comparable levels of both sugars and acids, with ratios ranging from 2.6-4.0, whereas the flesh shows ratios of 9.2-12.4. Proportionally, this is a shift of a factor of 3.1-3.9 between tissues of each cultivar. This change in ratio is largely driven by the discrepancy in citric acid content between tissues, as well as the slightly higher concentration of glucose in flesh when compared to both fluid and seeds. Interestingly, malic acid does not mirror citric acid in terms of tissue specific localisation, with non-significant differences in all but Angelle, between seeds and fluid. It is interesting to note that, although comparisons between cultivars were not conducted in this study, the salad tomato, Valkiria, contains more than twice the malic acid content of the other cultivars. This results in a non-significant rise in total sourness, due to its low concentration relative to citric acid, but may have some biological significance for the larger fruits.

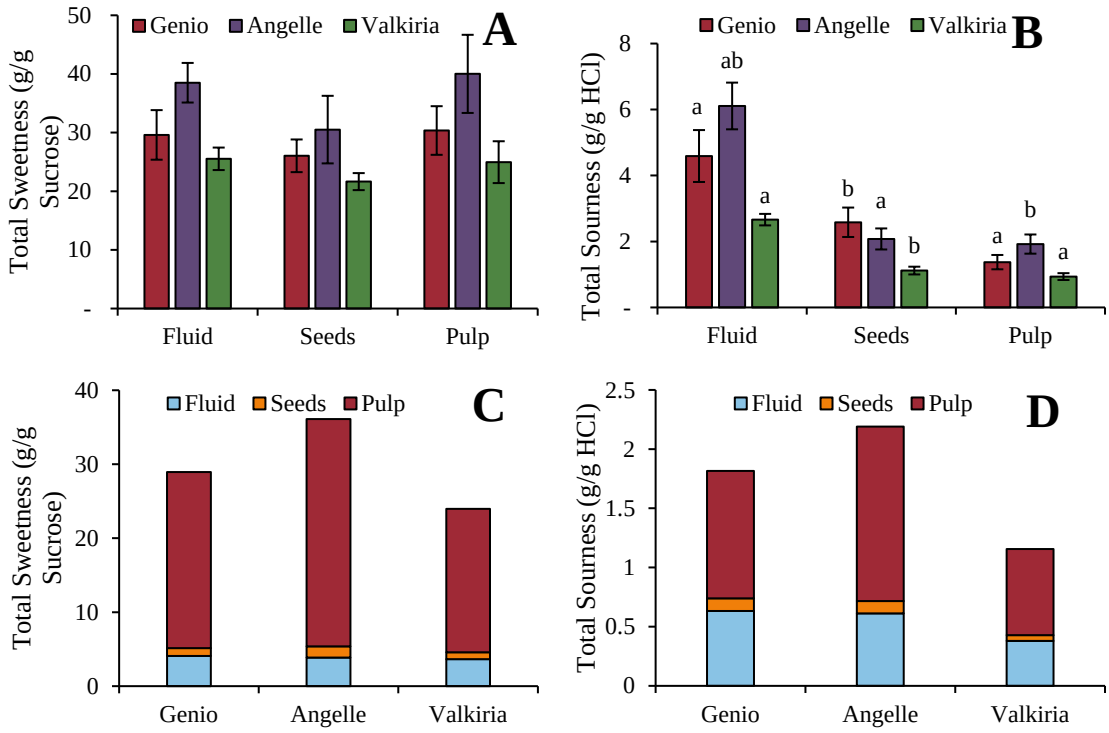


Figure 5.1 - Total sweetness and sourness for each cultivar and respective tissue types. **A –** The total sweetness provided by the glucose and fructose content of each tissue type, error bars represent standard deviation. **B –** Total sourness provided by each tissue type of each of the analysed cultivars, error bars represent standard deviation. **C** – Total sweetness of each cultivar with the proportion contributed by each of the tissue types visualised. **D -** Total sourness of each cultivar with the proportion contributed by each of the tissue types visualised. Significant differences as determined through pairwise comparisons following Kruskall-Wallis H test are represented by the same alphabetic character on a per cultivar basis. Comparisons between cultivars were not performed. Conversion factors used for both sweetness and sourness based on those defined by McLaughlin and Margolskee (McLaughlin and Margolskee, 1994). (n=18)

The differences in the distribution of sugars and organic acids result in different gustatory perception during the consumption of tissues, both individually and through the combined sensation provided by each tissue in whole fruits. **Figure 5.1A-D** shows the effect the sugar and acid concentration has on the overall taste of the tissue in question, as well as how much of a contribution each tissue makes to the taste of the whole fruit. In terms of the perceived sweetness, **Figure 5.1A**, this contribution of each tissue is not significantly different between the tissues of any of the cultivars, following correction for multiple comparisons. A similar trend can be observed across all three cultivars, with the seeds contributing slightly lower sweetness at equal weights, when compared to both the fluid and flesh, which present very similar levels of sweetness. The total sourness is very different, however, as can be seen in **Figure 5.1B**. In each cultivar there is a significant difference between the level of sourness provided by the fluid and flesh of the fruits. In addition, the fluid of Angelle tomatoes is the sourest tissue type when consumed in equal proportions, significantly sourer than both the flesh and seeds. The sweet/sour

sensation produced by consumption of the whole fruits of these cultivars can be seen in **Figure 5.1C-D**. Angelle fruits were both the sweetest and sourest of the cultivars analysed in this chapter, followed by Genio and then Valkiria. This matches up with fruit size, which is inversely proportional to the total sugars and acids assimilated during tomato ripening. In this case, a very small baby-plum cultivar was less than half the average fruit weight of the cocktail tomatoes, which are comparatively large when compared to other cherry/cocktail tomatoes studied previously in this thesis.

5.4.3 Amino Acid Composition of Each Tissue Type

Amino acids play essential roles in plant health, defence, respiration, amino group donation reactions, cellular regulation, reproduction, proliferation, enzyme and phytohormone formation and in the biosynthesis of many secondary metabolites (Hildebrandt *et al.*, 2015, Huang *et al.*, 2011, Kochevenko *et al.*, 2012, Tegeder, 2014). Arguably, most amino acids play a role in the quality of fresh tomato fruits, due to their intrinsic links to so many quality related processes. However, in terms of flavour, there are 4 main amino acids that are responsible for the formation of important volatile constituents of tomato and which are present at sufficient concentration to affect the perceived aroma and flavour. Leucine and isoleucine, and their α-keto acids, have been shown to be the direct precursors of 2- and 3-methylbutanal and methylbutanols (Kochevenko *et al.*, 2012). Methionine is responsible for the formation of methional, which is occasionally reported as detectable and important to the overall flavour of tomatoes, but which has not been a target in this study (Mayer *et al.*, 2008). Phenylalanine indirectly forms a number of volatile components through the formation and subsequent catabolism of cinnamic acid resulting in methyl salicylate, benzoic acid and eugenol and the production and breakdown of phenylacetaldehyde into 2-phenylethanol (Zhang *et al.*, 2015). Additionally, two amino acids, glutamic acid and aspartic acid are essential for the savoury umami sensation in fresh tomatoes, with glutamic acid presenting nearly 13 times greater umami sensation than equimolar amounts of aspartic acid (Yamaguchi *et al.*, 1971).

Table 5.5 - Concentration of amino acids that either directly influence flavour or act as precursors to important volatile constituents in the different tissue types of three supermarket bought tomato cultivars. Data presented is the mean of 6 fruits per cultivar (5 for Valrikia locule fluid) alongside the standard deviation of the biological replicates. Individual samples were analysed in triplicate prior to calculation of the mean. Statistically significant differences between tissues of the same cultivar are indicated by different alphabetic superscripts, as determined through Kruskal-Wallis-H and subsequent Post-Hoc analyses on all 18 replicates per group. Comparisons were only made between the tissue types of each cultivar, not intercultivar. Significance accepted at the $p \leq 0.05$ level following Bonferroni Corrections for multiple comparisons.

		Amino Acid Concentration (μg/g Fresh Weight)					
Cultivar	Tissue	LEU	ILE	MET	PHE	GLU	ASP
Angelle	Flesh	11.9^{a} ± 3.5	3.8^{a} ± 1.3	TR	106.3^{a} ± 28.3	4244^{a} ± 452	2404^{a} ± 194
	Locular Fluid	117.4^{b} ± 24.7	21.3^{b} ± 7.6	46.2^{b} ± 1.9	129.3^{a} ± 26.5	5986^{b} ± 1017	1545^{b} ± 256
	Seeds	245.7^{c} ± 10.9	53.5^{c} ± 3.8	56.2^{c} ± 2.8	219^{b} ± 14.8	3489^{a} ± 381	976^{c} ± 112
Genio	Flesh	4.3^{a} ± 3.6	2.5^{a} ± 0.4	TR	63.8^{a} ± 12.9	1587^{a} ± 379	1251^{a} ± 246
	Locular Fluid	43.5^{b} ± 16.8	0.8^{a} ± 0.1	TR	48.9^{a} ± 17.4	3615^{b} ± 301	1280^{a} ± 300
	Seeds	205.3^{c} ± 39.3	40.5^{b} ± 10.2	59^{b} ± 5.9	179.2^{b} ± 29.5	1525^{a} ± 313	834^{b} ± 101
Valkiria	Flesh	TR	TR	TR	42.3^{a} ± 6.4	1511^{a} ± 305	998^{a} ± 190
	Locular Fluid	21.4^{b} ± 7.4	TR	TR	36.6^{a} ± 7.4	5053^{b} ± 304	1679^{b} ± 310
	Seeds	159.1^{c} ± 28.6	26.7^{b} ± 7.1	47.6^{b} ± 2.9	141.7^{b} ± 19.7	2454^{c} ± 83	945^{a} ± 62

LEU Leucine, ILE Isoleucine, MET Methionine, PHE Phenylalanine, GLU Glutamic Acid, ASP Aspartic Acid

As can be seen in **Table 5.5**, the tissue specific distribution of the flavour-forming and umami amino acids is significantly different between the analysed tissues of each of the cultivars tested. Leucine, isoleucine, methionine and phenylalanine are significantly higher in seeds than in the flesh or locule fluid of each cultivar. Of these, all bar phenylalanine are also significantly higher in the locule fluid than in the flesh in Angelle fruits. This relationship is present for leucine in Genio and Valkiria fruits, but not in isoleucine and methionine, which were close to the limit of detection. The more abundant amino acids, glutamic and aspartic acid display a different relationship between tissues. Glutamic acid was significantly higher in the locule fluid than in both the flesh and seeds of each cultivar. The seeds presented between 40-60% of the glutamic acid compared to that of the locule fluid in each cultivar. In both Angelle and Genio fruits, the concentration of glutamic acid in the flesh and seeds was not significantly different. Aspartic acid similarly showed that the locule fluid contained a significantly higher concentration than that of the seeds for each cultivar. Angelle fruits contained the greatest concentration of aspartic acid in

the flesh, which was 60% higher than in the locule fluid. These results are in partial agreement of those of Oruna-Concha *et al.* who found that localisation of glutamic and aspartic acid differed significantly between the flesh (mesocarp and endocarp) and pulp (locule fluid, parenchyma and seeds) of 14 supermarket bought tomato cultivars. The authors reported similar concentrations of both amino acids in the pulp and flesh, to those reported here (Oruna-Concha *et al.*, 2007). This work expands on these findings by further categorising the pulp components and potentially highlighting the locule fluid as the primary source of the umami amino acids. As both the fluid and seeds were combined into a single 'pool' for analysis in their original paper, it is likely that a similar partitioning of these components was present in the fruit used for analysis. As with the sugars and organic acids, this highlights the importance of the locule fluid as a carrier of tastants during consumption of the fruits.

The total free amino acids present in the analysed tissues of the three cultivars can be seen in **Figure 5.2** below. The locule fluid of each cultivar contains the highest concentration of free amino acids on a weight for weight basis. The Valkiria fruits contained the least amino acids in the flesh, whereas Genio and Angelle contained the least in the seeds. This relationship seems to inversely correlate with fruit size, as the larger the fruits, the less assimilation of amino acids occurs in the flesh. Angelle consistently presented the highest total amino acids per tissue.

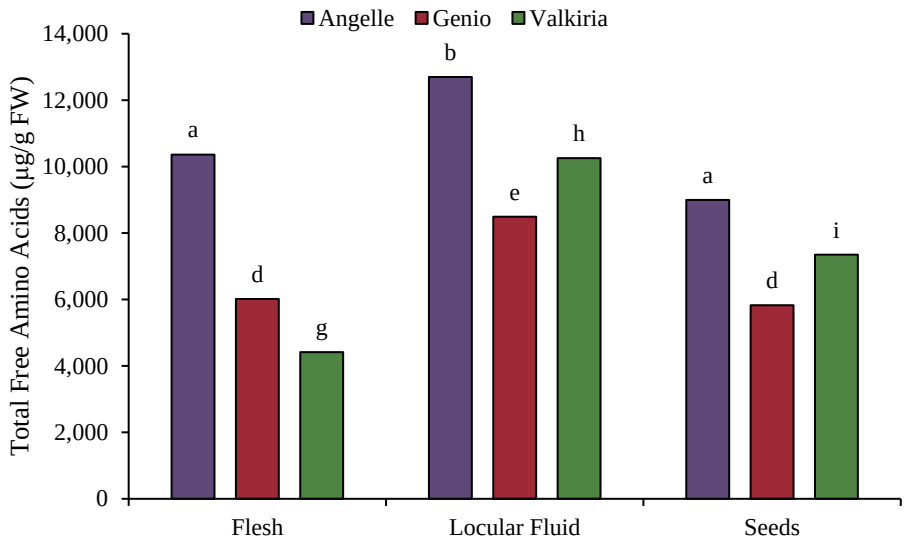


Figure 5.2 – The total free amino acid content of the flesh, locular fluid and seeds of three tomato cultivars. Total calculated as the sum of the means of 19 amino acids. Significance determined by Kruskal-Wallis-H and subsequent Post-Hoc pairwise comparisons. Significance only assessed on a per cultivar basis and accepted at the $p \leq 0.05$ level following Bonferroni Corrections for multiple comparisons. Actual values of each amino acid can be seen in **Table 5.5** and **Table 5.6**. (n=5 for Valkiria fluid, n=6 for all other populations).

The profile of additional amino acids across the tissues in each cultivar can be seen in **Table 5.5** below. This includes those amino acids which are not directly related to flavour or taste, or which

are at concentrations at which they would be unlikely to significantly alter the organoleptic profile of the fruits. A similar general trend of lower concentrations of most amino acids in the flesh of each cultivar, than in the locule fluid or seeds, can be observed. Asparagine and glutamine oppose this trend, generally accumulating in higher concentrations in flesh than in locule fluid or seeds, for each cultivar. Angelle fruits had comparable levels of asparagine between flesh and seeds, but significantly lower concentrations in the locule fluid. Genio fruits presented significantly higher concentrations of both amino acids in the flesh than either other tissue, with insignificant differences in glutamine between locule fluid and seeds and significantly lower asparagine content in the locule fluid, as opposed to the seeds. Valkiria fruits presented comparable concentrations of glutamine between the flesh and seeds, with significantly higher concentration in the locule fluid. The asparagine concentration of Valkiria fruits also differed from the other cultivars, with similar concentrations between the flesh and locular fluid and significantly higher concentrations in the seeds. The concentration of GABA in the fruits was also noteworthy, with significantly less present in the flesh of the fruits than in the seeds or locule fluid. This may due to the role of GABA in fruit ripening and the mechanisms by which it is catabolised to form other amino acids, including asparagine, aspartic acid, glutamine and glutamic acid, through which most other amino acids can be derived (Akihiro *et al.*, 2008, Takayama and Ezura, 2015). The localisation of residual GABA in and surrounding the seeds of tomato fruits may be due to this link to amino acid generation and nitrogen cycling, which would be essential to the germination, growth and survival of fertilised seeds. Furthermore, histidine was unreported in the fruit flesh of each cultivar studied, but it was detectable in the seeds and locule fluids. Similarly to GABA, histidine has been shown previously to play substantial roles in plant growth, proliferation and seed viability (Muralla *et al.*, 2007, Ingle, 2011).

Table 5.6 - Concentration of additional, non-flavour forming, amino acids in the different tissue types of three supermarket bought tomato cultivars. Data presented is the mean of 6 fruits per cultivar (5 for Valrikia locule fluid) alongside the standard deviation of the biological replicates. Individual samples were analysed in triplicate prior to calculation of the mean. Statistically significant differences between tissues of the same cultivar are indicated by the different alphabetic superscripts, as determined through Kruskal-Wallis-H and subsequent Post-Hoc analyses on all 18 replicates per group. Comparisons were only made between the tissue types of each cultivar, not intercultivar. Significance accepted at the $p \leq 0.05$ level following Bonferroni Corrections for multiple comparisons.

		Amino Acid Concentration (μg/g Fresh Weight)												
Cultivar	Tissue	ALA	GLY	VAL	THR	GABA	SER	PRO	ASP	GLN	LYS	HIS	TYR	TRP
Angelle	Flesh	140.6^{a} ± 22	15.3^{a} ± 0.7	2.5^{a} ± 1.9	109.8^{a} ± 6.1	218.5^{a} ± 44.5	164.7^{a} ± 26.5	62.6^{a} ± 14.2	397.1^{a} ± 71.9	2336.8^{a} ± 353.1	80.2^{a} ± 7	TR	35.9^{a} ± 1.6	28.9^{a} ± 4.5
	Locular Fluid	544.1^{b} ± 113.1	41.4^{b} ± 7.4	45.6^{b} ± 13.5	130.7^{b} ± 19.3	1305.5^{b} ± 331.6	301.2^{b} ± 62.9	77.6^{b} ± 14	192.8^{b} ± 46.6	1748.5^{b} ± 328.6	220.5^{b} ± 47.3	143.3^{b} ± 17.4	59.6^{b} ± 9.5	42.9^{b} ± 4.7
	Seeds	368.8^{c} ± 42.6	47.1^{b} ± 2	85.8^{c} ± 4.6	158.8^{c} ± 6.7	859.9^{c} ± 67.9	274.3^{b} ± 23.1	86.6^{b} ± 6.9	319^{a} ± 37.9	1070.2^{c} ± 128.3	358.4^{c} ± 25.6	155.7^{b} ± 9.2	130.5^{c} ± 4.1	44.4^{b} ± 2.9
Genio	Flesh	71.1^{a} ± 7.2	17.5^{a} ± 2.7	2.6^{a} ± 2	TR	176.9^{a} ± 72.6	124.1^{a} ± 18.8	43.8^{a} ± 9.9	505.6^{a} ± 127.6	2016.3^{a} ± 541.9	79.6^{a} ± 7.3	TR	44^{a} ± 11.1	26.8^{a} ± 4.4
	Locular Fluid	285.4^{b} ± 101.2	29.8^{b} ± 5.4	19.8^{b} ± 8.1	93.2^{a} ± 11.1	1380.7^{b} ± 369.6	166.4^{b} ± 20.4	21.4^{b} ± 5.1	157.8^{b} ± 30.6	1034.6^{b} ± 280.4	127.5^{b} ± 21.2	107.4^{b} ± 6.9	53.4^{a} ± 10.6	24.1^{a} ± 2.4
	Seeds	242.1^{b} ± 47.8	43.1^{c} ± 5.8	74.6^{c} ± 15	140.2^{b} ± 18.3	671.1^{c} ± 197.5	186.9^{b} ± 26	50^{a} ± 7.5	245.6^{c} ± 49.7	837.7^{b} ± 196.6	210.3^{c} ± 31.9	117.9^{b} ± 8.5	127.4^{b} ± 17	35.4^{b} ± 3.6
Valkiria	Flesh	53.1^{a} ± 12.4	15.5^{a} ± 1.7	TR	TR	379.8^{a} ± 151.8	116.9^{a} ± 34.1	21.5^{a} ± 5.6	195.7^{a} ± 36.7	969.1^{a} ± 274.2	58.2^{a} ± 2	TR	37^{a} ± 1.9	18.2^{a} ± 1.1
	Locular Fluid	202.5^{b} ± 48.1	24.2^{b} ± 2	5.4^{b} ± 3.2	TR	1306.5^{b} ± 238.9	152.2^{b} ± 10.4	6.2^{b} ± 2.7	150.8^{a} ± 25.1	1339.8^{b} ± 92.2	88.4^{b} ± 7.5	109.8^{b} ± 3.9	55.5^{b} ± 15.4	24.7^{b} ± 2.8
	Seeds	212^{b} ± 47.7	35.7^{c} ± 3	49.7^{c} ± 8.3	122.2^{b} ± 12.7	1335.3^{b} ± 275.5	175.8^{b} ± 13.9	32.8^{c} ± 6.8	237.5^{b} ± 27.7	975.4^{a} ± 80.8	168.4^{c} ± 24.4	113.3^{b} ± 4.5	90^{c} ± 10.5	28.4^{b} ± 2.1

ALA Alanine, GLY Glycine, VAL Valine, THR Threonine, GABA γ-Aminobutyric Acid, SER Serine, PRO Proline, ASP Asparagine, GLN Glutamine, LYS Lysine, HIS Histidine, TYR Tyrosine, TRP Tryptophan

5.4.4 Umami Contribution of Each Tissue Type

Although not as important as the sugar acid concentration and ratio on the overall acceptability of tomato fruits, umami is a significant gustatory sensation in both fresh and processed tomatoes. Due to umami and 'savoury' being relatively synonymous, it is an important counter point to the sweetness of tomatoes. The combination of umami and the sweet/sour balance moves tomatoes from sweet eating, like many fruits, to their culinary inclusion in many savoury dishes. As previously discussed, the sensation of umami is the combined intensity of glutamic and aspartic acids, as well as the monophosphate nucleotides of adenosine, guanosine, inosine and xanthine. It appears that cytidine and uridine monophosphates do not present any umami sensation (Yamaguchi *et al.*, 1971). It has been previously shown that umami nucleotides do not present any umami sensation in isolation, but instead present synergistic enhancement of umami amino acids (Li *et al.*, 2002, Yamaguchi *et al.*, 1971, Zhang *et al.*, 2008).

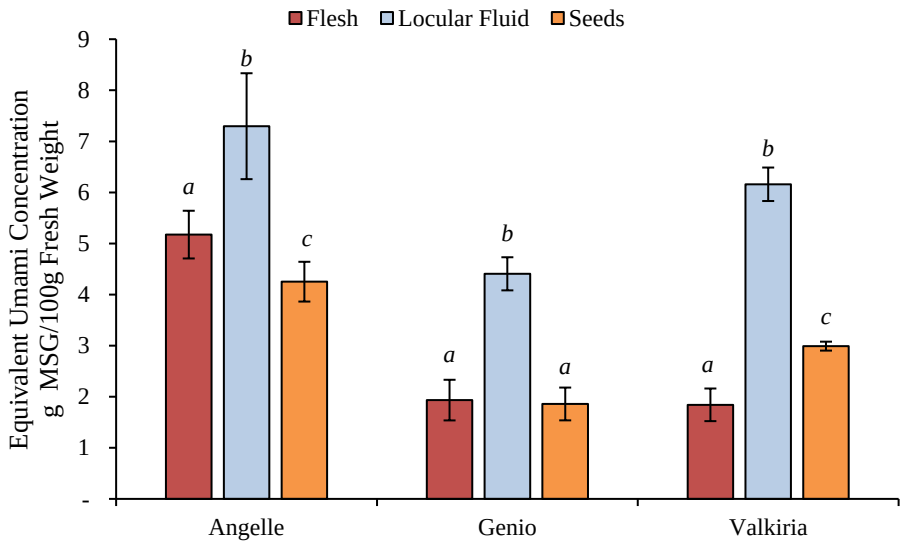


Figure 5.3 – Equivalent Umami Content (EUC) provided by the glutamic and aspartic acid content of each tissue of the studied cultivars, enhancement by monophosphate nucleotides has not been accounted for at this time. Umami content of each tissue represented as the required monosodium glutamate content per 100g of tissue to achieve the same umami intensity. Calculations based on those first described by Yamaguchi *et al.* and further expanded by Mau (Mau, 2005, Yamaguchi *et al.*, 1971). Error bars represent the standard deviation (n=10). Significance was determined by Kruskall-Wallis-H test and subsequent pairwise comparisons. Significantly different results are indicated by different alphabetic characters. Significance was accepted at the $p<0.05$ level following Bonferroni corrections for multiple comparisons (n=18).

Figure 5.3 above depicts the overall umami sensation provided by the amino acid content of each tissue for the three studied cultivars. The equivalent umami content is representative of the concentration of monosodium glutamate (MSG) required to provide the same umami intensity. It is immediately apparent that the locule fluid contributes the highest umami taste of the three specified tissues, which is observable in each of the studied cultivars. This would indicate that, in addition to the increased sweetness and sourness provided by the locule fluid, it also contributes

significantly to the savoury flavour of the tomatoes. However, this may be subject to change based on the localisation of nucleotides, specifically GMP and AMP, which may be present at sufficiently higher concentrations in the opposing tissues to change this relationship. The locule fluid appears to be responsible for a significant proportion of the gustatory experience of tomato, on a tissue weight for weight basis. However, there is between 4.2-4.5 times more flesh than fluid per fruit of each of the cultivars tested so, although the concentration is higher, the flesh will cumulatively provide more umami taste in a whole tomato. As previously mentioned, the liquid nature of the locule fluid is probably important to its immediate perception, whereas the flesh would need thorough mastication before all the compounds were liberated and made available to the taste pores.

5.4.5 Tissue Specific Potential for Volatile Formation

The formation of endogenous volatiles and those that form during tissue damage and disruption are essential to the overall flavour and aroma of fruits. Most of the volatiles responsible for the aroma and flavour of tomatoes are the latter, formed during damage, abscission of fruit or biological attack. This is notable by the lack of distinctive aroma of whole tomato fruits, as opposed to many other fleshy fruits which produce and exude endogenous volatiles as a method of attracting herbivores. Due to the different functions and chemical compositions of tissues in tomato fruits, volatile components are generated at different rates and intensities, dependant on tissue. Volatile organic compounds are widely produced by plants from most tissues, however the diverse roles that volatiles fill in plant defence, attracting pollinators, plant signalling and promoting consumption by herbivores, suggest localised production is likely (Dudareva *et al.*, 2013, Kesselmeier and Staudt, 1999). For example, C6 aldehydes are largely generated as a wound response, either to prevent damage by insects or by attracting animals to consume the fruits (Gardner, 1995, Hubert *et al.*, 2008, Utto *et al.*, 2008). Therefore, high concentrations of these compounds form from the flesh and surface of the tomato, where they can be rapidly released to ward off biological attack by microorganisms or insects or be used to indicate edible tissue. Many of the carotenoid-derived volatiles present fruity/floral aromas and are generally synthesised in fruit tissues as they ripen. The influx of these positive, appetising volatiles in ripe fruits also promotes consumption of ripe, fully developed fruits, enabling optimum mature seed dispersal. However, the pigments that are responsible for the generation of these compounds in tomato, including lycopene and numerous precursor compounds in the carotenoid biosynthesis pathway, are largely localised on the external surface of the fruits, where they can act as visual indicators of ripeness as well (Lewinsohn *et al.*, 2005, Simkin *et al.*, 2004, Vogel *et al.*, 2010). Therefore, the generation of carotenoid derived volatiles is likely to be lower in internal tissues, where there are less substrates available. The volatile generation of the flesh, locular fluid and seed tissues of the three analysed cultivars is displayed in **Table 5.7**.

Table 5.7 – Concentration of volatile compounds produced by each tissue (ppb). Data is the mean of 18 replicate samples ± the Standard Error of the Mean. Significance determined through Kruskall-Wallis-H tests and subsequent pairwise comparisons with Bonferroni corrections. In the case of one group being 'Not Detected' (N.D.) the remaining two groups were analysed using a Mann-Whitney-U test to determine significance. Significance is accepted at the $p<0.05$ level and denoted by different alphabetic characters for the results of the Kruskall-Wallis-H pairwise comparison and pairs of † for significantly different results as determined by Mann-Whitney-U.

Cultivar	Tissue	Isovaleraldehyde	1-Penten-3-one	Hexanal	*cis*-3/*trans*-2-hexenal	6-Methyl-5-hepten-2-one	*cis*-3-Hexen-1-ol	2-Isobutylthiazole	Methyl Salicylate	β-Ionone
Angelle	Flesh	54.5 ± 8.1 a	171.8 ± 29.3 a	3372.1 ± 242 a	658 ± 101.6 a	2382.2 ± 186.7 a	50.7 ± 8 a	169.5 ± 21.5 a	N.D	29.2 ± 1.7 †
	Fluid	54.3 ± 2.5 a	62.8 ± 1.8 a	2599.4 ± 111.4 a	177 ± 10.7 b	2681.3 ± 48.3 a	210.2 ± 28.5 b	94.9 ± 1.6 ab	N.D	10.1 ± 0.1 †
	Seeds	124.9 ± 3.1 b	50.7 ± 0.1 b	1013.7 ± 74 b	208.4 ± 9.6 ab	144.3 ± 4.2 b	198.5 ± 17.8 b	87.2 ± 1.4 b	N.D	N.D
Genio	Flesh	N.D	51.7 ± 3.4 †	1677.8 ± 119.2 ab	254.8 ± 19.1	412.7 ± 21.9 a	N.D.	83.2 ± 5.7 ab	175 ± 2.9 a	28.7 ± 0.2
	Fluid	28.8 ± 2.7 †	80.2 ± 2.6 †	2103.8 ± 175.9 a	266.7 ± 12.8	946.7 ± 72.7 b	263.9 ± 27.5 b	84.8 ± 3.4 b	22 ± 3.4 b	28 ± 3
	Seeds	67.9 ± 3.5 †	N.D.	1147.8 ± 107 b	296.5 ± 15.9	70.9 ± 2.7 c	143.7 ± 9.7 a	73.5 ± 1 a	58.4 ± 6.6 b	N.D
Valkiria	Flesh	145.3 ± 21.4 a	73.5 ± 4.7 †	835.2 ± 50.5 a	255.3 ± 21	775.3 ± 37.9 a	N.D.	286.6 ± 12.1 a	137.2 ± 0.9 a	30.8 ± 1.1
	Fluid	540.1 ± 35.9 b	117.3 ± 9.9 †	368.6 ± 39.4 b	235.8 ± 18.2	1580.5 ± 142 a	91.5 ± 1.5 †	664.6 ± 53.6 b	107.3 ± 0 ab	35.5 ± 0.4
	Seeds	448.2 ± 25.1 b	N.D.	867.1 ± 82.5 a	226.5 ± 11.2	268.6 ± 23.9 b	88 ± 2.4 †	111.6 ± 10 c	31.2 ± 6.5 b	N.D

Table 5.7 – Continued.

Cultivar	Tissue	*trans*-2-Pentanal	1-Penten-3-ol	1-Pentanol	Nonanal	*trans*-2-Octenal	6-Methyl-5-hepten-2-ol	*trans,trans*-2,4-Heptadienal	Linalool	*cis*-Geranylacetone	Benzyl Alcohol
Angelle	Flesh	77.6 ± 10.8 a	99.7 ± 7.4 a	36.5 ± 3.1 a	22.5 ± 3	13.8 ± 0.6 a	6.3 ± 0.3 a	19.5 ± 0.4 †	26.3 ± 1.6	74.4 ± 11.2 †	91.8 ± 6.8 a
	Fluid	62.5 ± 0.4 a	74.5 ± 2.9 a	48.2 ± 0.7 a	36.9 ± 1.3	27.7 ± 0.2 b	4.1 ± 0 b	37.5 ± 0.1 †	N.D	130.6 ± 1.2 †	107.6 ± 2.6 b
	Seeds	17.1 ± 0.1 b	47 ± 3.8 b	77.3 ± 3.6 b	N.D	3.2 ± 0.1 c	10 ± 0.5 c	N.D	N.D	N.D	76.3 ± 2.5 a
Genio	Flesh	38.4 ± 3.4 a	16.2 ± 2.2 a	3.2 ± 0.6 a	12.2 ± 0.3	17.2 ± 0.2 a	10.2 ± 0.2 a	20.1 ± 0.2 a	18.8 ± 0.5 †	5.9 ± 1 †	20 ± 0.5 a
	Fluid	67.9 ± 2 b	119.1 ± 11.8 b	43.3 ± 1.4 b	N.D	24.8 ± 0.8 b	3.6 ± 0.1 b	36.7 ± 1 b	8.6 ± 1.2 †	113.3 ± 4 †	N.D
	Seeds	48.8 ± 3.4 a	102 ± 5.1 b	91.4 ± 5 c	N.D	3.1 ± 0.1 a	3.4 ± 0 b	36.8 ± 0.1 b	N.D	N.D	57.9 ± 2.4 b
Valkiria	Flesh	46.4 ± 2.9 a	42.7 ± 4.6 a	7.3 ± 1.6 a	16.4 ± 0.8 a	16.3 ± 0.5 a	10.5 ± 0.4 a	20.3 ± 0.2 a	32.5 ± 1.3 †	113.5 ± 8.3 a	N.D
	Fluid	47.6 ± 4.4 a	46.9 ± 5.9 a	3.7 ± 0.9 a	29.6 ± 3.9 b	7.2 ± 0.6 b	3.6 ± 0.2 b	14.9 ± 0.7 b	138.5 ± 12.4 †	314.8 ± 26.9 b	17.8 ± 1 †
	Seeds	54.1 ± 2.1 b	112.5 ± 7.1 b	124.9 ± 13.5 b	37.2 ± 0.8 b	21.8 ± 1.6 a	5.8 ± 0.4 c	37.1 ± 1 c	N.D	54.2 ± 10.5 a	67 ± 2.3 †

Of the analysed volatiles in **Table 5.7,** two are derived from amino acids, isovaleraldehyde, otherwise known as 3-methylbutanal, and 2-isobutylthiazole. Isovaleraldehyde forms from leucine or derivatives of leucine, including its derivative α-ketoisocaproate, and the proposed routes of synthesis for 2-isobutylthiazole have been previously discussed, but are likely to also be from leucine or a derivative. These two compounds show inverse relationships when it comes to their production in flesh, fluid and seed tissues. In both Valkiria and Angelle fruits isovaleraldehyde was found to be present at significantly higher concentrations in seeds than in either other tissue. This was not the case for Genio, however, where the locule fluid liberated significantly higher concentrations. The concentration of leucine in these tissues correlated to the volatile production for Valkiria and Angelle, but not for Genio, where more the seeds contained significantly greater amounts than the fluid. Moreover, the formation of 2-isobutylthiazole was more comparable, with the flesh and the fluid contributing significantly more than the seeds. The flesh yielded more than the fluid for both Angelle and Genio, although this was not significant, whereas the opposite was true in Valkiria, where the fluid generated approximately 2.5 greater concentrations, which was significant. It has been previously shown that the catabolism of branched-chain amino acids for the purpose of volatile formation is not substrate limited (Kochevenko *et al.*, 2012). Therefore, the lack of universal correlation with precursor content, leucine in this case, is not surprising. It seems the generation of these volatiles is limited by compartmentalisation, enzyme inhibition or the *de novo* synthesis of the enzyme at specific developmental points. Different contributions from each tissue may be partly due to substrate availability, but also to enzyme activity.

The carotenoid derived volatiles include 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, β-ionone, linalool and *cis*-geranylacetone. Of these 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, *cis*-geranylacetone and β-ionone form from lycopene or its carotenoid derivatives, whereas linalool is derived from geranyl diphosphate (Schilmiller *et al.*, 2009, Lewinsohn *et al.*, 2005, Simkin *et al.*, 2004). The flesh and fluid of each of the three cultivars released significantly higher concentrations of 6-methyl-5-hepten-2-one and β-ionone than the seeds, which agrees with formation from carotenoids which are most abundant in the skin and flesh of tomatoes. The seeds contained non-detectable levels of β-ionone, linalool and *cis*-geranylacetone, probably due to limited substrate availability and the naturally low concentration of these volatiles in all tomato tissues. The tissue responsible for generating the highest concentration of 6-methyl-5-hepten-2-ol was cultivar specific, which the flesh contributing the most for Valkiria and Genio fruits, which was significant. However, the seeds of Angelle tomatoes contained significantly higher concentrations than the other tissues, which is unexpected as they probably contain the least substrate, based on coloration. The tissue specific formation of these volatiles seems, in part, to be driven by substrate localisation and availability, implying that the enzymes required for their synthesis are expressed in each tissue, but the rate of formation is dependent on both substrate

concentration and enzyme activity. It is possible that some quantities of these volatiles are endogenous, potentially forming from the photodegradation of lycopene and being either slowly exuded from the fruits or trapped internally prior to release upon tissue disruption. If that were the case, the locular fluid may act as a reservoir for the transient storage of some of these volatiles, prior to tissue damage/disruption upon consumption, perhaps explaining the lack of a strong aroma from undamaged fruits.

Lipid oxidation plays a significant role in the formation of volatiles that are integral to tomato flavour and aroma (Baldwin *et al.*, 2000, Buttery *et al.*, 1987, Feussner and Wasternack, 2002, Petro-Turza, 1986). Arguably the action of lipoxygenase and hydroperoxide lyases yields some of the most impactful tomato aroma compounds including hexanal, *cis*-3/*trans*-2-hexenal, *cis*-3-hexenol, 1-penten-3-one, 1-penten-3-ol, 1-pentanol, *trans,trans*-2,4-heptadienal and *trans*-2-pentental. Additional lipid derived volatiles include nonanal and *trans*-2-octenal, which have slightly different precursors and routes of synthesis. Formation of much of the lipid-oxidation family of volatiles is triggered by physical wounding or external sources of damage, such as biological attack or insect damage and therefore are common in the flesh of the fruit. However, the seeds of tomato, like most seeds contain high proportions of lipids when compared to other tissues, allowing for the synthesis of lipid oxidation products in the locular cavity by the seeds and fluid, as seen in

Table 5.7. Angelle and Genio fruits both generated significantly higher proportions of hexanal in the flesh and fluid than in the seeds. The flesh and seeds of Valkiria fruits were very comparable, however, yielding 2.5 times as much as the fluid. Angelle flesh generated approximately 2.5-3 times as much *cis*-3/*trans*-2-hexenal as the other tissues, but no significant difference was observed in the other cultivars. In all cultivars *cis*-3-henen-1-ol was least abundant in the flesh, below detectable levels for Angelle fruits. Significant differences were only apparent between the seeds and fluid of Genio fruit, where the fluid yielded approximately 60% more than the seeds. Of the C5 volatiles, 1-penten-3-one was highly cultivar specific, with Angelle fruits generating the most from flesh and fluid, Genio from flesh and seeds and Valkiria from seeds alone. Potentially this indicates a relationship with fruit size, with larger fruits generating higher proportions of 1-penten-3-one from the seeds/internal fruit environment than smaller fruits. A similar relationship is also observable in *trans*-2-pentanal and 1-penten-3-ol which derive from the enzymatic conversion of 1-penten-3-one (Petro-Turza, 1986, Zhang *et al.*, 2015). In addition, 1-pentanol is most abundant in seed tissues of all cultivars, which is significant, not showing the same over-expression in Angelle flesh as the other C5 compounds. This may be due to localisation of the relevant alcohol dehydrogenase to the seeds and parenchyma, however this is unconfirmed. Tomatoes contain approximately 25 times more linoleic acid than linolenic, with linoleic accounting for between 50-60% of the free fatty acid content (Guil-Guerrero and Rebolloso-

Fuentes, 2009). This relationship and concentration was also observed in tomato pomace, a by-product of tomato processing largely composed of seeds (Zuorro *et al.*, 2013). This is in agreement with the high concentration of hexanal that forms in tomatoes, as it is primarily derived from the sequential action of tomlox C and HPL on linoleic acid. The primary route of formation for the other C6 and C5 volatiles mentioned previously is through tomlox C and HPL cleavage of linolenic acid, which is far more substrate limited, which potentially explains the lower abundance of these volatiles. Interconversion of hexanal to *trans*-2-hexenal also occurs through the action of alcohol oxidoreductase/alcohol dehydrogenase, potentially explaining the higher abundance observed of the *cis*-3/*trans*-2-hexanal in these samples.

5.4.6 Sensory Analysis to Determine Preferred Sugar Acid Ratio

The importance of the ratio of sugar to acids and resulting sweet/sour balance in fresh tomatoes is one of the most powerful drivers of consumer preference. A number of studies have hypothesised that the improvement of commercial tomatoes and an increase in consumer acceptance can be primarily achieved by increasing the sugar/acid content (Jones and Scott, 1983, Stevens *et al.*, 1979). However, the ratio between sugars and acids, and therefore the sweet –sour balance of the fruits is debatably more important than the individual concentrations of each. It is difficult to determine what would constitute the optimal ratio of sweetness and sourness in tomatoes, mainly due to the variability in organoleptic preference and optimum intensities of each of the senses between consumers. However, altering the ratio of sweetness/sourness to increase acceptability and engagement of most of the population may be a valid method of improving consumer satisfaction with modern tomato cultivars.

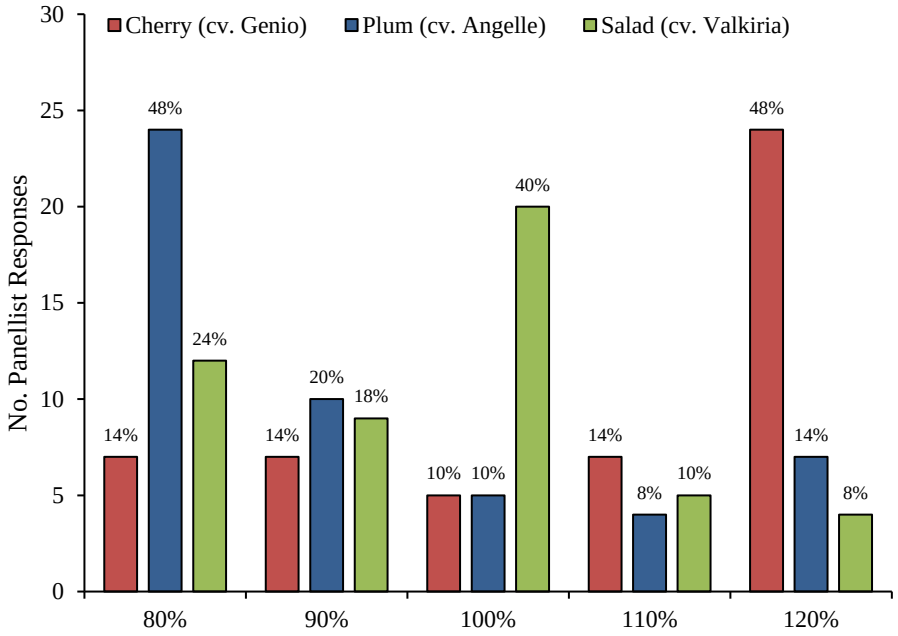


Figure 5.4 - Overall preference of simulated locular fluid samples containing between 80-120% of the native levels of citric and malic acids per cultivar. All solutions contained glucose and fructose at concentrations representative of native levels. Each solution also contained an equal aliquot of volatile spike to mimic tomato flavour and aroma. Data callouts represent the percentage of participants who preferred each sugar acid level (n=50).

The total preferred responses for each type of tomato solutions can be seen in **Figure 5.4**. The overall preference of participants differed based on the type of tomato the solutions were replicating. In cherry tomatoes a higher acid content was preferred by 48% of participants, suggesting that the taste suppression effect of acid on sugars was important to consumer perception, in solutions where there was a high sugar content. In plum tomatoes, the acidity was considered too high, with 48% of respondents suggesting that plum tomatoes with lower acid concentration would be more palatable than the native concentrations. The results were less significant in salad type tomato solutions, with only 40% of panellists suggesting that the current sugar/acid ratio was the most preferred. However, a combined 42% of respondents did suggest that lower acidity of between 10 and 20% would have improved the palatability of the solution. The results are less definitive in salad type as there is a lower amount of both hexoses and organic acids present, leading to a less intense taste overall.

These findings could be due to the high levels of acid natively present in the plum (*cv* Angelle) fruits used for this work, therefore this relationship would likely change in other plum tomato cultivars. The cherry (*cv* Genio) tomatoes had higher sugar content, but lower acids than the plum fruits suggesting that, after a certain threshold, increasing acidity negatively alters the flavour of the fruits, which is to be expected. However, based on the sensory analysis, the levels of sugar

and acids present in fresh tomatoes and the sensorial perception of sweetness and sourness are interlinked. Therefore, aiming to alter the content of either sugars or acids in relation to each other in fruits would lead to a reduction in palatability and consumer acceptance overall. This is because, in tomato, a certain level of acidity is required to reduce the overall sweetness of the fruit, to make it more palatable. By increasing either the sugars or acids, whilst not increasing the opposing one by as similar percentage, the balance would be modified and the fruits would taste either, over-sweet and bland, or sour and acidic. The sensory analysis of each tomato type was characterised by an incremental increase in sugar content. The results indicate that over-sweet tomatoes would not be well received and this further reinforces the concept that the taste suppression mechanisms which occurs in foods with high sugar/acid content are vital as sugar content increases. It has previously been demonstrated by Green *et al.* that there is a suppressive effect between sweetness and acidity at higher intensities, which helps to prevent either taste becoming overpowering and dominating the sensorial experience (Green *et al.*, 2010). Specifically, the presence of sweet compounds significantly reduced the acidity of binary sucrose/citric acid solutions. This effect was even more significant in tertiary solutions of sucrose/citric acid/sodium. The opposite effect, the suppression of sweetness by acidity, was also noted, but not to a significant degree. Over stimulation of gustatory and olfactory systems has been shown to reduce the total number of individual flavour-active components perceived. Therefore, in food systems, high levels of sweetness and/or acidity will have a suppressive effect on other flavour parameters.

5.5 Conclusions

Almost all previous chemical composition-based studies into the organoleptic properties of tomato utilise a whole tomato homogenate, as that is the best representation of the average sensorial experience of fresh tomato. This work indicates that this method may not adequately represent the complexity of tomato flavour and account for the mechanisms by which it is perceived during consumption. During consumption, full homogenisation of fruit tissue is only achieved flowing extensive mastication, so is not representative of the first few seconds of intense gustatory sensations which in tomato consumption, which are important to the impact of the sensory experience.

At this time there have been limited studies into the contribution of individual tissues of tomato fruit on the organoleptic properties during consumption. This appears to be the first instance where multiple gustatory sensations have been monitored alongside the complement of volatiles in these tissues of tomato fruits. In agreement with the initial hypothesis of the study, it has been confirmed that the locule fluid plays an essential role in multiple organoleptic aspects of tomato consumption, contributing the greatest sourness, umami and certain volatiles between the

analysed tissues as well as providing comparable sugar concentration to that found in the flesh. This importance of this is reinforced by the fact that the locular fluid is not bound by cell walls or membranes, allowing the fluid to immediately migrate into the taste pores on the tongue and to stimulate taste receptors, largely accounting for the immediate and intense sweet/sour taste that is characteristic of eating fresh tomatoes. Moreover, following the initial, intense gustatory sensation provided by the locular fluid, the prolonged and lingering gustatory experiences possibly derive from the sequential release of further sugars, acids and umami compounds from the flesh and seeds during mastication and cellular disruption. In addition, the volatile complement of the locular fluid may be more rapidly volatilised, due to the liquid state providing high surface areas for thermal transfer and thus enabling rapid temperature acclimatisation and subsequent retronasal detection of volatile aromas and, therefore, flavour. The importance of the locule fluid on the sensorial properties of fresh tomato may also explain why larger fruits which are often cut or prepared prior to use, are often less well liked than smaller fruited cultivars that are eaten whole. During the preparation of larger tomato fruits, large proportions of the locule fluid are probably lost following cutting, thereby dramatically supressing the initial burst of gustatory sensation that is so desirable in smaller fruits. This is compounded by the general decrease in sugar and acid content in these larger fruits, further lowering their sensory impact.

The sensorial study carried out as part of this work confirms the importance of the sugar acid ratio and also indicates that it is not absolute, but rather flexible based on the intensity of both sensations. Cultivars that presented higher concentrations of both sugars and acids, such as the baby-plum ‘Angelle’, could be improved by lowering the acidity slightly whilst maintaining higher sugar levels. The opposite is true of low sugar and acid cultivars such as the salad type ‘Valkiria’ which could be potentially improved by more acidity, due to the low gustatory impact of the cultivar as a whole. It can also be seen that this relationship is not absolute for all sensory participants, with small numbers disagreeing with the majority. This may indicate that breeding strategies aiming to achieve this altered sugar/acid balance may improve crops for most consumers, whilst moving new cultivars further from the sweet/sour composition desired by some other consumers.

5.6 Future Work

Quantification of monophosphate nucleotides would give a better understanding of the level of umami provided by each tissue. It is apparent from the amino acid data that significant umami will derive from the locular fluid, more so than the seeds themselves. However, certain nucleotides, primarily GMP and AMP in tomato, modify the way umami is perceived and therefore may change this proposed relationship.

Further investigations into the tissue distribution and composition of tomato fruits and identifying cultivars with higher percentages of locule fluid may help to understand this tissue further, together with its organoleptic properties and ways in which fruit can be manipulated through breeding. The benefit of higher proportions of locule fluid may be outweighed by a dilution of the taste-active compounds that are present, therefore methods to increase the amount of sugars/acids may improve the initial impact of tomato taste.

Further understanding of how impactful the initial 'burst' of locule fluid is to tomato flavour could be achieved, through the use of sensory analysis preference and intensity testing on tomato fruits with and without the locular fluid. The locular fluid accounts for 15-20% of the total fruit weight, so removal of the tissue would have to be counteracted by the addition of additional flesh. Ideally, this could be achieved by deconstructing tomatoes into solid components (flesh and seeds) and the fluid and then creating samples with different proportions of the locule fluid present. Samples without fluid that consisted of 100% flesh would probably present with significantly lower impact of sweetness and sourness upon consumption but, after a period of mastication, sugars and acids would be liberated from the flesh and seeds and the sweetness and acidity would probably increase.

6 Generation of Flavour Active Compounds During On-Truss Ripening of Three Cherry Tomato Cultivars

6.1 Chapter Abstract

The effect of fruit ripening on the synthesis of many of the most important flavour and taste-active components of fresh tomato were monitored using an on-truss design. Truss positions per cultivar were selected to give fruits at 'Mature Green', 'Breaker/Turning', 'Orange', 'Light Red', 'Red' and 'Table Ripe' stages. Ripening progression correlated with rising glucose, fructose, citrate, glutamate and aspartate, in each cultivar, all of which provide gustatory impact in fresh tomato. The relationship between these compounds and the routes of biosynthesis were explored using previously published findings, which showed the interlinked nature of their biosynthetic pathways, central to which was the combined glycolysis, citric acid cycle and GABA shunt. Additionally, increased production of hexanal, trans-2-pentenal, 1-penten-3-ol, 6-methyl-5-hepten-2-one, β-ionone, isovaleraldehyde and 2-isobutylthiazole was observed throughout the ripening process. The development of these aroma compounds at the later stages is indicative of their roles in creating appealing aroma and flavour, promoting fruit consumption and seed dispersal. Many proposed approaches to improving modern cultivars suggest increases in specific compounds, namely sugars/acids and certain volatiles, but based on the work discussed in this chapter, this may be difficult to achieve without having knock-on effects of related biochemical pathways and the generation of other quality-defining compounds.

6.2 Introduction

The fruiting bodies of flowering plants are as diverse as the plant species themselves; however, functionally they fill the same role of protecting developing seeds during maturation and dispersing fully mature seeds following the culmination of ripening. The process of ripening in fleshy fruits, such as tomato, is controlled by a multitude of genes throughout seed and fruit maturation, followed by ripening of the fruit itself. The full complement of biochemical changes during ripening are not comprehensively understood. However, it is a highly complex process that effects the entire organ of the plant. Seed development and dispersal are the primary goals of the plant, enabling them to propagate and survive as a species, but the method of seed dispersal can be very different between plant species. Some plants disperse their seeds directly upon death, reseeding with their offspring and fertilising the immediate area through decomposition of the parent plant (Klee and Giovannoni, 2011). Others use environmental methods of seed dispersal such as wind, water, transference by animals (externally) or direct physical expulsion from the plant. However, some plant species evolved to produce organs that are attractive to animals for ingestion and consumption, forming a synergistic relationship between the animal and plant (Giovannoni, 2004). Fruits that have developed for this purpose often have appealing colours, are soft and fleshy when ripe, contain flavour-active compounds and have good nutritional value which attracts animals and provides a benefit during consumption. Seeds are protected from digestion and may be excreted over much wider ranges than could be achieved by the plant itself, reducing competition between seeds and increasing the likelihood of survival of the offspring.

The appeal of fruit in the human diet stems from the desirable combination of macro nutrients, with high carbohydrate content for energy, minimal fat and a reasonable amount of protein (~3.5%). In addition, many fruits contain respectable levels of fibre, vitamins, minerals and antioxidants, which are important inclusions in the human diet (Guiné *et al.*, 2010). The composition of these nutrients is highly species-specific, with diverse nutritional profiles provided by different fruits or fruit tissues. In addition to the nutritional benefit of fruit consumption, most fruits present characteristic organoleptic properties that synergise with other food groups, allowing for the creation of dishes consisting of fruits alongside meats, grains, fats or various combinations of ingredients. The combination of sugars, acids, umami components and volatiles provide a highly desirable sensorial character that is well-liked by consumers (Guiné *et al.*, 2010). Both the nutritional content and the organoleptic profiles of most fruits change and improve throughout fruit ripening, with under-ripe, immature fruits being both nutritionally deficient and unpalatable. Therefore, the process of ripening of many different fruits has been extensively studied as it is intrinsically linked to the generation of quality in the resulting foodstuff. Due to the complexity of the ripening process of fleshy fruits, it is an essential step in understanding the

development of compounds known to influence the final quality of the crop. The biosynthetic pathways, precursor compounds and genes and enzymes responsible for the formation of such desirable end products is of significant interest in modern cultivar breeding and novel, genetic-led, crop improvement strategies (Tieman *et al.*, 2017, Klee and Giovannoni, 2011, Keurentjes, 2009, Giovannoni, 2004, Causse *et al.*, 2004). However, the reduction or lack of a compound may not only be due to the presence or absence of genes or enzymes responsible for its generation, but rather an interruption in a related biosynthetic pathway, potentially limiting the required precursor. Due to the significant biochemical shift in fruit composition during fruit ripening, much information can be gained about the generation of such important, quality defining compounds in fruits of differing ripeness. Different rates of production of quality-defining compounds at different developmental or ripening stages may indicate the activity of responsible enzymes, biosynthesis of precursors or presence of inhibiting conditions.

Commercially, the process of fruit maturation and ripening converts an inedible crop of limited value to a desirable food source and is therefore a pivotal step in adding significant value. At present, commercial tomato cultivation, including harvesting, uses visual assessment of fruit ripeness, coupled with predefined colour ranges, set by retailers, to determine the optimum harvest point for crops. This method presents several benefits and drawbacks. Predefined colour charts ensure that retailers only receive fruits within their, 'personalised' acceptable colour range, reducing the likelihood of product recalls and ensuring that fruit can be made available to consumers shortly after harvest. However, harvesting fruits too early, prior to full development, can hamper the generation of important quality-defining components and their related attributes, which has been previously demonstrated (Kader *et al.*, 1977, Betancourt *et al.*, 1977, Arias *et al.*, 2000b). Additionally, colour development in tomato fruits is rapid up until the orange/light red stage, following which, colour development slows. However, other biochemical processes, such as sugar, acid and umami generation and volatile potential may still be in flux during these later transitions, which would not be accurately represented in the fruit surface colour. Many cherry tomato cultivars are harvested, shipped and sold on-truss, as this is often perceived as being of higher quality to consumers and therefore adding value to the crop. Due to this practice, whole trusses are harvested at the same time, with obviously unripe fruits removed prior to sale. However, of the remaining fruits that are considered 'ripe' and suitable for sale, there may be significant compositional differences which lead to altered sensorial experiences during consumption.

6.3 Experimental Design

The ripening profile of various quality-determining compounds across three commercial cherry tomato cultivars was ascertained at 6 different truss positions per cultivar. The selected positions corresponded with the most commonly differentiated ripening stages of fresh tomatoes, as seen in **Table 6.1** below.

Table 6.1 – The truss position of fruits used in this study. Mean fruits per truss vary between cultivars, therefore different positions were selected to spread out the sampled fruit along the trusses. Final position was 12 for PIC, 14-16 for AXI and 14-17 for JUA, but was always classified as Mature Green.

		Position on Truss		
Sample Number	Ripening Stage	Piccolo (PIC)	Axiani (AXI)	Juanita (JUA)
1	Table Ripe (TR)	1	1	1
2	Red (R)	3	3	4
3	Light Red (LR)	4	5	6
4	Orange (O)	6	8	8
5	Breaker/Turning (BR/T)	8	11	10
6	Mature Green (MG)	Final	Final	Final

Examples of the fruit colouration and ripening profiles of each of the cultivars is displayed in **Figure 6.1**, with fruits used in this study annotated alongside. Ten replicate trusses per cultivar were used, all of which showed very comparable ripening progression and colouration gradient along the truss. The 'final' position was fruit 12 for each Piccolo truss, however Axiani and Juanita trusses were less predictable, setting more varied numbers of fruit. Therefore, the 'final' position for Axiani includes truss positions 14-16 and truss positions 14-17 for Juanita, depending on truss length.

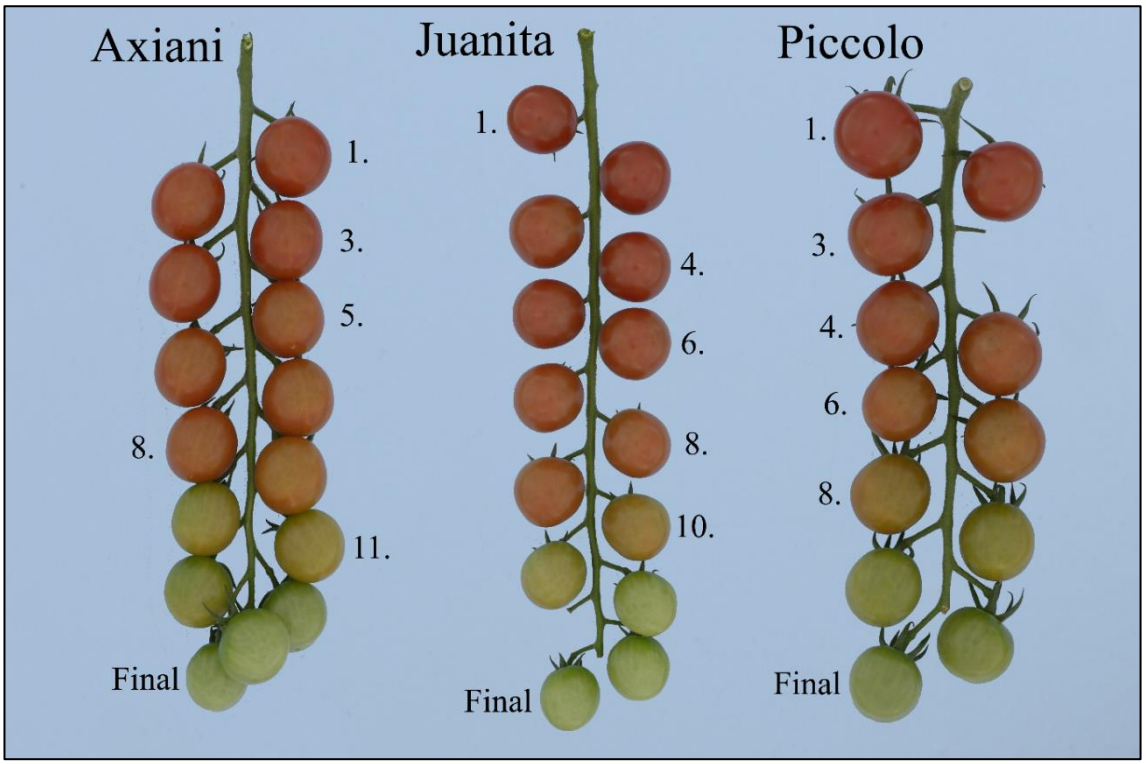


Figure 6.1 – Typical, on-truss ripening profile of Axiani (left), Juanita (middle) and Piccolo (right) fruits used in this study. Positions used for each cultivar are annotated and previously explained in **Table 6.1.**

Most often, studies into the compositional differences of ripening of tomatoes have been conducted on fruits that have been categorised visually as belonging to specific ripening stages (Raffo *et al.*, 2002, Gautier *et al.*, 2008, Sorrequieta *et al.*, 2010, Riley *et al.*, 1996, Akihiro *et al.*, 2008). However, the links between fruits of different ripening stages that occur on the same truss have not been well examined. In previous work by Arias *et al.* hydroponic tomato fruits *cv.* 'Laura' were shown to contain significantly higher amounts of various important compounds including ~30% more lycopene and 7.6% more soluble solids when ripened on-truss as opposed to those that had been removed, which agrees with previous findings from other studies' (Betancourt *et al.*, 1977, Arias *et al.*, 2000b, Kader *et al.*, 1977). Therefore, on-truss ripening is likely to be important to the continued accumulation of important photoassimilate precursors, such as carbohydrates and amino acids. Moreover, the competitive allocation of assimilate in sink tissues, in this case fruits on a truss, has been shown to be a function of fruit activity and respiration. Fruits higher on a truss develop first, and therefore, may sequester higher proportions of assimilate than later developing, less active fruits, indicating fruit position may be a factor in the total import during development and subsequent ripening (Bertin and Gary, 1990, Bertin, 1995). In addition, an on-truss design enabled standardisation of environmental conditions such as plant health and age, lighting/shading, heat and nutrient availability to a greater extent than using fruits from various trusses and plants.

6.4 Methods and Materials

Methodologies used in this chapter have been previously described in Chapter 2 of this thesis. These include colour analysis by DigiEye, sugar and organic acid quantification by enzymatic assay spectrophotometric detection, free amino acid determination by EZ:FAAST derivatisation and GC-MS analysis and the quantification and estimation of volatile composition by HS-SPME-TOF-MS. All statistical analysis was carried out using SPSS 24.

The presented data for hexanal and *cis*-3/*trans*-2-hexenal is semi-quantitative due to the overloading of the mass spectrometer due to high abundance. Lower sample weights did not alleviate this issue until only 100 mg was used, which gave very irreproducible results and resulted in most other volatiles being present at trace levels or entirely undetectable. Full quantification will follow using dilutions once validated. Additionally, the *cis*-3/*trans*-2-hexenal figure is a sum value of both compounds. It was proven that the SPME procedure and, specifically, the high temperature fibre desorption, resulted in *trans*-2-hexenal fully isomerising to *cis*-3-hexenal. This was replicated with 3 sets of brand new, unopened standards. Therefore, both compounds are represented within the observed *cis*-3-hexenal.

6.5 Results and Discussion

6.5.1 Visual Changes to Tomato Fruits Throughout Ripening

Visually, typical tomato fruits change drastically as ripening progresses, transitioning from vibrant green to a strong red colour which can be darker or lighter depending on cultivar. Maturing tomato fruits are green due to the presence of chlorophyll. It has been previously established that although maturing tomato fruits contain chlorophyll and are photosynthetically active, they do not significantly contribute to the entrapment of carbon dioxide in the way that primary source tissues such as mature leaves do. Rather, the photosynthesis within mature fruits appears to directly determine seed set and generation (Lytovchenko *et al.*, 2011). Following the formation and setting of seeds within 'Mature Green' MG fruits, chlorophyll begins to degrade, and chloroplasts are converted into pigment storing chromoplasts. During this degradation, the green colour of the fruits fade, leaving them briefly pale green/white before the fruit kick-starts the synthesis of pigments, primarily carotenoids. This paling of the fruit, up until approximately 10% of the surface is flushed orange, is recognised as the 'Breaker' stage, following which ripening can fully progress, even after removal from the plant. **Figure 6.2** below, shows the ripening colour gradient of the fruits used in this study. MG fruits, represented by dark green points, are the most different in terms of L*a*b* colour values, which is to be expected. Light green points represent the 'Breaker/Turning' (BR/T) class of fruits, which is the most variable stage of fruit ripening, as significant metabolic and biochemical changes are in progress. It has been previously shown that

the biosynthesis of many important compounds starts during the conversion to Breaker/Turning fruits, including the formation of some sugars, acids, amino acids, pigments, antioxidants and the potential for volatile synthesis, which will be further discussed later in this chapter (Takayama and Ezura, 2015, Sorrequieta *et al.*, 2010, Riley *et al.*, 1996, Raffo *et al.*, 2002, Gautier *et al.*, 2008, Bramley, 2002, Baldwin *et al.*, 1991b, Alexander and Grierson, 2002). Colouration of fruits following the 'Turning' stage, is driven by further synthesis of carotenoids, resulting in increasing positive a* values which correspond to red colours and slight lowering of b* values, due to the loss of blue colour present in the green chlorophyll and presence of more yellow tones provided by various carotenoids. The colour transition from 'Orange' (O) to 'Table Ripe' (TR) is relatively linear and significantly less extreme than that of the earlier ripening stages. It is apparent in **Figure 6.2** that there is a slight overlap of 2-3 samples between the 'Breaker/Turning' and 'Orange' classes, due to the difficulty in selecting trusses which showed the perfect, ripening profile in the designated fruit positions.

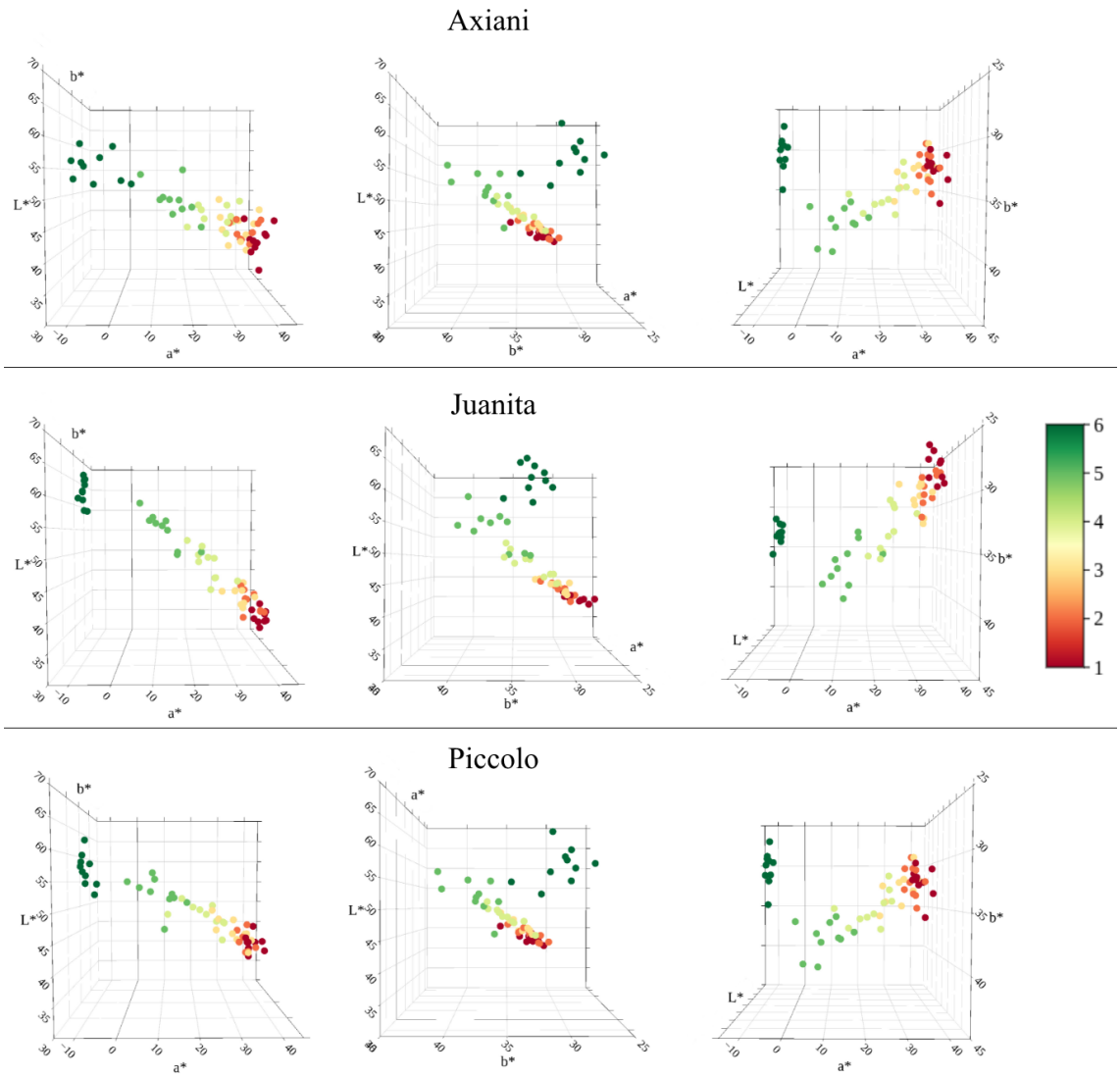


Figure 6.2 – Colouration of fruits at the selected positions on each of the trusses used in this study. Colour was determined using L*a*b* values. Marker colouration is indicative of ripening stage, not representative. Ripening stages are 6=Mature Green, 5=Breaker/Turning, 4=Orange, 3=Light Red, 2=Red and 1=Red Ripe, visualised green to red, respectively. Plots represent the 10 replicate trusses used per cultivar.

6.5.2 Development and Generation of Flavour-Active Compounds Throughout Ripening

From a commercial standpoint, ripening marks a rapid change in sensorial and nutritional quality of the fruits. Therefore, the development of flavour in ripening fruit is of paramount importance to the industry and consumers alike. As with most food products, the overall flavour of fresh tomatoes is dependent on the generation of at least 40 flavour-active compounds and the ratio of many of those to each other. Understanding the stages at which these compounds develop during ripening as well as determining whether there is scope for increasing the total quantity or manipulating the ratio of flavour-active compounds is a possible method for improving the flavour of new cultivars. In addition, due to the reliance of visual assessment of tomato ripening in commercial growing, the differences in fruit composition between later ripening stages may be difficult to predict based on visual inspection alone. Better understanding of the point at which

organoleptically important compounds are generated may inform retailers of expected limits of variability or be sufficient to indicate a change in fruit selection in the future. Of the 5 taste modalities, umami, sweetness and acidity are the most important in fresh tomato flavour. The amount of glutamate, aspartate and certain monophosphate nucleotides are responsible for the umami, glucose and fructose for sweetness and citrate and malate for acidity. In addition, the aroma and flavour profile of fresh tomato changes as the fruit develop and ripen, with significant changes in the content of multiple character impact volatiles. These changes are essential to the desirable flavour and taste of ripe fruits and therefore the progression of synthesis and better understanding of the limiting factors for their generation may be important targets for future crop improvement and genomic trials.

6.5.3 Shifts in the Amino Acid Profile of Ripening Tomato Fruits

Amino acids are one of the primary dry matter import targets for maturing tomato fruits, along with carbohydrates, primarily sucrose and organic acids (Valle *et al.*, 1998). During tomato fruit development, amino acids are translocated from source tissues such as roots and leaves, via the phloem, to the fruits. Valle, Boggio and Heldt measured the composition of phloem en-route to maturing tomato fruits, quantifying 14 amino acids, 46.1% of which was contributed by glutamine and glutamic acid (Valle *et al.*, 1998). Glutamine and glutamic acid both play important roles in the generation of many other amino acids, as well as driving the GABA, or γ-aminobutyric acid, shunt, which is closely associated with the citric acid cycle and a key component in both carbon and nitrogen metabolism (Umbarger, 1978, Sorrequieta *et al.*, 2010, Michaeli and Fromm, 2015). The free amino acid composition of the three studied cultivars can be seen in **Table 6.2**, **Table 6.3**, **Table 6.4** for Axiani, Juanita and Piccolo respectively. As expected, MG fruits of each of these cultivars amassed large proportions of GABA during fruit development. In agreement with the findings of Gallego *et al.*, GABA is present at higher concentrations than glutamic acid prior to the breaker/turning fruits. However, glutamine rather than GABA was the most abundant amino acid prior to the onset of ripening. This contrasts with the findings of Sorrequieta *et al.* who noted that GABA accounted for 40% of the free amino acids in MG 'Micro-Tom' fruits (Sorrequieta *et al.*, 2010). In the present work, GABA accounted for 11.9, 13.5 and 22.5% of the free amino acid content in mature green Piccolo, Axiani and Juanita respectively. The reasons for lower GABA concentrations are not immediately apparent, but may be either due to prior catabolism of GABA to feed the citric acid cycle and generate energy in the form of ATP and proteogenic amino acids which may be used for the synthesis of the enzymes responsible for the various biochemical shifts in ripening tomato fruit (Takayama and Ezura, 2015, Bouché and Fromm, 2004).

Table 6.2 – Free Amino Acid composition of Axiani tomato fruits at different stages of ripening. Data presented is mean of 10 replicate fruits ± standard deviation. Statistical significance was determined by Kruskal-Wallis H test and subsequent pairwise comparison Post-Hoc analysis. Significance accepted at the $p<0.05$ level following Bonferonni Correction for multiple comparisons. Significantly different pairs are indicated by the same alphabetical character, a lack of a character indicates no pairwise significance to other classes.

		Amino Acid Composition (µg/g Fresh Weight)															
Cultivar	Ripening Stage	ALA	GLY	VAL	LEU	ILE	THR	GABA	SER	PRO	ASN	ASP	GLU	PHE	GLN	LYS	TRP
Axiani	Table Ripe (TR)	83.1 ± 30.1	15.1 ± 4.2	26.2 ± 10.5 a	74 ± 29.1 a	32.2 ± 13.4 a	106.2 ± 35.9 a	301.8 ± 93.5 a	306.5 ± 98.8 a	111.2 ± 31.9 a,c	283.8 ± 117.4 a	1125.7 ± 240.4 a	3722.9 ± 1456.6 a	194.2 ± 48.2	1476.6 ± 473.7	123.6 ± 53.6	10.6 ± 3.2
	Red (R)	91.9 ± 30.3	15.3 ± 4	26.6 ± 10.3 b	77.3 ± 19.8 b	34.4 ± 13.4 b	124.7 ± 43.3 b	352.8 ± 86 b	320.5 ± 69 b	109.5 ± 39.5 b,d	307.9 ± 84.1	1214.8 ± 266.5 b	4483.9 ± 766.6 b,e	189.6 ± 42.9	1657.4 ± 543.8	142.7 ± 39.2	9.4 ± 2.2
	Light Red (LR)	83.8 ± 28.5	15.6 ± 4.2	29.4 ± 10.5 c	86.6 ± 29.8	37.5 ± 13.5 c	122.4 ± 37.3 c	451.9 ± 110.9 c	323.4 ± 93.4 c	83.2 ± 29.1	287.2 ± 114.5 b	1039.8 ± 196.4	3605.9 ± 1348 c	194.8 ± 46.9	1569.2 ± 446.3	139.3 ± 50.8	9.1 ± 3
	Orange (O)	71.7 ± 20.1	14.8 ± 2.7	28.8 ± 7.2 d	83.2 ± 17.7	37.8 ± 7.6 d	115.7 ± 30 d	630.7 ± 92.2 a	277.2 ± 89	69 ± 18.7	281.7 ± 82.7 c	924 ± 248.5	3532.4 ± 1049.1 d	180.2 ± 43.6	1449.8 ± 417.4	122.4 ± 37.7	7.8 ± 3.2
	Breaker/Turning (BR/T)	61.3 ± 13.3	15.7 ± 3.3	37.3 ± 12.6	90.4 ± 22.7	61.7 ± 21.2	184 ± 63.5	827.1 ± 123.3 a,b	346.1 ± 91.1	49.8 ± 15.2 a,b	417.1 ± 171	862.3 ± 223.7	2375.4 ± 836.9 e	174.9 ± 38.4	2010.8 ± 673.7	148.4 ± 55.7	8 ± 2.5
	Mature Green (MG)	59.5 ± 13.7	21.5 ± 6	99.5 ± 36.8 a,b,c,d	129.6 ± 32.3 a,b	130.7 ± 45.7 a,b,c,d	350.2 ± 114.9 a,b,c,d	1065.8 ± 222.7 a,b,c	502.5 ± 69.6 a,b,c	50.3 ± 15.5 c,d	677.9 ± 260.3 a,b,c	768.6 ± 195 a,b	644.4 ± 172.1 a,b,c,d	233.6 ± 72.7	2995.5 ± 1211	176.1 ± 70.5	13 ± 4.3

ALA Alanine, GLY Glycine, VAL Valine, LEU Leucine, ILE Isoleucine, THR Threonine, GABA γ-Aminobutyric Acid, SER Serine, PRO Proline, ASP Asparagine, ASP Aspartic Acid, GLU Glutamic Acid, PHE Phenylalanine, GLN Glutamine, LYS Lysine, TRP Tryptophan

Table 6.3 – Free Amino Acid composition of Juanita tomato fruits at different stages of ripening. Data presented is mean of 10 replicate fruits ± standard deviation. Statistical significance was determined by Kruskal-Wallis H test and subsequent pairwise comparison Post-Hoc analysis. Significance accepted at the $p<0.05$ level following Bonferonni Correction for multiple comparisons. Significantly different pairs are indicated by the same alphabetical character, a lack of a character indicates no pairwise significance to other classes.

		Amino Acid Composition (µg/g Fresh Weight)															
Cultivar	Ripening Stage	ALA	GLY	VAL	LEU	ILE	THR	GABA	SER	PRO	ASN	ASP	GLU	PHE	GLN	LYS	TRP
Juanita	Table Ripe (TR)	83.9 ± 17.6 a	14.8 ± 3.7	19.2 ± 6.4 a	55.1 ± 15.6	28.1 ± 10.9 a,b	86.6 ± 29.1 a	460.4 ± 89.2 a,b	202.3 ± 44.5	53.2 ± 19.4 a,b,c	185.7 ± 67.6 a	1117.4 ± 302.4 a,b,c	5524.4 ± 1319.3 a,b,c	136.6 ± 35.7	949.6 ± 295.1	146.2 ± 39.4 a	6 ± 2.6
	Red (R)	61.9 ± 16.5 b	12.3 ± 2.7 a	19.6 ± 12.3 b	50.1 ± 19.3 a	32.2 ± 20.8 c	98.4 ± 30.2 b	586.7 ± 149.4 c	160.5 ± 35.9 a	34.7 ± 8.5 d	182.7 ± 92.3 b	876.7 ± 235	3759 ± 1402.4 d	138.5 ± 36.9	1228.8 ± 568.1	114.9 ± 41.1	4.3 ± 1.2
	Light Red (LR)	62.1 ± 14.8 c	13.1 ± 2.8	19.8 ± 5.9 c	52 ± 12.7 b	32.3 ± 9.3 d	94.4 ± 34.1 c	652.4 ± 160 d	181.8 ± 31.3 b	35.8 ± 12.8 e	254.1 ± 126.7 c	854.2 ± 220.4	3947.2 ± 1049.9 e	129.2 ± 27.6	952.2 ± 301.4	137 ± 27.1	4.7 ± 2.4
	Orange (O)	44.5 ± 12.1	12.1 ± 2.4 b	21.2 ± 7.2 d	49 ± 12.7	45.8 ± 16.2	108 ± 31.8	669.8 ± 120.5 e	164.2 ± 51.8 c	25.5 ± 11.8 a	199.1 ± 103.8	667.1 ± 204.7 a	2831.4 ± 982.8 a,f	125.4 ± 29.5	1049.6 ± 519.3	117.2 ± 41.8	4.7 ± 2.4
	Breaker/Turning (BR/T)	52.9 ± 9.6	14.6 ± 2.4	31.9 ± 8.5	67.2 ± 13.9	55.9 ± 18 a	126.8 ± 45	933.1 ± 230.6 b	202.2 ± 38.9	16.8 ± 4.4 b,d,e	271.1 ± 67.9	652.7 ± 122.2 b	1565.6 ± 504.7 b	152 ± 51	1339.3 ± 326.2	129.9 ± 31.3	3.7 ± 1.2 a
	Mature Green (MG)	56 ± 12.9 a,b,c	18.7 ± 6 a,b	69.8 ± 20.6 a,b,c,d	79.7 ± 21.4 a,b	82.6 ± 28.1 b,c,d	174.9 ± 56.1 a,b,c	1203.1 ± 180.5 a,c,d,e	273.2 ± 52.8 a,b,c	23.4 ± 4.7 c	364.4 ± 104.1 a,b,c	561.2 ± 183.8 c	446.6 ± 91.4 c,d,e,f	172.3 ± 39.6	1727.5 ± 595.1	96.8 ± 27.4 a	7.1 ± 2.1 a

ALA Alanine, GLY Glycine, VAL Valine, LEU Leucine, ILE Isoleucine, THR Threonine, GABA γ-Aminobutyric Acid, SER Serine, PRO Proline, ASP Asparagine, ASP Aspartic Acid, GLU Glutamic Acid, PHE Phenylalanine, GLN Glutamine, LYS Lysine, TRP Tryptophan

Table 6.4 – Free Amino Acid composition of Piccolo tomato fruits at different stages of ripening. Data presented is mean of 10 replicate fruits ± standard deviation. Statistical significance was determined by Kruskal-Wallis H test and subsequent pairwise comparison Post-Hoc analysis. Significance accepted at the $p<0.05$ level following Bonferonni Correction for multiple comparisons. Significantly different pairs are indicated by the same alphabetical character, a lack of a character indicates no pairwise significance to other classes.

		Amino Acid Composition (µg/g Fresh Weight)															
Cultivar	Ripening Stage	ALA	GLY	VAL	LEU	ILE	THR	GABA	SER	PRO	ASN	ASP	GLU	PHE	GLN	LYS	TRP
Piccolo	Table Ripe (TR)	46.8 ± 17.8	8.3 ± 1.9 a	10.4 ± 7.4 a	24.3 ± 9.7 a,b,c	12 ± 6 a,b,c,d	6 ± 19 a	283.3 ± 103.9 a	243 ± 75.7	49.5 ± 11.2 a	375.6 ± 114.9 a	1397.1 ± 276.8 a,b,c	5556.4 ± 1431.5 a,b	151.7 ± 50.5	1886.7 ± 694.1	87.7 ± 23.9	6.6 ± 3.3
	Red (R)	64.3 ± 20.4	13.3 ± 3.6	22.2 ± 6.8	75.4 ± 15.7 a	29.4 ± 6.7 e	60.2 ± 42.9 b	335.7 ± 93.9 b	272.6 ± 86.5	60.1 ± 21.7 b,d,e	418.6 ± 142.4 b	1298 ± 198.9 d	5021.7 ± 1364.2 c	157.5 ± 35.1	2213.9 ± 460.9	153.3 ± 53	3.4 ± 2.2
	Light Red (LR)	56.9 ± 19.2	13.4 ± 4.1	20.7 ± 6.4 b	57.8 ± 26.4	32 ± 9.5 a	55.5 ± 51.4	412.1 ± 122.6 c	263.4 ± 91.1	46.7 ± 11.9 c	503.3 ± 165.2	1194.7 ± 223 e	4930 ± 760.9 d	133 ± 52.6	2235.7 ± 749	128.6 ± 48.8	4 ± 3.1
	Orange (O)	45.4 ± 17.3	13.2 ± 3.7	21.2 ± 10	79 ± 33.6 b	34 ± 12.3 b	59.3 ± 44.6	429.8 ± 132.9 d	259.2 ± 82.3	30.8 ± 11.1 d	545.3 ± 124.2	980.2 ± 182.8 a	3986.8 ± 548.2	112.7 ± 44.1	2001.3 ± 793.5	128.2 ± 38	2.6 ± 2.1
	Breaker/Turning (BR/T)	47.9 ± 19.4	12.4 ± 5.2	24.5 ± 9.4	62.7 ± 29.4	37.1 ± 15.8 c	79.6 ± 52.5	544.4 ± 104.2 a	292.6 ± 90	31.3 ± 9.2 e	633.1 ± 283.3	934.7 ± 297.8 b	3219.4 ± 728.8 a	129.6 ± 37.4	2377.5 ± 860.8	136.4 ± 45.5	3.3 ± 3.7
	Mature Green (MG)	46.8 ± 14.9	14.2 ± 3.5 a	44 ± 16 a,b	85 ± 33.3 c	64.1 ± 24.4 d,e	109.4 ± 67.2 a,b	803.5 ± 184 a,b,c,d	359.1 ± 118.1	15.8 ± 4.8 a,b,c	839.8 ± 383.4 a,b	780.1 ± 254.5 c,d,e	994.1 ± 483.8 b,c,d	129.7 ± 50.6	2346.5 ± 695.1	112.2 ± 33.8	2.1 ± 2.5

ALA Alanine, GLY Glycine, VAL Valine, LEU Leucine, ILE Isoleucine, THR Threonine, GABA γ-Aminobutyric Acid, SER Serine, PRO Proline, ASP Asparagine, ASP Aspartic Acid, GLU Glutamic Acid, PHE Phenylalanine, GLN Glutamine, LYS Lysine, TRP Tryptophan

6.5.3.1 The Biosynthesis and Catabolism of γ-Aminobutyric Acid (GABA) and Subsequent Synthesis of Important Amino Acids

GABA, or γ-aminobutyric acid, is a non-proteogenic amino acid found in a number of plants, and many animals, especially mammals (Takayama and Ezura, 2015). In plants GABA biosynthesis is also thought to be linked to biotic and abiotic stressors, namely drought or salinity stressing, extreme temperatures, hypoxia, phytohormones, soil pH imbalance, biological attack (viral, fungal or bacterial) and physical damage, including consumption by herbivores (Ramesh *et al.*, 2015, Shelp *et al.*, 1995). Furthermore, endogenous GABA concentration has shown an inverse relationship with root growth and proliferation (Renault *et al.*, 2013) and high levels of GABA have also been shown to disrupt the proper formation of pollen tubes in flowering plants, leading to infertility (Palanivelu *et al.*, 2003).

In tomatoes, GABA is of particular importance in the maturing and ripening fruits. It has been previously demonstrated that GABA biosynthesis is significantly more active during fruit maturation and that, by the mature green stage, GABA is the most abundant free amino acid (Sorrequieta *et al.*, 2010, Akihiro *et al.*, 2008). Upon the onset of ripening, this is followed by near complete catabolism to form some of the most abundant and important free amino acids in tomato fruit; glutamic acid, aspartic acid, glutamine and asparagine (Akihiro *et al.*, 2008, Takayama and Ezura, 2015). Biosynthesis of GABA in higher plants is primarily through the combined action of Tricarboxylic acid cycle (TCA) and the GABA shunt, which can be seen in **Figure 6.3** (Akihiro *et al.*, 2008, Takayama and Ezura, 2015, Baum *et al.*, 1996). Initially, glutamic acid is converted to GABA through the action of glutamate decarboxylate (GAD), releasing CO_2 (Baum *et al.*, 1996, Takayama and Ezura, 2015). Baum *et al.* noted that only GAD enzymes containing a calcium /calmodulin binding domain (CaCBD) had been found in plants. It was shown that an unbound CaCBD site has an inhibitory effect on the action of GAD, preventing GABA formation, but that the binding of Ca^{2+}/CaM blocks this inhibitory action (Takayama and Ezura, 2015). Akihiro *et al.* have previously isolated three genes responsible for the transcription of GAD from 'Micro-Tom', SlGAD1, SlGAD2 and SlGAD3. By suppressing each gene individually, plants synthesised significantly lower levels of GABA when SlGAD2 and SlGAD3 were suppressed. Additionally, suppression of all three genes in single plants resulted in just 10% of GABA when compared to the wild type (Akihiro *et al.*, 2008, Takayama and Ezura, 2015). GABA accumulation has been shown to accelerate in salt stressed plants and fruits stored in low O_2 or <10% CO_2 conditions postharvest. This indicates that SlGAD2 and SlGAD3 are affected by environmental stressors (Deewatthanawong *et al.*, 2010, Takayama and Ezura, 2015).

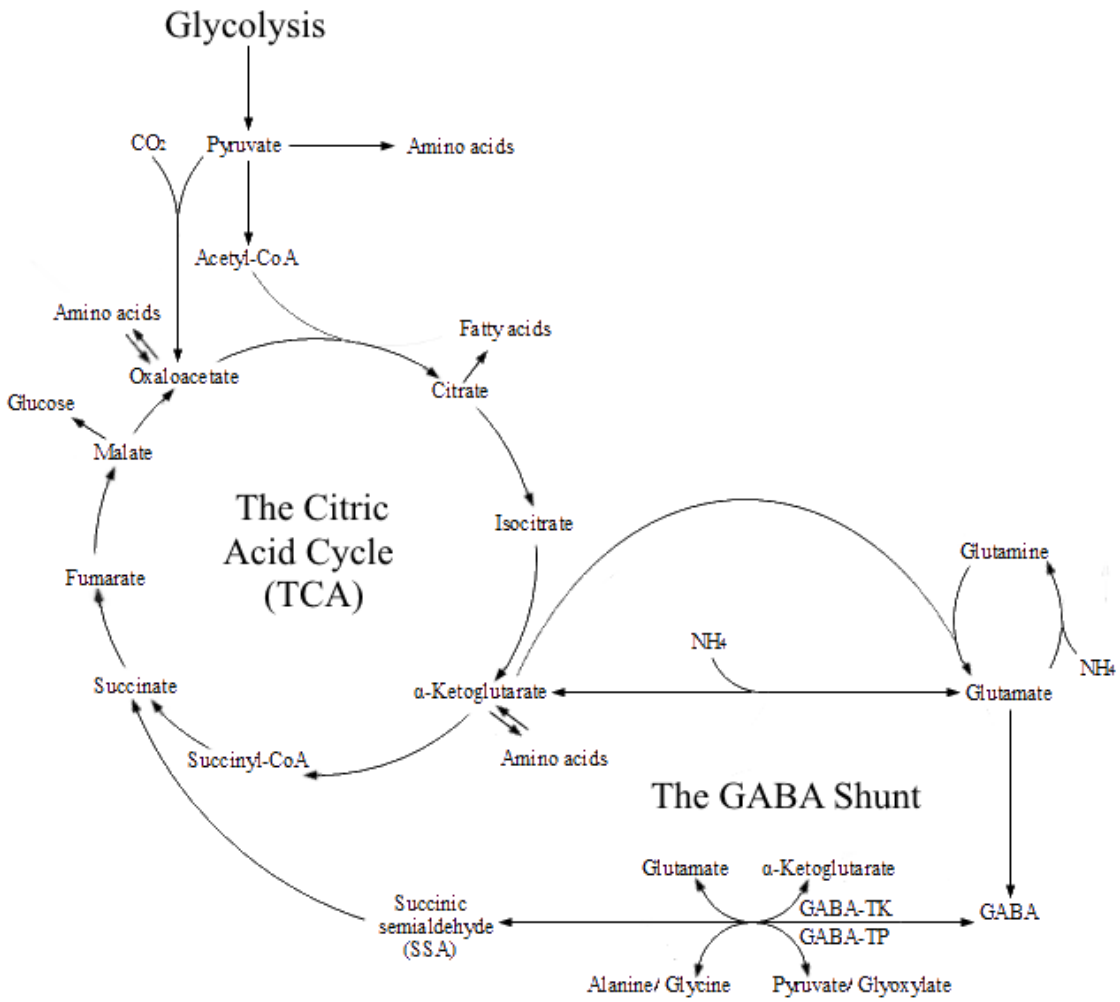


Figure 6.3 – Schematic representation of the interactions between the Citric Acid Cycle (TCA) and the GABA Shunt. The combined system plays a role in amino acid, lipid, organic acid and sugar synthesis. Adapted from (Voet and Voet, 2004, Takayama and Ezura, 2015).

Catabolism of GABA occurs through transamination by GABA-T enzymes, either GABA-TK which is dependent on α-ketoglutarate or GABA-TP which is pyruvate/glyoxylate dependant, generating alanine or glycine respectively. GABA-TK reactions involve the donation of an amino group to α-ketoglutarate, generating glutamate. GABA-TP reactions generate alanine using pyruvate or glycine using glyoxylate. Bouché and Fromm noted that although Palanivelu *et al.* demonstrated that *Arabidopsis* knockouts for GABA-TP had 100-fold increases in GABA content in the flowers, a corresponding increase in GABA concentration was not observed in other organs of the plant and therefore concluded that GABA-TP although present in plants, may be isolated to specific regions, such as the flowers (Bouché and Fromm, 2004, Palanivelu *et al.*, 2003, Takayama and Ezura, 2015). It has previously been demonstrated that tomato is one of the plants that has both GABA-TK and GABA-TP activity. Akihiro *et al.* have identified a significantly increased level of GABA-TK activity in tomato fruits after the breaker stage, which is contrary to many of the previous plant species studied in which GABA-TK is significantly lower than

GABA-TP activity (Akihiro *et al.*, 2008). This explains the characteristically high levels of glutamic acid in ripe tomato fruits, which is not seen in other fruits. Contrary to these findings, Koike *et al.* found that only GABA-T1, the mitochondrial enzyme, was active in tomato fruit through the use of knockout transgenic plants. Furthermore, the authors suggested that GABA-T1 to T3 action is active throughout the vegetative state of plant development, but that only GABA-T1 activity was significant in the flowers and fruits of the plant (Koike *et al.*, 2013).

The conversion of GABA to glutamate or its biosynthesis from α-ketoglutarate via the TCA and GABA shunt then enables the biosynthesis of aspartate and its derivatives. This widely studied route of formation occurs via transamination of glutamate to aspartate (Umbarger, 1978). Alternatively *de novo* synthesis of aspartate from pyruvate is also possible, and may be the route of formation prior to the onset of ripening, when glutamate is less abundant, as seen in **Table 6.2**, **Table 6.3** and **Table 6.4**. Aspartate is then responsible for the formation of asparagine, methionine, threonine, lysine and isoleucine. The concentration of aspartate and glutamate increase together throughout ripening, but aspartate accumulates to a much lesser extent than glutamate. In the studied cultivars the aspartate content increased between the Mature Green (MG) and Table Ripe (TR) stage by 54%, 79% and 99% for Axiani, Piccolo and Juanita fruits respectively. Due to its role in the synthesis of methionine, and to a lesser extent isoleucine, which utilises pyruvate as a precursor as well as a similar biosynthetic route as other pyruvate derived amino acids, aspartate is also important in volatile generation. Of volatiles that have been previously reported to influence the overall aroma and flavour or tomatoes, three have links to aspartate or derivatives; methional from methionine in addition to 2- methylbutanal/ol from α-ketomethylvaleric acid which is the direct precursor in isoleucine (Kochevenko *et al.*, 2012, Mayer *et al.*, 2008, Mathieu *et al.*, 2009).

6.5.4 Accumulation of Transitory Starch and Sucrose in Maturing Tomato Fruits and Subsequent Catabolism to Glucose and Fructose During Ripening

Sugars contribute up to 65% of total soluble solids content in ripe tomato. Each tomato fruit on a plant acts as a competing sink for the accumulation of photoassimilate, but the developmental stage and activity level of each fruit determines the proportion delivered to each sink (Yelle *et al.*, 1988). The activity level of a fruit is dependent on the processes involved in the import of carbon-rich compounds into maturing fruits; chiefly this encompasses transport and unloading of sucrose from phloem, hydrolysis of sugars and storage of carbohydrate as starch in maturing fruits (Ho *et al.*, 1982, Walker and Ho, 1977, Yelle *et al.*, 1988). Thereby, the more 'active' fruits will amass more photoassimilate and therefore generate higher levels of sugars when fully ripened. This suggests that proximal fruits on a truss are likely to contain higher assimilate and subsequently synthesised metabolites, including sugars, acids and amino acids, than more distal fruits at the same stage of ripening. The sugar content of ripe tomatoes primarily consists of glucose and

fructose, with minute traces of other sugars such as sucrose. Sucrose is present at higher concentrations in mature green tomato fruits, but is broken down into its constituents, glucose and fructose, during the ripening process (Schouten *et al.*, 2016, Damon *et al.*, 1988, Beckles, 2012).

Source-Sink relationships consist of two independent functions within the plant. The 'Source' tissues, primarily photosynthetically active tissues, such as mature leaves, where environmental CO_2 is transformed into sucrose, the primary carbohydrate photoassimilate (Osorio *et al.*, 2014). 'Sink' tissues can vary, but are primarily defined as tissues which cannot harness environmental carbon, this includes fruits, stems, roots or tubers etc. The interconnection between these processes may be essential to the understanding of the potential sugar accumulation in ripe fruits. It has been previously shown that tomato fruits act as the primary sinks for carbohydrate in fruiting plants, and that glucose and fructose contribute approximately 50% of the dry matter (Ho and Hewitt, 1986, Walker *et al.*, 1978, Ho, 1996). This is particularly prevalent in fruits between 10-40 DAA, during which time the maturing fruit import the majority of the dry matter present in fully ripe fruits. Indeed, final fruit size has been closely correlated to the amount of imported assimilates, and therefore increased importation of sucrose in developing fruits increases biomass of ripe fruits (Ho, 1996). However, Ho concluded that, although the amount of assimilation of sucrose plant-wide may limit overall carbohydrate transfer to fruits, the potential yield of the crop is more closely related to fruit number and mass (Ho, 1996). Carbohydrate translocation from the leaves to the immature fruits is in the form of sucrose, the primary carbohydrate photoassimilate in tomato plants. Following the transfer and accumulation of sucrose in immature fruits there is a transient build-up of starch, thought to act as a reservoir of accessible carbohydrate following maturation and throughout ripening (Schaffer and Petreikov, 1997). In addition, the conversion of sucrose to starch has been proposed as a method to maintain the steep sugar import gradient, enabling further, rapid import of carbohydrates into fruits which remain attached to the plant (Luengwilai and Beckles, 2010, Petreikov *et al.*, 2009, Beckles, 2012). In ripe fruits, the sucrose and starch are catabolised into glucose and fructose almost in entirety, with negligible sucrose or starch detected in ripe fruits (Wang *et al.*, 1993, Schaffer and Petreikov, 1997). The rate at which sucrose is imported to the fruits is correlated with the concentrations of sucrose available in the leaves (Walker *et al.*, 1978). It was proposed by Walker *et al.* that the enzymes that are responsible for the conversion of sucrose within developing fruits to hexose monosaccharides, namely acid invertase and sucrose synthase, are probably responsible for the regulation and control of importing sucrose to fruits. Increasing the rate of conversion of sucrose to hexoses could increase the total amount of sucrose imported to the fruits, therefore potentially increasing the total monosaccharide content following ripening. Wang *et al.* concluded that sucrose synthase was correlated to the import of sucrose into developing fruits, but that acid invertase, although involved in the production of glucose and fructose, was not rate limiting (Walker *et al.*, 1978).

Following the onset of ripening, the breakdown of sucrose and starch for the purposes of generating high concentrations of monosaccharides is an essential process in increasing the palatability, nutritional value and appeal of commercial tomato fruits. As previously discussed, the sugar content of tomato fruits is one of the most important factors in the overall liking, acceptance and saleability of tomatoes. Therefore, the generation of these metabolites, mechanisms of action and limiting steps are of significant interest with regards to crop improvement. The generation of monosaccharides throughout the ripening of Axiani, Piccolo and Juanita fruits was monitored and presented in **Table 6.5**. All three cultivars show dramatic sugar development throughout the ripening process, with a greater than 3-fold increase shown by Piccolo and Axiani fruits, and a ~2.3-fold increase in total sugars in Juanita. Similar development of reducing sugars was shown by Gautier *et al.* in 'Cervil' cherry tomatoes, with the authors reporting approximately a 3.7-fold increase between MG and TR fruits, which is very comparable with the 3.6-fold increase observed in Piccolo in this work (Gautier *et al.*, 2008). In contrast, Raffo *et al.* only observed a 27% rise in glucose and fructose concentration throughout ripening of 'Naomi' cherry tomatoes. It is worth noting, however, that the most under-ripe fruit analysed in their study was labelled as 'Green-Yellow', and therefore is likely to be more comparable to the 'Breaker/ Turning' fruits used in the current work (Raffo *et al.*, 2002). The reported values for glucose and fructose in 'G-Y' fruits are comparable to these findings, however there is significantly less sugar development in the following stages of ripening. Based on the development of sugars alone, it is apparent that the first three positions of each of the cultivars are largely indistinguishable, with 'Table Ripe' fruits containing slightly higher concentrations of both glucose and fructose than 'Red' or 'Light Red' fruits, although this rise was not significant. The most active phase of sugar generation for each of the cultivars was between 'Mature Green' and 'Light Red' fruits, after which the rate of generation slowed. This is probably due to substrate limiting as sucrose and starch stocks in the fruit are depleted. The ratio of glucose to fructose was between 0.7-1.0, apart from Piccolo 'Breakers' which amassed fructose at a faster rate than glucose, causing the ratio to drop to 0.6. The observed relationships between glucose and fructose generation throughout ripening are in agreement with those found previously, including the total sugars at the 'Table Ripe' stage (Raffo *et al.*, 2002, Gautier *et al.*, 2008, Baldwin *et al.*, 1991b). Following the catabolism of sucrose, glucose and fructose are liberated in equimolar amounts. However, the slight imbalance between fructose and glucose that is observed is probably due to the biological uses of both sugars. Glucose is widely consumed in various metabolic pathways, including aerobic respiration, glycolysis to generate pyruvate, ATP and NADH or incorporation into the synthesis of other compounds. Indeed, a proportion of glucose produced through sucrose and starch catabolism will be further incorporated into the Citric Acid Cycle (TCA), assisting in the generation of organic acids during fruit ripening. Fructose is also utilised as a substrate in several biosynthetic pathways, including glycolysis, but, its consumption is less ubiquitous than that of glucose.

6.5.5 The Citric Acid Cycle - Organic Acid, Consumption, Synthesis and Interconversion

Organic acids play important roles in modification of cellular pH, redox balance, generation and use of ATP and the donation of protons for membrane pumps and gradients (Igamberdiev and Eprintsev, 2016). Organic acids form primarily from the citric acid cycle or tricarboxylic acid cycle (TCA), acting as transient intermediates in carbohydrate catabolism. Moreover, the TCA is an essential biosynthetic pathway for the generation of a number of the metabolites that have been studied in this chapter. Compounds involved as substrates, intermediates or products of the TCA include sugars, organic acids and various amino acids, in addition to the various reactive intermediates and energy carriers such as ATP, NADH and Coenzyme A (CoA). The progression of ripening in tomato fruits marks the change in behaviour of the predominant organic acids, citric and malic. During fruit development, immature fruits import various forms of carbon organic acids being one such example (Walker and Ho, 1977). Throughout the maturation of tomato fruits, organic acid content increases, probably as methods of energy and carbon storage (Igamberdiev and Eprintsev, 2016). During ripening, organic acids are used as substrates for respiration and interconverted through the action of the TCA (Knee and Finger, 1992). The operation of the TCA in ripening tomatoes is flexible, able to operate as a closed system, which is the cyclic mode of metabolite generation. In this configuration, the metabolic cycle continues to utilise each product in the cycle as an intermediate in the subsequent step. However, the TCA can also 'open' into a non-cyclic pathway, controlled by 'valves' for malic and citric acid (Igamberdiev and Eprintsev, 2016).

Table 6.5 – Concentration of glucose, fructose, citric acid and malic acid present in the three analysed tomato cultivars at different stages of ripening. Ratios of between the pairs of sugars, acids and the total acids and sugars are also shown. Data presented is mean of 10 replicate fruits ± standard deviation. Statistical significance was determined by Kruskal-Wallis H test and subsequent pairwise comparison Post-Hoc analysis. Significance accepted at the $p<0.05$ level following Bonferonni Correction for multiple comparisons. Significantly different pairs are indicated by the same alphabetical character, a lack of a character indicates no pairwise significance to other classes.

Cultivar	Ripening Stage	Glucose (mg/g)	Fructose (mg/g)	Total Sugars (mg/g)	G/ F Ratio	Malic Acid (µg/g)	Citric Acid (mg/g)	Total Acids (mg/g)	C/ M Ratio	Sugar/ Acid Ratio
Axiani	Table Ripe (TR)	27.5 ± 5.3 a,b	29.1 ± 4.3 a,b	56.6 ± 8.5 a,b	1 ± 0.2	474.7 ± 73.9 a,b,c	7.2 ± 1.1 a	7.6 ± 1 a	15.6 ± 3.9 a	7.5 ± 1.2 a,b,c
	Red (R)	23.4 ± 3.8 c,d	25 ± 3.6 c	48.3 ± 6.5 c,d	0.9 ± 0.2	511.8 ± 101.1 d,e,f	6.9 ± 0.8 b	7.4 ± 0.8 b	14 ± 3 b	6.6 ± 1.3 d,e,f
	Light Red (LR)	23.8 ± 3.8 e,f	26.1 ± 2.3 d,e	50 ± 4.8 e,f	0.9 ± 0.1	670.6 ± 164.9	8.2 ± 1	8.8 ± 1	12.9 ± 3.6 c	5.7 ± 1 g
	Orange (O)	19.8 ± 4.5 g	20.9 ± 3.1	40.7 ± 6.9	0.9 ± 0.2	826.4 ± 170.6 a,d	11.1 ± 1.1 a,b,c	11.9 ± 1.2 a,b,c	13.9 ± 3 d	3.5 ± 0.7 a,d
	Breaker/Turning (BR/T)	14.1 ± 3.4 a,c,e	17.3 ± 2.4 b,e	31.5 ± 5.2 a,c,e	0.8 ± 0.2	878.1 ± 141.3 b,e	8.5 ± 1.5 d	9.4 ± 1.5	10 ± 2.4	3.4 ± 0.5 b,e
	Mature Green (MG)	8.9 ± 2.1 b,d,f,g	10.5 ± 2.5 a,c,d	19.3 ± 4.2 b,d,f	0.9 ± 0.2	923.4 ± 159.8 c,f	5.9 ± 1.8 c,d	6.8 ± 1.8 c	6.4 ± 1.8 a,b,c,d	3.1 ± 1.5 c,f,g
Juanita	Table Ripe (TR)	19.8 ± 3.8 a,b	23.9 ± 2.4 a,b	43.7 ± 5.3 a,b,c	0.8 ± 0.1	398.5 ± 62.4 a,b	5.5 ± 0.9 a	5.9 ± 0.9 a	14 ± 2 a,b	7.5 ± 1.6 a,b,c
	Red (R)	18.5 ± 3.1 c,d	22.9 ± 3.1 c,d	41.4 ± 5.7 d	0.8 ± 0.1	424.8 ± 73.2 c,d	5.7 ± 0.7 b	6.1 ± 0.7 b	13.9 ± 3.4 c	6.8 ± 1.1 d,e
	Light Red (LR)	15.5 ± 4.8 e	20.8 ± 3 e	38.4 ± 9.7 e	0.7 ± 0.2	485 ± 96.5 e,f	5.8 ± 1 c	6.3 ± 1.1 c	12.2 ± 2 d	6.3 ± 2.2 f
	Orange (O)	15.7 ± 3.3 f	17.4 ± 2.2	30.5 ± 6.5 a	0.8 ± 0.3	588.2 ± 92.5	9.4 ± 1.3 a,b,c,d	10 ± 1.3 a,b,c,d	16.5 ± 4.3 e,f	3.1 ± 0.8 a,d,f
	Breaker/Turning (BR/T)	11.2 ± 3.4 a,c	14.2 ± 2.7 a,c	29.4 ± 8.5 b	0.9 ± 0.3	825.6 ± 129.7 a,c,e	6.8 ± 1.3 e	7.6 ± 1.4 e	8.4 ± 1.9 a,e	4.1 ± 1.6 b,e
	Mature Green (MG)	8.5 ± 2.3 b,d,e,f	11.8 ± 1.5 b,d,e	20.3 ± 3.2 c,d,e	0.7 ± 0.2	886.4 ± 142 b,d,f	4 ± 1.4 d,e	4.9 ± 1.4 d,e	4.7 ± 1.8 b,c,d,f	4.6 ± 2.2 c
Piccolo	Table Ripe (TR)	25.6 ± 5.1 a,b	29.7 ± 4.7 a,b	55.3 ± 8.4 a,b,c	0.9 ± 0.1	408.7 ± 68.7 a,b,c	6.7 ± 1.2 a	7.1 ± 1.2 a	16.8 ± 3.8 a,b	8 ± 1.9 a,b,c
	Red (R)	22.5 ± 5.8 c,d	24.7 ± 4 c,d	47.2 ± 9.2 d,e	0.9 ± 0.2 a	456.8 ± 54 d,e	6.8 ± 1.1 b	7.2 ± 1.1 b	15 ± 3.2 c	6.6 ± 1.4 d,e,f
	Light Red (LR)	17.2 ± 4.5 e	23.3 ± 3.9 e	40.5 ± 7.3 f	0.7 ± 0.2	514.4 ± 99.1 f,g	7.3 ± 1.4	7.8 ± 1.3	14.8 ± 4.5 d	5.4 ± 1.7 g
	Orange (O)	15.7 ± 3.5	19.7 ± 2.6	35.4 ± 5.7 a	0.8 ± 0.1	696.4 ± 105.1 a	10 ± 1.9 a,b,c	10.7 ± 1.9 a,b,c	14.7 ± 3.6 e	3.3 ± 0.5 a,d
	Breaker/Turning (BR/T)	10.4 ± 3.3 a,c	16.8 ± 2 a,c	27.2 ± 4.9 b,d	0.6 ± 0.2 a	821.3 ± 159.2 b,d,f	8.3 ± 1.3 d	9.1 ± 1.4 d	10.4 ± 2.5 a	3 ± 0.7 b,e
	Mature Green (MG)	7 ± 1.9 b,d,e	9.9 ± 3 b,d,e	16.5 ± 3.7 c,e,f	0.7 ± 0.3	884.5 ± 138 c,e,g	5.1 ± 1.6 c,d	6 ± 1.5 c,d	6 ± 2.5 b,c,d,e	3 ± 1.2 c,f,g

During operation of the open TCA, citric and malic acids can be exported from the metabolic pathway, preventing their consumption and allowing for them to be transported out of the mitochondria and to other, subcellular, storage locations, specifically the vacuole (Igamberdiev and Eprintsev, 2016). Conversion to the incomplete 'open' TCA occurs when the redox level increases, allowing for accumulation of acidic species to counteract the increase redox potential of the environment. The TCA 'valves' are mechanisms by which malic and citric acids can be sequestered or diverted to other biochemical pathways. This includes the conversion of citric acid to α-ketoglutarate via isocitrate, enabling an acceleration in the generation of amino acids (Igamberdiev and Eprintsev, 2016).

The concentrations of both citric and malic acid in ripening Piccolo, Axiani and Juanita fruits can be seen in **Table 6.5**. There was an obvious decline in cellular malic acid between MG and TR fruits of each of the three cultivars. More than 50% of the malic acid present in MG fruits was sequentially catabolised throughout the progression of fruit ripening. The rate at which endogenous malic acid decreased was universal across each of the cultivars studied, with a final concentration at full fruit ripeness of between ~400-475 µg/g fresh weight, which is in agreement with the previous findings presented in Chapter 4. Citric acid behaved differently to malic acid, with rapid accumulation between MG and O fruits, followed by a reduction in LR, R and TR fruits. Between MG and TR fruits there was an approximate rise of 22%, 31% and 37% in Axiani, Piccolo and Juanita fruits respectively. Axiani fruits had the highest concentration of citric acid at MG and TR, but showed the least development. In contrast, Juanita fruits displayed the opposite, presenting with little citric acid at MG (4 mg/g FW), but proportionally increasing, by the most during ripening. This may indicate a level of product-based inhibition on citric acid accumulation, whereby higher concentrations within the fruit inhibit or slow the rate at which further accumulation takes place, potentially as a method of preventing over-acidification of cells which may disrupt other biochemical processes and lead to developmental issues in the ripening fruits. The ratio of the generation of sugars and acids throughout ripening appears to be relatively similar between the cultivars, with sugar/acid ratios of 7.5 for both Axiani and Juanita and 8.0 for Piccolo. This is in agreement with previous sensory analysis data presented in Chapter 4, whereby Piccolo is considered sweeter than the other two cultivars, and Axiani is more acidic, which acts as a counter to the sweetness. Although the ratio of sugars/acids is the same between Axiani and Juanita fruits, the absolute values of these components are not, with Axiani fruits containing approximately 30% more sugars and acids than those present in Juanita. Piccolo and Axiani fruits accumulated very comparable concentrations of sugars. Nevertheless, it is the decrease in fruit acidity is that separates the cultivars, both in terms of chemical composition and sensorial impact.

The composition of these two acids presented in **Table 6.5** is in agreement with the literature, with citric acid predominant over malic in ripe fruits. Variance in the progression of

accumulation/breakdown of these acids in ripening fruit has been also previously reported. Most authors agree that malic acid accumulates throughout fruit maturation, with a subsequent and continued decrease during fruit ripening (Baldwin *et al.*, 1991b, Knee and Finger, 1992, Davies *et al.*, 1981). The journey of citric acid in relation to ripening progression is less apparent. Citric acid has been previously demonstrated to increase, decrease and be unchanged between MG and TR fruits of various cultivars (Baldwin *et al.*, 1991b, Davies, 1966, Knee and Finger, 1992, Thorne and Efiuvwevwere, 1988). Thorne and Efiuvwevwere monitored the generation of organic acids in tomato fruits stored and ripened at different temperatures. It was found that fruits chilled at 5 and 7 °C, accumulated citric acid, whereas those at 12 or 19 °C were unchanged or decreased following 9 days of ripening, a trend that continued for the full 21 days of monitoring. The authors did not observe the same trend with malic acid, which decreased over the course of full experiment. This indicates that the accumulation of citric acid can be influenced by environmental conditions, such as fruit temperature, but malic acid is less subject to external interference. Carrari *et al.* on the other hand, showed a rapid decrease in citric acid content from fruit set, development and maturation until the breaker stage, at which point there is a spike in citric acid content which gradually decreases as the fruit fully ripen (Carrari *et al.*, 2006). Moreover, Davies, Hobson and McGlasson also showed an increase from 1.9 to 2.3 mg/g FW between mature green and 'green-yellow' fruits, eventually decreasing to 2.0 mg/g FW at full fruit ripeness. Both these profiles are very comparable to that observed in the current work, i.e. the rapid accumulation at the 'Breaker' transition and subsequent decrease at the culmination of ripening. The reasons for citric acid development varying to such a significant degree are not immediately apparent. The trends observed here and by Carrari *et al.* and Davies, Hobson and McGlasson all utilised fruits that were ripened on-plant and then harvested for chemical analyses, whereas Thorne and Efiuvwevwere experimental design required off-plant ripening under various controlled conditions. Potentially, early removal of trusses/fruits from the plants removes the possibility for additional carbohydrate import, which, in-turn, starves the TCA cycle of glucose derived pyruvate. In this case, lower activity of the TCA cycle would continue to use the malate present in matured fruits, but would likely not assimilate additional citric acid via the citrate 'valve' of the incomplete TCA, with more resources directed to amino acid synthesis.

In fully ripened fruits malic acid accounts for between ~5-30% of the total organic acid content, highly dependent on cultivar and tomato type (Baldwin *et al.*, 1991b, Raffo *et al.*, 2002, Rosales *et al.*, 2011, Anthon *et al.*, 2011, Schouten *et al.*, 2016). Larger fruited cultivars, including 'salad' and 'beefsteak' varieties, amass a greater proportion of malic acid as compared to smaller fruited cultivars, both on a per gram basis and as a proportion of total acids. The reason for preferential malic acid production and suppressed citric acid generation in larger fruit types does not appear to have been well explained. Although the biosynthesis of both citrate and malate are interlinked, both have different roles in fruit development and function, beyond solely pH regulation and

sensorial impact. Malate has been previously shown to be integral to proper starch biosynthesis in maturing fruits (Centeno *et al.*, 2011). Centeno *et al*. showed that, through the use of antisense, transgenic tomatoes, suppression of malate synthesis during tomato development led to increase in the conversion of imported sucrose to starch. The reason for this was attributed to a reduction in the redox state of fruits, with reduced organic acids available to modify the cellular pH, leading to reduction of AGPase activity in the plastids. The opposite effect was shown in a transgenic line with increased malate dehydrogenase (MDH) activity and concomitant increase in malate production. Increased malate led to a reduction in both starch accumulation and resulting sugars at full ripeness (Centeno *et al.*, 2011). As previously discussed, reduced conversion of sucrose to starch would lower the import gradient for further carbohydrate accumulation, potentially reducing the total available sugars following ripening. Interestingly, increased malate accumulation in larger fruited cultivars may also lead to slower/reduced import of carbohydrates, partially accounting for the lower sugar content in the ripe fruits of such cultivars. Unfortunately, this study focused solely on the progression of ripening in cherry tomato cultivars and has therefore been unable to further examine this phenomenon in detail. In addition, the Centeno *et al.* also noted a decreased resistance to both *B. cinerea* and opportunistic pathogenic infection during fruit over-ripening. Although the mechanism behind the gain/loss of resistance in relation to malate levels was not fully determined, previous claims that cell wall degradation was the cause of the resistance increase was not considered to be responsible in this case (Cantu *et al.*, 2008, Saladié *et al.*, 2007).

6.5.6 Generation of Volatile Components of Tomato Flavour and Aroma During Ripening

The changes in volatile production in ripening tomato fruits are directly tied to the requirement of seed dispersal by the plant. Many of the volatiles that improve the palatability and appeal of tomato fruits are significantly lower in under ripe fruits (Rambla *et al.*, 2014, Simkin *et al.*, 2004, Speirs *et al.*, 1998, Baldwin *et al.*, 1991b). Indeed, it is possible that the complement of volatiles produced by various plant species from nutritionally valuable compounds has evolved as a method of encouraging consumption of fruits and seeds by signalling to the natural consumers of the plants the presence of a rich nutrient source (Rambla *et al.*, 2014). The majority of flavour-active volatiles, formed from amino acid and fatty acid catabolism, are not present at significant levels in under ripe fruits due to limited precursors and compartmentalisation of required enzymes (Baldwin *et al.*, 1991b). Klee proposed that subcellular compartmentalisation and separation of substrates and the enzymes responsible for their catabolism to generate flavour active volatiles occurs to a greater extent in under-ripe fruits. Cell wall loosening is more apparent in the later stages of fruit ripening, therefore compartmentalised enzymes and precursors have greater opportunity to interact following the relaxation of cellular structure. The same process occurs upon tissue disruption or damage during fruit preparation or consumption, which generates

a complement of desirable, flavour-active volatiles (Klee, 2010, Rambla *et al.*, 2014). Substrate limitations apply to almost all volatiles that are present at above their odour detection threshold in ripe fruits. The concentration of many of the volatiles most important to tomato flavour produced throughout the ripening of Axiani, Juanita and Piccolo fruits can be seen in **Table 6.6** and **Table 6.7.**

Table 6.6 – Volatile compounds (part 1) produced by Axiani, Juanita and Piccolo fruits at the six different ripening positions used for this study. Concentrations represented as ppb. Data presented is the mean of ten replicate fruits per position ±SEM. Significant differences were determined using Kruskall-Wallis-H test with subsequent pairwise comparisons. Significance accepted at the $p<0.05$ level following Bonferroni correction for multiple comparisons.

Cultivar	Ripening Stage	Isovaleraldehyde	1-Penten-3-one	Hexanal	cis-3-Hexenal	6-Methyl-5-hepten-2-one	cis-3-Hexen-1-ol	2-Isobutylthiazole	Methyl Salicylate	β-Ionone
Axiani	Table Ripe (TR)	52 ± 8.1 a	38.8 ± 10.5	2520.5 ± 155 a,b,c	1375.2 ± 102.4	74.8 ± 21.2 a	61.6 ± 16	98.3 ± 24.2 a	38.5 ± 4.6	17.1 ± 0.3
	Red (R)	50.4 ± 8.4 b	47.6 ± 10.8	2326.7 ± 260.2 d	1403.2 ± 155.4	50.8 ± 14	49.3 ± 13.7	79.2 ± 17.3 b	43.6 ± 4.2	17 ± 0.1
	Light Red (LR)	53.3 ± 9 c	37.8 ± 9.5	2180.1 ± 235.4 e	1585.9 ± 102.4	43.7 ± 13.1	46 ± 8.7	50.7 ± 11.7	39.6 ± 4.3	16.5 ± 0.1
	Orange (O)	41.1 ± 7.6	34.5 ± 7	1214.1 ± 201.2 a	1067.2 ± 156.5	30.8 ± 8.7	56.7 ± 16	17.4 ± 3.9 a,b	44 ± 3.7	16 ± 0
	Breaker/Turning (BRT)	21.1 ± 3.5	38.8 ± 7.5	1198.4 ± 174 b	1460 ± 120.1	16.4 ± 4.2	49.6 ± 9.6	N.D.	38.6 ± 4.3	N.D.
	Mature Green (MG)	12.3 ± 1.7 a,b,c	28.5 ± 6.7	532.8 ± 118 c,d,e	1343.7 ± 226	9.5 ± 1.8 a	45.1 ± 7.9	N.D.	34.9 ± 4.8	N.D.
Juanita	Table Ripe (TR)	295.6 ± 36 a,b,c	64.7 ± 5.6	2239.3 ± 109.5 a,b	1843.3 ± 69	207.1 ± 32.4 a,b	42.6 ± 1.5	195.3 ± 38.8 a,b	32.5 ± 4.5	17.3 ± 0.5
	Red (R)	197.8 ± 26.1 d,e	65.7 ± 4.8	2115.4 ± 129.5 c,d	1843 ± 100.1	175.8 ± 31.5 c,d	44.9 ± 1.8	70.3 ± 21.4	25.4 ± 0.7	16.8 ± 0.1
	Light Red (LR)	188.9 ± 22.9 f,g	58.1 ± 3.9	1928.4 ± 103.1 e	1776.8 ± 70.8	142.3 ± 20.9 e,f	42.4 ± 1.1	35.2 ± 5.9 a	24.3 ± 0.7	N.D.
	Orange (O)	84.3 ± 12.7 a	58.1 ± 3.9	1742.8 ± 94.4	1743.4 ± 96.9	78.1 ± 15.2	44.5 ± 2.4	15.6 ± 5.2 b	N.D.	N.D.
	Breaker/Turning (BRT)	24.8 ± 3.5 b,d,f	51.6 ± 7.3	1275.5 ± 131 a,c	1726.5 ± 141	25.4 ± 3.9 a,c,e	44.2 ± 4	N.D.	34.8 ± 4.7	N.D.
	Mature Green (MG)	11.5 ± 1.7 c,e,g	53.1 ± 2.7	1043.3 ± 60 b,d,e	1723 ± 129.9	14.1 ± 0.6 b,d,f	60.4 ± 11.2	N.D.	39.9 ± 4.3	N.D.
Piccolo	Table Ripe (TR)	81.3 ± 11.7 a,b	59.4 ± 6.2	1660.7 ± 99.5 a,b	1526.6 ± 141.6	124.4 ± 7.7 a,b,c	38.5 ± 2	66.1 ± 7.3 a	33.6 ± 4.8	16.7 ± 0.1
	Red (R)	70.1 ± 13.4 c	57.5 ± 6.3	1428.4 ± 189.5 c	1495.9 ± 178.7	104.5 ± 12.6 d,e	62 ± 22.7	48.6 ± 6	31.1 ± 4.6	N.D.
	Light Red (LR)	80.4 ± 13.6 d,e	52.7 ± 3.2	1446 ± 97.3 d	1482.9 ± 127.3	104.4 ± 13.4 f,g	41.4 ± 2.9	43.1 ± 6.6	34.1 ± 4.8	N.D.
	Orange (O)	52.4 ± 6.1 f	53.8 ± 2.9	1242.1 ± 72.2	1441 ± 90	53.4 ± 6.1 a	44.6 ± 3.8	27.5 ± 6 a	21.8 ± 0.2	N.D.
	Breaker/Turning (BRT)	27.3 ± 2.2 a,d	60.4 ± 3.5	1073.7 ± 101.9 a	1710.6 ± 124.4	30.1 ± 2.1 b,d,f	48.6 ± 3	36.6 ± 0.3	28.4 ± 0.1	N.D.
	Mature Green (MG)	12.7 ± 0.6 b,c,e,f	48.4 ± 3.6	593.3 ± 104.1 b,c,d	1215.1 ± 201.1	14.3 ± 0.5 c,e,g	63.8 ± 8.1	N.D.	28.3 ± 4.2	N.D.

Table 6.7 – Volatile compounds (part 2) produced by Axiani, Juanita and Piccolo fruits at the six different ripening positions used for this study. Concentrations represented as ppb. Data presented is the mean of ten replicate fruits per position ±SEM. Significant differences were determined using Kruskall-Wallis-H test with subsequent pairwise comparisons. Significance accepted at the $p<0.05$ level following Bonferroni correction for multiple comparisons.

Cultivar	Ripening Stage	trans-2-Pentanal	1-Penten-3-ol	1-Pentanol	Nonanal	trans-2-Octenal	6-Methyl-5-hepten-2-ol	trans,trans-2,4-Heptadienal	Linalool	cis-Geranylacetone	Benzyl Alcohol
Axiani	Table Ripe (TR)	31.5 ± 5.7	62.6 ± 14.9	38.1 ± 8.4	18.8 ± 5	7.7 ± 1.2	1.2 ± 0	9.7 ± 2.4	15.4 ± 0.3	N.D.	26 ± 6.4
	Red (R)	30.2 ± 5.3	58.5 ± 13.2	32.6 ± 5.7	20.6 ± 5.1	6.6 ± 1.1	N.D.	8.9 ± 2.5	13.5 ± 1.4	N.D.	20.4 ± 3.1
	Light Red (LR)	27.9 ± 4.4	54.3 ± 11.1	33.6 ± 4.7	19.5 ± 5.1	5.7 ± 1.1	N.D.	9.1 ± 2.6	13.5 ± 1.5	N.D.	17.5 ± 2.1
	Orange (O)	19.3 ± 2.7	36.3 ± 7.7	33.1 ± 9.3	21.5 ± 5	3.9 ± 2.1	N.D.	10.8 ± 2.7	13.4 ± 1.4	N.D.	28.2 ± 7.8
	Breaker/Turning (BRT)	21.5 ± 2.7	31.2 ± 5.8	28.4 ± 5.8	19.6 ± 5.2	5.9 ± 1.9	N.D.	11.8 ± 2.3	13.8 ± 1.2	N.D.	15 ± 1.8
	Mature Green (MG)	17.1 ± 2.1	18.6 ± 3.3	33.4 ± 6.5	21.4 ± 5.1	N.D.	N.D.	10.7 ± 2.4	15.1 ± 0.7	N.D.	17.9 ± 0
Juanita	Table Ripe (TR)	36.9 ± 3.4	71.5 ± 6.3	40.3 ± 2.6	9.1 ± 2.1	9.7 ± 2.1	1.6 ± 0	4.4 ± 1.2	N.D.	N.D.	41.4 ± 11.5
	Red (R)	35.3 ± 3.2	71.6 ± 6.3	37.8 ± 2.4	12.2 ± 3.6	6 ± 2.2	1.3 ± 0.1	4.8 ± 1.3	N.D.	N.D.	59 ± 8.8 a
	Light Red (LR)	31.5 ± 3.1	62.1 ± 6.8	35.3 ± 3	7.9 ± 2.2	5.3 ± 1.9	N.D.	3.9 ± 1.3	N.D.	N.D.	66.3 ± 10.7 b
	Orange (O)	29.5 ± 4.3	54.7 ± 7.5	33.5 ± 5.1	4.1 ± 1.5	6.6 ± 2.9	N.D.	3.6 ± 0.9	N.D.	N.D.	47.2 ± 4.1
	Breaker/Turning (BRT)	27.1 ± 3.3	46.6 ± 8.6	32.2 ± 4.4	15.4 ± 4	12 ± 0.3	N.D.	5.3 ± 1.8	11.3 ± 2.2	N.D.	16.7 ± 3.4 a,b
	Mature Green (MG)	22.5 ± 2.7	54.6 ± 6.9	53.3 ± 13.2	3.6 ± 1.2	8.7 ± 1.8	N.D.	3.4 ± 0.8	N.D.	N.D.	N.D.
Piccolo	Table Ripe (TR)	23.6 ± 6.1	54.5 ± 8.6 a	18.6 ± 4	N.D.	1.3 ± 0.1	N.D.	2.5 ± 0.1	17.6 ± 1.7	83.1 ± 23.2	89.5 ± 11.2
	Red (R)	24.4 ± 4.7	56.3 ± 7.4 b	21.8 ± 2.5	N.D.	0.5 ± 0.1	N.D.	2.4 ± 0.2	16.5 ± 2.9	73.1 ± 15.9	104.1 ± 14 a,b
	Light Red (LR)	19.2 ± 4	45.6 ± 6.2	16.8 ± 3.3	N.D.	N.D.	N.D.	2.7 ± 0.1	25.4 ± 3.5	119.1 ± 35	113.7 ± 14.8 c,d
	Orange (O)	15.9 ± 4.4	37.2 ± 4.3	14.5 ± 2.8	N.D.	N.D.	N.D.	2.3 ± 0.2	24 ± 5.4	61.9 ± 12.9	88.5 ± 14.5
	Breaker/Turning (BRT)	20.4 ± 4.5	39.4 ± 6	20.5 ± 4.4	N.D.	N.D.	N.D.	2.6 ± 0.2	33.8 ± 7.1	125.8 ± 37.8	46.2 ± 9 a,c
	Mature Green (MG)	18.4 ± 2.4	19.9 ± 3.7 a,b	23 ± 3.8	N.D.	N.D.	N.D.	2.6 ± 0.1	36.5 ± 3.7	101.9 ± 29.6	5.1 ± 1.1 b,d

The generation of carotenoids, following chloroplast-chromoplast conversion and biosynthesis of carotenoids, enables the formation of carotenoid derived volatiles such as 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, *cis*-geranyl acetone, β-ionone, β-damascenone, geranial and citral (Rambla *et al.*, 2014, Simkin *et al.*, 2004, Bauchet *et al.*, 2017, Buttery and Ling, 1993, Zhang *et al.*, 2015). As can be seen in **Table 6.6** and **Table 6.7** the concentration of carotenoid derived volatiles shows a progressive increase during the ripening process. The concentrations of β-Ionone and 6-methyl-5-hepten-2-ol were both undetected in 'Mature Green' and 'Breaker' fruits, only increasing to the point of quantification in 'Orange' or 'Light Red' fruits, dependant on cultivar. On the other hand, 6-methyl-5-hepten-2-one was detectable at all stage of ripening for all cultivars. Previously Lewinsohn *et al.* claimed that 6-methyl-5-hepten-2-one could form from the degradation of lycopene, prolycopene, δ-carotene, and neurosporene (Lewinsohn *et al.*, 2005). However, their study focused on ripe fruits and non-standard carotenoid mutants. It seems likely that the formation of 6-methyl-5-hepten-2-one in green fruits is from the breakdown on phytoene, the first compound in the carotenoid biosynthesis pathway and present in mature green fruits (Fraser *et al.*, 2007). Structurally, there are only minor discrepancies between phytoene, neurosporene and all *trans*-lycopene, therefore the same fragmentation of the molecule following hydroxylation would probably produce 6-methyl-5-hepten-2-one. In addition, the fragment likely to form 6-methyl-5-hepten-2-one occurs more frequently in phytoene than in the more unsaturated carotenoids, as seen in **Figure 6.4**.

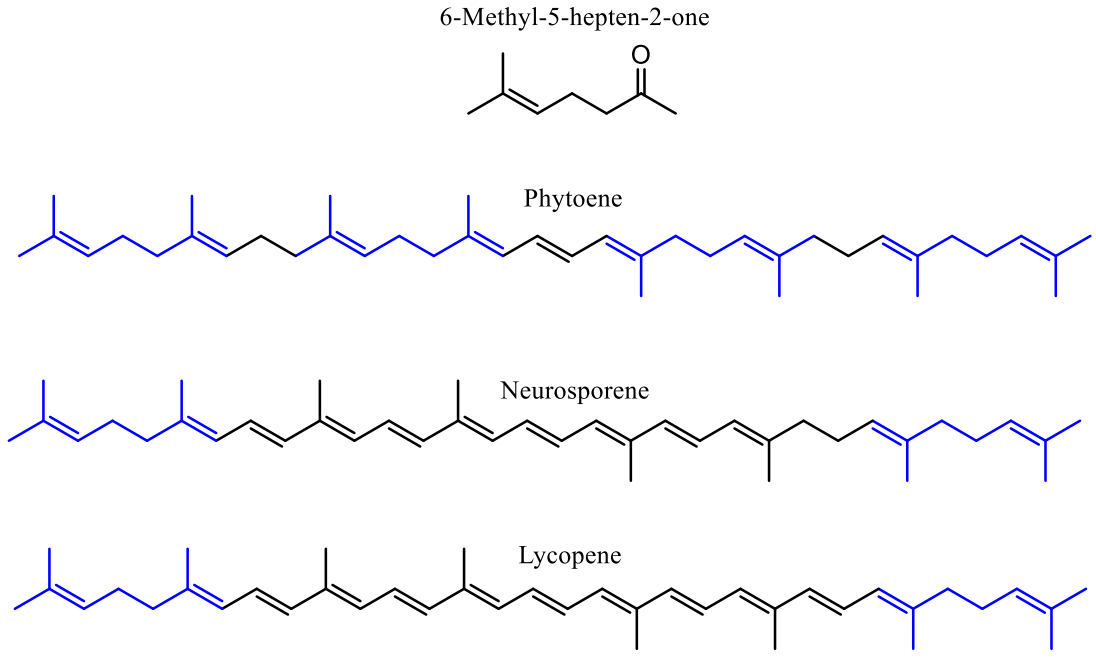


Figure 6.4 – Potential fragments (blue) of phytoene, neurosporene and all *trans*-lycopene that are the likely structural precursors to 6-methyl-5-hepten-2-one, following hydroxylation.

The synthesis of amino acid derived volatiles is suppressed in maturing fruits, including mature green fruits, prior to the onset of ripening. Most of the amino acid derived volatiles in tomato

fruits form from branched chain or aromatic amino acids, which are not present at significant levels prior to ripening. This is largely due to the strong bias for GABA synthesis over most other amino acids during fruit maturation. As the primary route of nitrogen metabolism in green fruits is focused on the production of GABA, the synthesis of other amino acids is stunted. However, as discussed previously, the catabolism of GABA to generate other amino acids may reduce substrate limitations on the formation of amino acid derived volatiles. However, based on the amino acid profiles presented previously in **Table 6.2**, **Table 6.3** and **Table 6.4**, this does not appear to be the case. The main amino acid derived volatiles form from the degradation of branched chain amino acids (BCAA), valine leucine or isoleucine or related compounds, namely 2-methylbutanal, 3-methylbutanal, 2-methylbutanol, 3-methylbutanol, 2-isobutylthiazole. Of these, only 2-isobutylthiazole and 3-methylbutanal (isovaleraldehyde) were quantified in this study. As can be seen in **Table 6.6**, the concentration of both these volatiles increases significantly as ripening progresses. However, this does not appear to be due to free substrate, as the concentration of both leucine and isoleucine decreases consistently throughout the ripening process. There may be a number of potential limitations that prevent accumulation of isovaleraldehyde early in ripening. The formation of the amino acid derived volatiles may not be substrate dependant after all, and instead my require the catabolism of GABA to generate proteogenic amino acids to allow for the synthesis of enzymes involved in the generation of volatile components. Such suggestions have been previously postulated with the generation of 2-phenylethanol and 2-phenylacetaldehyde from phenylalanine in tomatoes (Tieman *et al.*, 2006a). In their study, Tieman *et al.* noted that it was the activity of the enzymes responsible for the synthesis of the volatiles and not the endogenous phenylalanine substrate that limited their formation. This was observed in both a 'wild type' and a *Solanum pennellii* introgression line, IL8–2-1, which had been previously demonstrated to generate concentrations of the target volatiles that were 1,000 times greater than that of wild type (Tieman *et al.*, 2006a). Moreover, in a further study by Kochevenko *et al.* it was proven that it is in fact, not BCAA that act as substrates for the formation of these volatiles, but the intermediate α-keto acids that are synthesised during the generation or catabolism of BCAA (Kochevenko *et al.*, 2012). Therefore, the potential secondary limitation may be synthesis of amino acids following the increase in GABA catabolism during ripening, which results in more α-keto acid generation, increasing the available pool of substrate. However, both Tieman *et al.* and Kochevenko *et al.* conclude that availability of substrates is not the limiting factor and that, although biosynthesis of amino acids liberates the required substrates and potentially synthesises the required enzymes, there must be additional, downstream regulation of their biosynthesis. One of these mechanisms may be the inhibition of the enzymes involved in the conversion of the amino acid to the volatile. Inhibition of this conversion in under-ripe fruits would be beneficial to the plant, ensuring amino acids are available for use rather than lost as volatiles. Additionally, production of appetising volatiles prior to full

seed development and viability may lead to premature predation and reduce the rate at which plants can proliferate.

An additional route of carbon consumption in developing fruits is in the synthesis of fatty acids. Fatty acids can be synthesised within the plastids of cells, with a versatile substrate pool that includes pyruvate and glucose-6-phophate from glycolysis, and malate and citrate from the TCA cycle (Rawsthorne, 2002, Voet and Voet, 2004). Fatty acids synthesis begins with the production of malonyl-CoA through the conversion of acetyl-CoA. As much of the carbon substrate is derived from either glycolysis or the TCA cycle, fatty acid generation is reduced at early ripening stages. In addition to this, the lipoxygenase enzymes involved in the catabolism of fatty acids and generation of important volatiles such as hexanal, hexenals and hexanols and some C5 volatiles are less active in immature fruits (Chen *et al.*, 2004, Zhang *et al.*, 2015, Shen *et al.*, 2014). Lipoxygenases in tomato have been extensively studied and, therefore, have been named for the plant as tomlox A-E. Although these enzymes fill similar roles in the breakdown of fatty acids, specifically linoleic and linolenic acids, they are not homologous. The tomlox A, B and E proteins are 77% related, whereas tomlox C and D only share 47% of the same amino acid sequence as the tomlox A protein (Chen *et al.*, 2004). The expression of genes responsible for the transcription of each lipoxygenase has been shown to be dependent on tissue, developmental stage, ripening and function (Chen *et al.*, 2004, Griffiths *et al.*, 1999, Heitz *et al.*, 1997). Griffiths *et al.* investigated the expression and activity of tomlox A, B and C in ripening tomato fruits, discovering different methods of control for each. Tomlox A was found to decrease throughout fruit ripening, but to a lesser extent in a low-ethylene mutant indicating exudation of ethylene in ripening fruit inhibits expression of this isoform. On the other hand, both tomlox B and C increased throughout ripening, with upregulation driven by ethylene. Tomlox C was thought to have an additional, developmental control due to a spike in expression following the 'Breaker' stage (Griffiths *et al.*, 1999). Heitz *et al.* showed a distinct difference in function between tomlox C and D. Tomlox D was shown to be present in leaves, but not in fruits and the opposite effect was seen for tomlox C. The authors concluded that tomlox D was responsible for wound-induced, octadecanoic pathway for the synthesis of volatiles used in plant defence, whereas tomlox C was involved in the synthesis of desirable aroma compounds in ripening fruits (Heitz *et al.*, 1997). It seems likely that the generation of the C6 and C5 aroma volatiles in 'Mature Green' fruits, as seen in **Table 6.6** is due to the activity of tomlox A and B, which are both present and active in green fruits. Following the 'Breaker' stage, tomlox C expression and increasing activity throughout ripening, coupled with the ongoing synthesis of *de novo* fatty acids, accounts for the increasing production of the desirable flavour and aroma volatiles that are important to fruit flavour and quality.

6.6 Conclusion

As has been discussed in this chapter, the synthesis of the primary, quality-defined compounds associated with fresh tomato are strongly affected by the ripening process. Moreover, the routes of biosynthesis of sugars, amino acids and many volatiles are intrinsically interlinked through common pathways and precursors, including the citric acid cycle and GABA shunts.

The generation of many compounds important to the quality of three cherry tomato cultivars at 6 different ripening stages was studied using an on-truss approach. It was seen that sugar development increased between 'Mature Green' and 'Table Ripe' fruits relatively linearly, whist the ratio of glucose to fructose was comparable between cultivars, and it was closer to 1-1 in later ripening stages. This may be due to the consumption of glucose through respiration or glycolysis in the earlier developmental phases, which would allow for the synthesis of additional compounds such as amino acids or lipids, and lower the ratio, which was lowest in Piccolo 'Breaker' fruits at 0.6. Acids, including citric and malic acid, displayed contrasting relationships throughout ripening. Malic acid was highest in 'Mature Green' fruits, steadily decreasing throughout ripening. Citric acid, however, increased sharply from 'Mature Green' to the 'Breaker/Turning' or 'Orange' and then slowly decreased until 'Table Ripe' fruits, ending at approximately 120-130% of the concentration in 'Mature Green' fruits.

Amino acids largely behaved as previously reported in the literature, except that GABA was not the most abundant amino acid in 'Mature Green' fruits, which was glutamine. It has previously been shown that glutamine and glutamate account for more than half of the imported amino acids in the phloem going to developing fruits, so the accumulation of glutamine may be due to a slower rate of conversion of glutamine to GABA. As expected the catabolism of GABA throughout ripening was concomitant with the synthesis and accumulation of a number of important amino acids, namely glutamate, aspartate, proline and alanine. Of these, the most significant increase was seen in glutamate which increased by 5.6, 5.7 and 12.4 times between 'Mature Green' fruits of Piccolo, Axiani and Juanita, respectively. Although Juanita fruits appear to synthesis glutamate at a faster rate than the other cultivars, this is due to much lower concentration in 'Mature Green' fruits, with concentrations in 'Table Ripe' fruits closely comparable to those in Piccolo.

Differences in the expressed volatiles throughout ripening progression was also examined. The amino acid derived volatiles isovaleraldehyde and 2-isobutylthiazole both showed strong increases over the ripening gradient used in this study. Based on the findings of previous work, it seems that this is not due to substrate limitations, even though it coincides with amino acid generation from GABA catabolism, but rather is due to under expression or inhibition of the enzymes responsible for their synthesis. Lipid volatiles were varied, Hexanal, *trans*-2-pentanal

and 1-penten-3-ol showed progressive increases throughout ripening, whereas *cis*-3/*trans*-2-hexenal, cis-3-hexenol, 1-pentanol, nonanal, *trans*-2-octenol and *trans,trans*-2,4-heptadienal remained largely unchanged. Routes of formation of these compounds are quite interlinked, particularly the C5 and C6 compounds that are generated through the action of tomlox C, primarily. However, the downstream enzymic conversion of the resulting fatty acid hydroperoxides utilises much more specific enzymes, including hydroperoxide lyases, alcohol dehydrogenases and enal isomerases for the synthesis of each compound. Therefore, the different profiles seen during ripening, may related to specific enzyme expression, inhibition or compartmentalisation, which was beyond the scope of the current work.

Due to the interlinked nature of many of the flavour or taste-active components of fresh tomato, it may be difficult to enhance the formation of specific compounds without causing a 'knock-on' effect on the rest. For example, higher sugar content would probably lead to increased activity of the TCA and GABA shunt, yielding greater acid and amino acid generation, whilst potentially also synthesising additional fatty acids. In some ways this may be desirable, as both amino acids and organic acids are essential to good flavour in tomato. However, works have previously shown negative associations between consumer liking and acceptance and certain volatiles, including those derived from amino acids. Therefore, increasing the metabolic rate and biosynthesis of compounds, across the board, may actually lead to greater off-flavours. Careful monitoring of the chemical composition of new hybrids, particularly those using genomic approaches, will be essential to ensure that higher quality cultivars are produced in the future.

6.7 Future Work

This work focused on the progression of ripening throughout cherry tomato cultivation. It was apparent that the chemical composition was highly variable in the transient stages of ripening, particularly 'Breaker/Turning' fruits. However, it also showed that there were subtle differences in the initial three fruit positions 'Table Ripe' to 'Light Red'. In many situations fruits in these positions would be sold together, often on-truss. This indicates that it may be worth investigating the different composition of fully ripe fruits, based on their position on the truss. As fruits act as sinks for imported assimilate throughout development, and fruits showing higher metabolic activity sequester a higher proportion of the available assimilate, lower fruits on the truss may be 'starved' of assimilate during maturation by the more developed preceding fruits. This would explain how a punnet of visually comparable tomatoes occasionally contains fruits with less desirable organoleptic profiles, particularly low sugars, high acids and bland taste.

Full quantification of hexanal and *cis*-3/*trans*-2-hexenal using a dilution and aliquoting procedure, once validated, will provide further differentiation of the level of lipoxygenase activity during

ripening as well as enable better understanding of the impact of the green/grassy aromas on the flavour and aroma of ripe fruits. This work is ongoing and the new data will be available within the next month.

Profiling of the nucleotide content of developing tomato fruits would add significant value to the work conducted so far in this chapter. Nucleotide metabolism accounts for a large part of the utilisation of nitrogen within the plant that is not accounted for by the accumulation of GABA and subsequent synthesis of the amino acid complement in tomatoes. The synthesis of nucleotides is closely linked to amino acid synthesis, as it relies on ammonia liberated from the conversion of glutamine to glutamate. In addition, the monophosphate nucleotides contribute to the organoleptic experience of fresh tomato flavour, by modifying the intensity of the umami flavour contributed by glutamate and aspartate and are therefore are crucial to for understanding the savoury flavour of fresh tomatoes.

A further review into the generation of organic acids previously reported in the literature, taking into account cultivars, growth and harvest conditions, external factors and experimental designs may be able to elucidate the reasons behind the non-uniform trajectory of citric acid in tomato ripening.

7 The Metabolomic Classification of Ten Tomato Cultivars and Cultivar Specific Biomarker Identification by LCMS/MS

7.1 Chapter Abstract

Tomatoes (*Solanum Lycopersicum cv.*) from 10 commercial UK cultivars were grown in glasshouses, at Thanet Earth, Kent, throughout 2015. Of these, one cultivar, 'Elegance' was sampled twice once as a summer harvest and once in winter. The tomatoes were snap frozen in liquid nitrogen, homogenised and stored at -80 °C before being sub-sampled for LC-MS analysis. The polar metabolites were extracted by the addition of acidified, ice-cold methanol (±0.1.25% FA) following the procedure specified by De Vos *et al.* and analysed on a LC-Orbitrap-MS system. The resulting peak table was separated per cultivar and binary 'cultivar vs. not cultivar' classes drawn up, allowing for analyses using multivariate receiver operating characteristic (ROC) curves. The top 25 features that clearly separated each cultivar from the others were extracted and those that were upregulated in the cultivar in question were classified as biomarkers of that cultivar. Biomarkers of all 11 experimental classes were recombined and the peak table analysed by PCA, showing extremely good separation and clustering of all cultivars. The only clusters with significant overlap were the winter vs summer harvests of 'Elegance', which also grouped close to 'Temptation'. The commonalities shared between the two populations of 'Elegance' tomatoes were tested further, independent of other cultivars. By the same method, a list of 74 mass spectral features that were significantly different between winter and summer crops was isolated. LC-MS/MS analysis of the biomarkers of each of the cultivars tentatively identified 7 compounds, 6 of which play key roles in plant health, quality or flavour. Tentative LC-MS/MS identifications were achieved using a workflow incorporating SIRIUS, Sum formula Identification by Ranking Isotope Using mass Spectrometry.

7.2 Introduction

Metabolomics was first used in the 1970's, after advances in analytical equipment allowed for simultaneous, large scale detection and quantification of significant portions of the metabolome. Prior to this, the theory of metabolites being used as 'markers' for health and nutrition conditions or disorders had been theorised for centuries. This is apparent by the assessment of bodily fluids as a method of detecting sickness in patients as early as 2000 BC, whereby, the sweetness of urine was used as a predictor of diabetes (Gebregiworgis and Powers, 2012). The concept of metabolomics remained a diagnostic tool primarily focused on the assessment of biological fluids until early in the 20th century, when the first mass spectrometer was developed at the University of Cambridge. It wasn't until the 1970's that metabolomic studies moved on to the analysis of a wider spectrum of metabolites and in a single analysis; heralded by Pauling *et al.* who monitored over 500 metabolites in human breath and urine as indicators of diet and nutritional state of a subject (Pauling *et al.*, 1971). The term 'Metabolomics' was not coined until 1994, when it joined the genomics, transcriptomics and proteomics as a bioinformatics tool and a quantitative assessment of system biology (Bennett and Wallsgrove, 1994). Untargeted metabolomic profiling and fingerprinting is becoming more commonplace in food analysis and assessment. Applications in the food industry range from adulteration of foodstuffs (Cubero-Leon *et al.*, 2014), detection of pathogens, assessment of genetically modified crops, predicting quality/sensory attributes and effects of processing (Weimer and Slupsky, 2013).

Tomatoes are one of the most extensively researched plants in the world, due to both commercial significance and its classification as a 'model fleshy fruit' for the purposes of plant and fruit development research. The tomato genome is one of the 450 fully sequenced, plant genomes, and was the 17th plant genome to be fully sequenced (The Tomato Genome Consortium, 2012, NCBI, 2018). A domesticated tomato hybrid, Heinz 1708, was used for the sequencing of the genome, as well as one of its closest wild relatives *S. pimpinellifolium*. By comparing the two, the manuscript demonstrated only a deviation of 0.6% in nucleotide content, suggesting intrinsic similarities between the two, even after the selective hybridisation for commercialisation of *S. lycopersicum* (Knapp and Peralta, 2016). This indicates that following centuries of commercialisation and hybridisation, tomatoes retain a significant genetic similarity to the related 12 wild species included in the *Solanumn* genus. The focus of modern tomato breeding is shifting away from traits such as shelf-life and uniformity of fruits and refocussing on the reintroduction of traits responsible for the generation of desirable flavour active compounds. It has been hypothesised that hybridisation of commercial *Solanum lycopersicum* with some of its genetic relatives/ancestors or 'heirloom' cultivars may help to reintroduce some of the traits associated with the formation of flavour active compounds (Jones and Scott, 1983, Klee and Giovannoni, 2011, Tanksley and McCouch, 1997, Tieman *et al.*, 2017). Moreover, understanding what

distinguishes and separates commercial tomato cultivars, both in terms of flavour and metabolites, is an important tool, which could be introduced as a method of screening for promising future hybrids. To that end, metabolomic profiling and classification of tomato cultivars is a valuable technique which has not yet been fully explored or utilised.

Metabolomic classification is a valuable tool for identifying the biochemical between similar experimental groups, whether caused by underlying genetic variation between similar cultivars or environmental factors. The importance of understanding the biosynthetic pathways responsible for the formation of compounds, which are of interest to humans, cannot be overstated. Plants provide wide-ranging nutritional benefits in the diet, providing vitamins, minerals, antioxidants and non-dietary fibre, as well as the required macronutrients (Carrari *et al.*, 2006). Additionally, consumer acceptability of a plant or fruit as a food source is dependent on a complex variety of compounds. Primarily, this is based on the sensorial perception of the foodstuff and the presence of those compounds, which positively or negatively correlate to acceptable sensorial experience (Carrari *et al.*, 2006). Elucidating the biosynthetic pathways involved in the formation of certain compounds in plants will assist in further breeding endeavours and streamline the creation of more nutritional and flavoursome cultivars in the future. Through understanding the pathways responsible for the formation of desirable compounds, as well as pathways that yield negatively associated metabolites it may be possible to replace 'anecdotal' and 'trial and error' breeding methods with a more scientific and targeted approach to crop and cultivar development. By understanding which existing genetic stock is most desirable for hybridisation, with the aim of producing cultivars that are high in positively associated metabolites, whilst suppressing the formation of metabolites that negatively correlate with acceptance, significantly reduces the number of hybridisations that breeders conduct. Efficient breeding and hybridisation strategies increases the profitability of the crop as a whole, as a more acceptable and valuable product can be produced with less investment of resources and time. However, achieving this level of sophistication and accuracy in hybridisation requires much more information about the existing cultivars and greater understanding of how traits transfer to the resulting hybrids. Indeed, targeted hybridisation to pass on genes and desirable traits has been the staple practice of agriculture for decades. However, the complementation of genetic mapping of new hybrids with the metabolomic profile provides a deep understanding of how the genetic ancestry and chemical composition of new hybrids results in these desirable and undesirable traits. Compounds directly related to positive sensorial attributes in fresh tomato flavour consist of taste and flavour active compounds. There are known to be over 400 different volatile components in fresh tomato flavour, although it is likely that only 15-30 are present at levels over the odour detection threshold and therefore directly influence the perceived flavour. Additionally, sugars, acids, amino acids and nucleotides are responsible for the sweet, sour and umami flavours that are commonly associated with increased consumer acceptance.

One of the most significant obstacles to progress in small molecule analysis is the ultimate isolation, identification and structural elucidation of targets/biomarkers. This is due to the reliance on experimentally derived spectra of standard compounds for identity confirmation (Böcker *et al.*, 2009). Moreover, the resulting spectra from different instrument configurations is often not directly comparable, limiting the number of compounds that can be confirmed by this method. Fortunately, powerful computational tools for *in silico* prediction are emerging, bypassing the need for direct comparison of fragmentation patterns and allowing for identification of *de novo* compounds and biomarkers. SIRIUS is a good example of such a software, capable of combining isotopic pattern analysis, predictive fragmentation trees and automated database matching (Dührkop *et al.*, 2013). Isotopic pattern analysis relies on the natural abundance of stable isotopes for each element common in biomolecules, specifically CHONPS.

This chapter will focus on the differentiation of the ten primary tomato cultivars following a comparable experimental design to Chapter 4. The studied cultivars were 'Elegance', 'Temptation', 'Campari', 'P194', 'DR2', 'Sunstream', 'Juanita', 'Axiani', 'Piccolo' and 'Oranjestar'. Discrimination between the analysed cultivars was achieved using a LC-MS workflow for small molecule profiling. In addition, upregulated, mass spectral features that drive the clustering relationships of the dataset have been isolated and subjected to fragmentation by LC-MS/MS. The resulting spectra were compared to those available online in mzCloud, a biomolecule database for high resolution compound identification. In addition to this data analysis route, SIRIUS, an open access framework for *de novo* formula and structure elucidation was used to predict the identity of a number of metabolites not present in the available databases.

7.3 Metabolite Profiling as a Means of Cultivar Classification and Differentiation

7.3.1 Data Processing and Cultivar Biomarker Selection

The objective of this work was to classify and separate cultivars using multivariate approaches to all of the reproducible mass spectral features present in the collected data set. For the purposes of the initial cultivar classification section of this Chapter, the two harvests of Elegance fruits, ELE and EL2, are treated as separate cultivars. The dataset for this Chapter was collected over 12 days of constant, sequential LC-MS analyses of 452 samples was, therefore, extremely complex, initially consisting of 9,305 mass spectral features. Therefore, the dataset was simplified by changing the approach to biomarker selection. Identical copies of the peak table were made for each cultivar, whereby each cultivar was designated by name, for example 'AXI' and all other samples were recoded as 'Not AXI', thereby converting the question from trying to determine which features separated each class from 10 other, individual classes, to a binary separation. As samples were either separated into the cultivar group or into the opposing 'not the cultivar' group the most influential features that caused the separation between the two clusters could be extracted

from the 9,305 total features. By separating samples into binary classes, Multivariate Receiver Operating Characteristic (ROC) curves were employed to determine the best degree of separation between the sample populations. ROC curves are powerful tools for the purposes of binary classification, able to calculate and visualise classification rates of multiple models based on incrementally increasing numbers of utilised features. Prior to ROC analyses, outlying samples were assessed and removed from the dataset as they would be damaging to the binary class ROC analysis. Outliers were determined by PLS-DA and visual inspection. Outliers were only removed if they were due to instrument error (dummy injections, low sum area, etc.). A total of 4 samples were omitted from the downstream data processing methodology. The "Biomarker Analysis" module of MetaboAnalyst 4.0 (http://www.metaboanalyst.ca) was used to calculate multivariate ROC curves of each of the sample populations. Data uploading was performed identically to that previously described in Chapter 2, including missing values, filtering, normalisation, transformation and scaling of the datasets. The PLS-DA algorithm was used for both classification and feature ranking in the Multivariate ROC curves, with the number of latent variables set to 3 for each cultivar. The results of each ROC curve can be seen in **Table 7.1** along with the upregulated features accepted as biomarkers for each cultivar. To ensure each experimental class was treated identically, the best fitting model for each was determined and the same one was applied to each cultivar. The aim was to achieve the best classification rate per cultivar, whilst including as many significant features which contributed to the separation as possible and without overfitting the model. Although perfect classification rates would be desirable, the cost is inclusion of features which provide significantly lower power in terms of classification, which reduces the effectiveness of data reduction techniques such as this.

Table 7.1 – Classification rates of Receiver Operating Characteristic (ROC) curves of each of the binary cultivar classes. Iteration of the ROC model used for biomarker determination included 25 features per cultivar, upregulated features in the cultivar class were selected as biomarkers. Confusion matrix shows the classification rates of the ROC model with 0 representing 'cultivar' and 1 representing 'not cultivar' groups.

	Confusion matrix						
	Correct Classification		Incorrect Classification		ROC Statistics		Upregulated Biomarkers
Cultivar	0-0	1-1	0-1	1-0	AUC	CI	
AXI	33	368	0	0	0.999	0.990-1.000	15
PIC	34	365	0	2	0.951	0.884-1.000	20
JUA	38	361	1	1	0.978	0.922-1.000	17
ORA	36	365	0	0	1.000	1.000-1.000	22
SUN	41	359	1	0	0.998	0.997-0.999	18
DR2	36	361	4	0	0.997	0.988-1.000	11
CAM	38	323	40	0	0.965	0.900-0.992	0
TEM	33	366	1	1	0.992	0.955-1.000	24
194	36	359	6	0	0.998	0.990-1.000	14
ELE	39	348	14	0	0.991	0.987-0.999	2
EL2	33	361	7	0	0.992	0.973-1.000	1

Therefore, the 25 most important features in the correct classification of each binary cultivar ROC were selected as being the drivers in correct classification. Of these, only those that were upregulated in the cultivar class (higher peak areas in 'cultivar' class than 'not cultivar' class) were considered biomarkers of the cultivar. This decision was made to avoid crossover between sample classes, whereby, the same feature may be an upregulated driver in one and a downregulated driver in another. Additionally, 'biomarkers' are commonly defined compounds whose presence is indicative of a more general, global trait, in this case, the cultivar of the fruit. Therefore, only the upregulated compounds of each cultivar were taken forwards and pooled together. The resulting list contained 134 biomarkers, 10 of which were biomarkers of more than one cultivar. Of these 10, 5 were upregulated in both 194 and SUN fruits, the other 5 were upregulated in both JUA and DR2 fruits. All other features were only present in the top 25 most important features of a single cultivar each.

7.3.2 Metabolomic Profiling of Cultivars using ROC Biomarkers

Biomarker determination using ROC curves highlighted those features that were most powerful in separating and clustering each cultivar as compared to all other samples. However, this technique did not describe the distribution of the individual cultivar clusters visually. Visual assessment of multivariate data, analysed through data reduction techniques such as principal component analysis (PCA), can be very valuable in terms of showing the interconnectivity of the

experimental classes in the dataset. In this case, understanding what made a cultivar different only addressed half the question, the clustering distribution of the cultivars based on the extracted biomarkers showed which cultivars shared common links and which were the most different/unique. Therefore, the extracted peak table containing the 134 features thought to be biomarkers of at least one cultivar was uploaded to MetaboAnalyst 4.0 using the 'Statistical Analysis' node. The resulting PCA plot can be seen in **Figure 7.1** below.

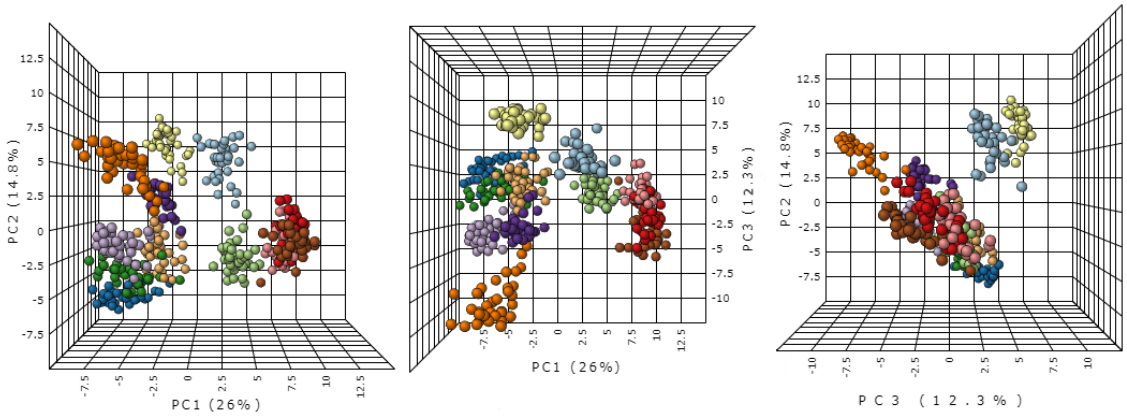


Figure 7.1- 3D PCA plots of upregulated features considered to be cultivar biomarkers through ROC analysis. P194/Carmaque, Axiani, Campari, DR28090TC/Strabena, Elegance 2nd Crop, Elegance, Juanita, Orange Cherry, Piccolo, QC's, Sunstream, Temptation.

The first 3 principal components account for 53.1% of the variability within the dataset, with 26%, 14.8% and 12.3% explained by PC1-3 respectively. Classification of samples was correct for each cultivar with the only overlap being observable between whole clusters rather than wrongly classified individual samples. The positions of cultivar clusters are indicative of similarities or differences between the concentrations of the included biomarker features in each cultivar. The most distinct cluster is also the only 'tangerine' type tomato, Orange Cherry. ORA samples clustered away from all other cultivars, being most similar to both the QC and PIC groups, but with only 2 samples close to the QC cluster. A second interesting group is that of P194 and Sunstream, both of which are distinctly different from the other cultivars on PC2 and PC3. As expected, the replicate harvests of ELE and EL2 showed a strong overlap, both presenting with very similar distributions over all three principal components. Interestingly, Temptation fruits also shared a strong similarity with ELE and EL2 fruits, with slight overlapping of clusters between all three populations. However, this overlap was not mirrored in the sample clustering seen in the heatmap shown in **Figure 7.2**, where the only misclassified samples belonged to ELE and EL2 sample populations. Difficulty in separating samples of ELE and EL2 is expected as both populations comprise the same cultivar, harvested at different seasons and therefore cultivar specific traits are likely to be present in both experimental classes.

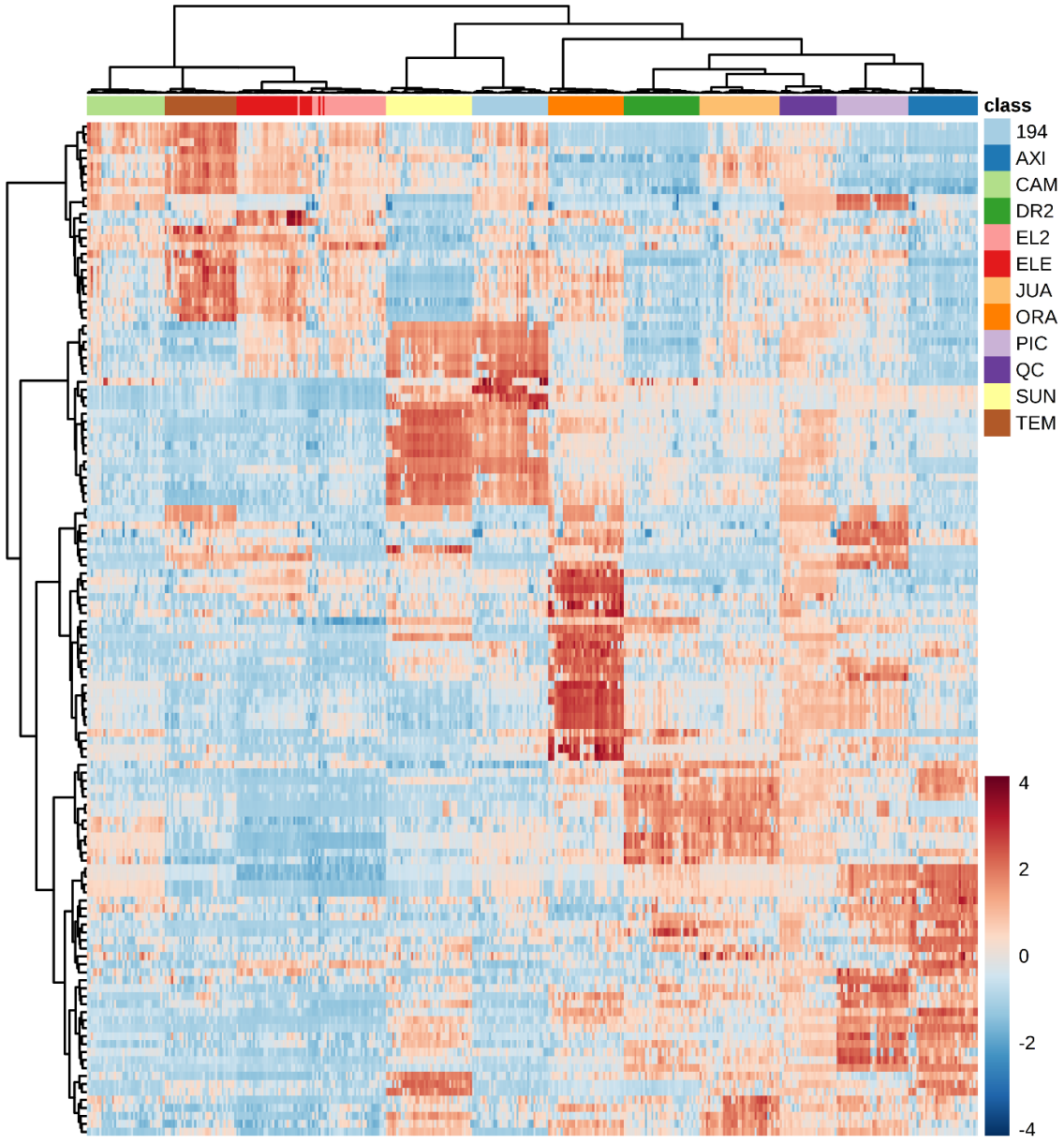


Figure 7.2 – Heatmap of the 134 mass spectral features that were upregulated in at least one cultivar. Samples are represented as colour coded columns and are not force-grouped, correctly clustering based on similarity apart from ELE and EL2. The 134 mass spectral features are displayed in rows clustering based on inter-relatedness.

Examination of the heatmap is a good method of discerning the differences in the experimental classes, particularly as the sample clustering is so accurate. The interrelated experimental classes can be further scrutinised through the up- or downregulation of the included metabolites with the aim of determining the cause of the overlap between classes. For example, the populations of ELE and EL2 are the only two with overlapping samples, but the two classes are very similar in terms of the metabolites present (rows), with a handful of metabolites showing strong concentrational shifts. This could be indicative of specific pathways that are more active in certain seasons, either due to light quality, nutrition, plant age or plant stressing. It is also clear that the features that drive the interrelatedness of TEM and ELE/EL2 are largely due to higher concentrations of the

metabolites present in ELE/EL2 in the TEM samples. This indicates that there are probably strong links between the biochemistry of Temptation and Elegance fruits, with the concentration difference in the upregulated metabolites potentially being driven by difference in final fruit weight. Temptation fruits used for this study weighed 68.9±10.4 g as opposed to 106.0±20.4 g of the Elegance fruits. Certain metabolites, including sugars and organic acids, have been previously shown to be inversely correlated to fruit size (Beckles, 2012). Therefore, TEM fruits displaying higher concentrations of metabolites common to both TEM and ELE/EL2, may be due to the 35% reduction in fruit size. Significant differences are notable between ORA and the remaining cultivars. The upregulated compounds that are present in ORA fruits occur at far higher concentrations than in any other cultivar, with the most similarity shared with PIC. As ORA is the only 'tangerine' type of tomato, it is likely that these compounds are related to the shifts in the biochemistry of the 'tangerine' type versus the standard red tomatoes. Some of these differences may be related to the biosynthesis of carotenoids, which is the most obvious difference in 'tangerine' type fruits; however there are likely to be other biosynthesis pathways that are interrupted or upregulated in these non-standard ripening mutants. Strong interrelatedness between 194 and SUN is apparent, as well as between DR2 and JUA; both pairs shared 5 upregulated features, which is well represented in the similarities visible in **Figure 7.2**. Both pairs consist of quite different cultivars, 194 is a medium salad/cocktail paired to a plum tomato, SUN. Moreover, the plum tomato DR2, is closely related to JUA, a cherry type. It is an unexpected finding that there are closer similarities between these pairs than there are between both plum-type tomatoes, but there are clearly compositional differences in the fruit that are not reflected in tomato type, appearance or size.

7.3.3 Compositional Changes in Elegance Fruits Harvested in Summer (EL2) vs Winter (ELE)

Elegance was used as a control cultivar in this study, being present in both summer and winter harvests. Although both ELE and EL2 clustered together in the previous PCA containing all the analysed cultivars, there were slight differences between the two. To determine which features were causing the differences between the two seasons both harvests of Elegance were compared against each other. The original peak table containing all 9,305 features was separated into all ELE samples and all EL2 samples. The reproducibility and abundance of the features was assessed per class, with a 100,000 peak area and 20% relative standard deviation threshold applied. Features that met these requirements in at least one class were retained, those features that did not meet one or both of the thresholds were eliminated from the analysis. A peak table containing the 106 remaining features was constructed and PCA and t-test functions of MetaboAnalyst were used to investigate the significant differences observable between the two harvests.

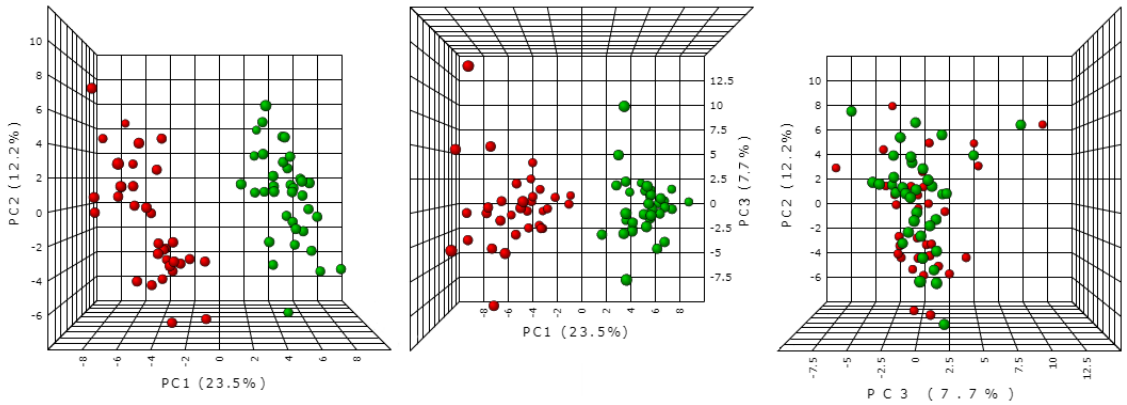


Figure 7.3 – 3D PCA of the 106 extracted features present in the Elegance crops. ● = ELE (n=37), ● = EL2 (n=33).

The PCA in **Figure 7.3** shows the clustering of both classes. Following removal of those features more strongly associated with non-Elegance cultivars, clear differences between the summer, EL2, and winter, ELE, crops are observable. The principal components explain of 43.1% of the total variability in the dataset, with 23.5%, 12.2% and 7.7% explained by PC1-3 respectively. As there was clear separation and good clustering of the two sample populations, the t-test function of MetaboAnalyst was applied to discern which of the features were driving the separation of the two classes. To account for potentially non-normal distribution of data, particularly that of certain features, a Wilcoxon-Rank-Sum (or Mann-Whitney-U) test was performed as a non-parametric alternative to the t-test. Of the 106 features included in the dataset, 74 were significantly ($p \leq 0.05$) different between the summer and winter Elegance harvests. The differences in the most significant features as determined through Wilcoxon-Rank test can be seen in **Figure 7.4**. Of the 74 most significant features, 61 were extremely significant, $p < 0.001$, 10 were highly significant, $p \leq 0.01$ and the remaining 3 were significant at the $p \leq 0.05$. Additionally, of the top 10 features, 8 of them were upregulated in the winter crop, ELE, and only 2 in the summer crop, EL2. Indeed, of the 74, only 29 of the compounds are present at higher concentrations in EL2 fruits. As with the concentrational difference between Temptation and Elegance fruits, discussed previously, this may be driven by fruit size, with ELE fruits 17 g heavier, on average, than those of the summer EL2 crop, at 113.7±12.8 g and 96.3±12.2 g respectively. The reasons for the ~15% decrease in fruit size of the summer crop are difficult to determine. However, identifying the metabolites that are in flux between the two populations may shed light on the biosynthesis pathways which are most affected by seasons.

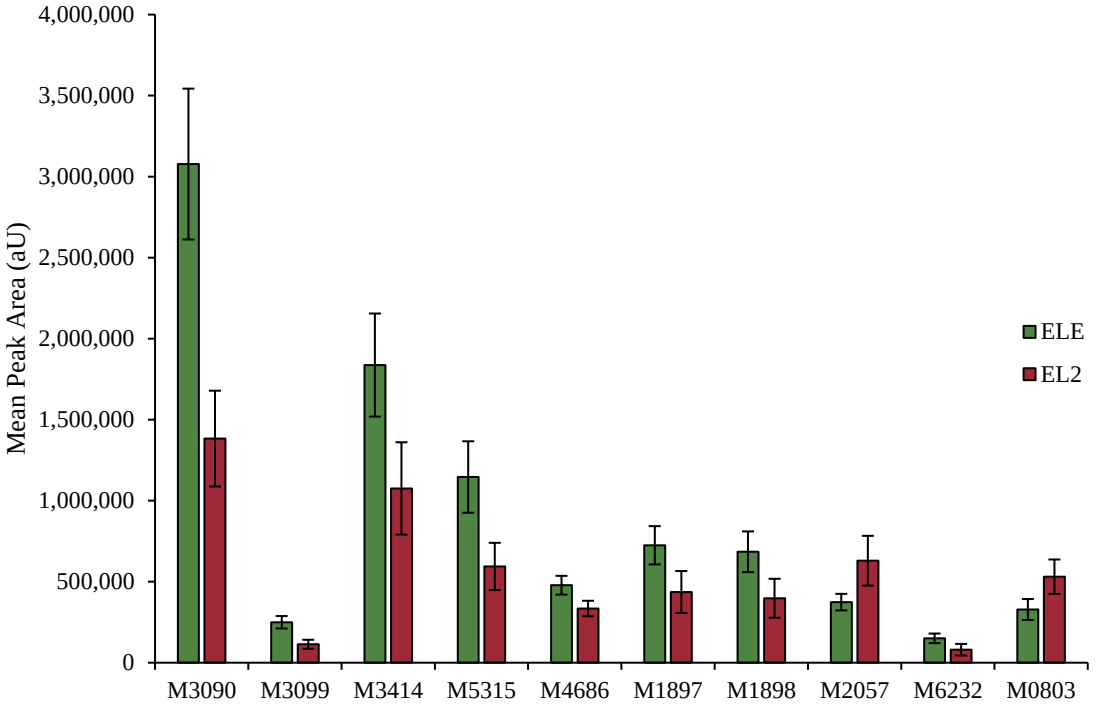


Figure 7.4 – Mean peak areas of the most significantly different mass spectral features, as determined by Wilcoxon-Rank-Sum test. Significance decreases from left to right ($p<0.001$).

The effect of growth and harvest seasons on tomato fruit saleability and quality is of significant value to the commercial tomato growing industry, particularly in the UK where greenhouses are a necessary measure to ensure year-round production. These results demonstrate that there is a significant change in the metabolite profile of Elegance fruits of a winter crop versus a summer crop. Therefore, the primary drivers in this phenomenon are important targets for characterisation and identification. Following identification and characterisation of the significant metabolites, the biosynthetic pathways and related compounds can be elucidated. This will, in turn, provide a good basis to understanding the seasonality of Elegance tomatoes and the pathways most susceptible to interruption through climatic effects. Due to the importance of understanding seasonal effects on crop quality, the features that were significantly different between both harvests of Elegance are shown in **Appendix 4**. The object of this study was not to determine which metabolites were upregulated based on the growth and harvest season of Elegance, so these features were not targets for MS2 analysis and fragmentation. However, subsequent MS2 revisions should focus on identifying these compounds and their biosynthesis pathways and roles in fruit viability, health and quality.

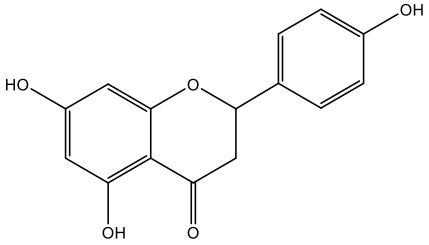
7.4 Cultivar Specific Biomarker Identification through SIRIUS Matching

All 134 of the mass spectral features which were deemed to be strongly influential in the correct classification of the 11 different cultivars were taken forward for MS2 isolation and fragmentation. Due to the similarities between cultivars, as well as the unknown genetic relationships between modern, hybrid cultivars, there was some overlap between strongly upregulated compounds in several cultivars. Therefore, some of the compounds that were isolated by MS2 are not biomarkers of a single compound, but increased abundance is highly associated with specific cultivars. Many other compounds were more strongly represented in specific cultivars, as can be seen in **Appendix 5**. However, of these, some were not suitable for isolation, either due to degradation or reduction in peak area, comparable coeluting mass peaks or inability to correctly isolate the molecular ion. Of the 134, 7 had strong identification matches using the SIRIUS workflow. Of these, 6 had defined roles in quality of both plant health and nutrition or flavour.

7.4.1 Biomarkers Directly Influencing Final Fruit Quality

This section focuses on compounds that influence the overall fruit quality, both in terms of growth, health and viability and in relation to fruit quality upon consumption, including health promoting benefits, nutrition, flavour, taste and structure/mouthfeel.

7.4.1.1 M6835 - Naringenin

Proposed Molecular Structure	
Name / IUPAC Name	Naringenin /
Molecular Formula	$C_{15}H_{12}O_5$
Exact Mass	272.06847
Mass±H	273.07575
Detected Accurate Mass	273.07708
Isotope Score / Fragmentation Score	5.72 / 29.58
Total Explained Intensity	91.32%
SIRIUS Score	92.79%

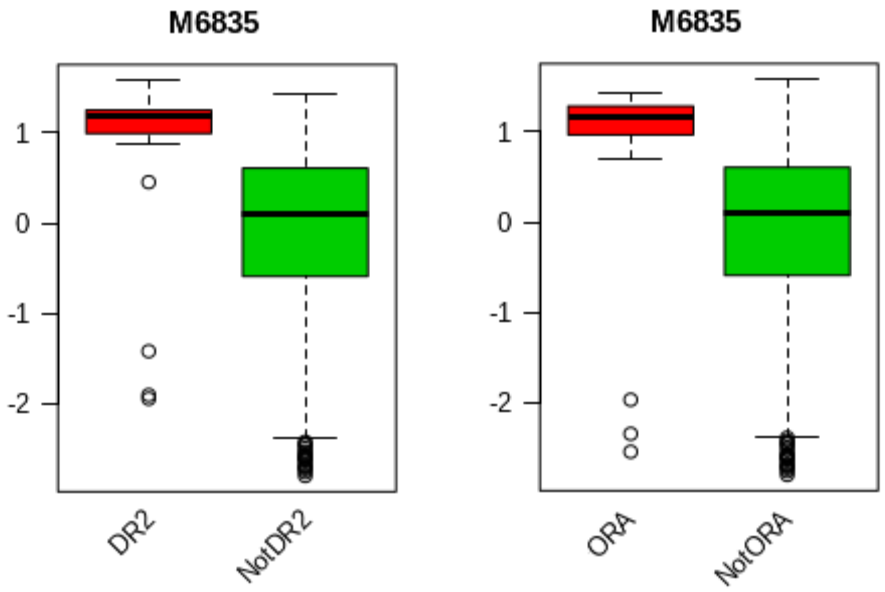


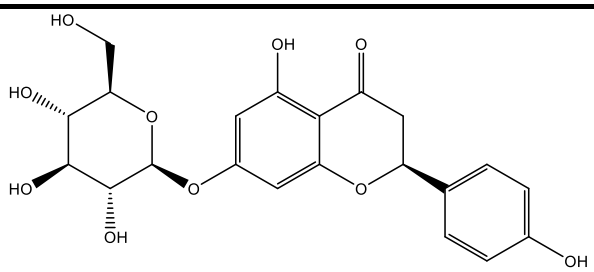
Figure 7.5 – Loading values of mass spectral feature M6835 in ORA and DR2 populations compared to all other samples. This feature was upregulated in two cultivars out of the 11 analysed.

Naringenin is a flavonone derived from the phenylpropanoid pathway (PPP), which originates with the catabolism of phenylalanine. The phenypropanoid pathway is responsible for the formation of several groups of compounds vital to plant health and viability, but that also confer several nutritional or organoleptic traits upon consumption. The PPP is responsible for the biosynthesis of lignin, a vital component for the formation of cell walls and structural integrity of the whole plant. In addition, the PPP leads to the synthesis of flavonoids, coumarins, lignans stilbenes, anthrocyanins and condensed tannins, amongst others (Fraser and Chapple, 2011, Winkel, 2006). Many of these compounds play important roles in plant health and function, providing antioxidative protection, biological defence or pigmentation for attraction of pollinators or seed dispersal. Moreover, following consumption, many of these compounds confer benefits to the consumer, including nutritional benefits and modification of the organoleptic experience. Previous published work indicates a slight reduction in the instance of coronary heart disease following inclusion of higher concentrations of flavonoids in the diet (Fraser and Chapple, 2011). In addition, narignenin and some of its derivatives contribute a bitter sensation upon consumption, providing characteristic tastes of certain foods, including grapefruit, oranges, cumin and peppermint (Yao *et al.*, 2004).

Naringenin has been previously reported at 4.50-12.55 mg/Kg in 'Remate' and 'Daniella' tomatoes, where it accounted for just 1.03% and 3.30% of the total phenolics, respectively (Martínez-Valverde *et al.*, 2002). Mitchell *et al.* reported higher concentrations in 'Halley 3155' tomatoes, at 30.2 mg/Kg over a 10 year monitoring period. In both studies, there all of the studied cultivars presented with ~2-3 times more quercetin than naringenin, quercetin being a derivative of naringenin. This may indicate that the activity of the flavonoid biosynthesis

leads to the temporary formation of naringenin as an important intermediate to the formation of various other phenolic secondary metabolites.

7.4.1.2 M6435 - Prunin

Proposed Molecular Structure	
Name / IUPAC Name	Prunin / (2S)-5-hydroxy-2-(4-hydroxyphenyl)-7-([(2S,3R,4S,5S,6R)-3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxy)-3,4-dihydro-2H-1-benzopyran-4-one
Molecular Formula	$C_{21}H_{22}O_{10}$
Exact Mass	434.12130
Mass±H	435.12858
Detected Accurate Mass	435.12994
Isotope Score / Fragmentation Score	6.06 / 65.37
Total Explained Intensity	95.96%
SIRIUS Score	91.63%

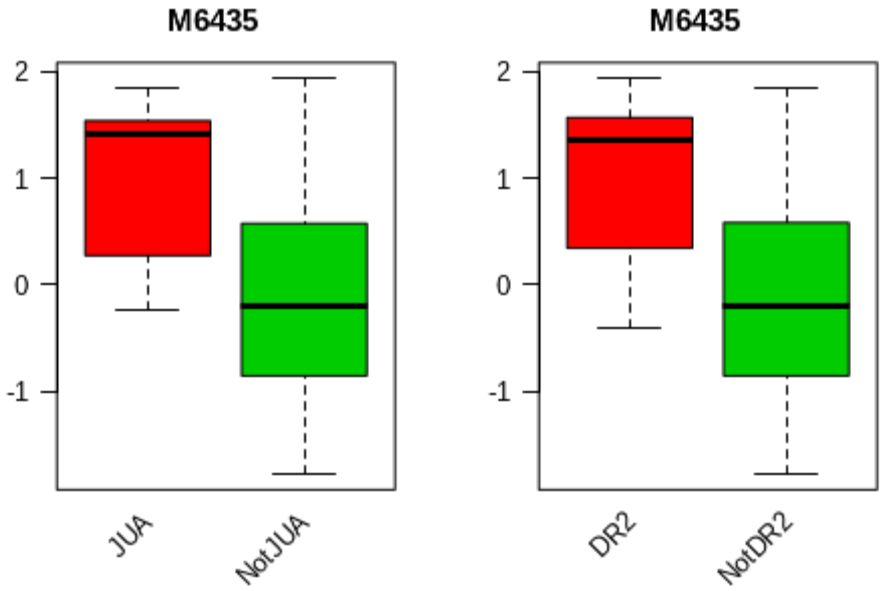
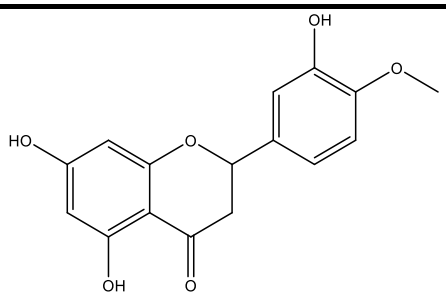


Figure 7.6 – Loading values of mass spectral feature M6435 in JUA and DR2 populations compared to all other samples. This feature was upregulated in two cultivars out of the 11 analysed.

Vallverdú-Queralt *et al.* recently isolated, fragmented and confirmed the identity and presence of prunin in ripe tomato homogenates (Vallverdú-Queralt *et al.*, 2010). Prior to this, manuscripts published by Tohge and coworkers and Slimestad and Verheul both reference the initial discovery of prunin in tomatoes, which was made by Miki and Akatsu in 1972, but published in

Japanese (Miki and Akatsu, 1972, Slimestad and Verheul, 2009, Tohge *et al.*, 2014). Prunin is more commonly associated with ripening citrus fruits, believed to be an intermediate flavonone derived from narigenin, which, in turn, forms from phenylalanine, as described above (Bilbao *et al.*, 2007, Frydman *et al.*, 2004). In citrus, the existence of prunin is thought to be fleeting as it is converted into either the neohesperidoside or rutinoside, dependant on species. The neohesperidoside flavanones are extremely bitter in taste, contributing significantly to the overall bitterness of grapefruit and pomello, whereas the flavourless rutinoside flavanones form more abundantly in orange or mandarin, for example (Frydman *et al.*, 2004, Winkel, 2006, Crozier *et al.*, 2009).

7.4.1.3 M7537 – Hesperetine/Hesperetin

Proposed Molecular Structure	
Name / IUPAC Name	Hesperetine/ Hesperetin /
Molecular Formula	$C_{16}H_{14}O_6$
Exact Mass	302.07904
Mass±H	303.08632
Detected Accurate Mass	303.08780
Isotope Score / Fragmentation Score	3.30 / 24.84
Total Explained Intensity	85.26%
SIRIUS Score	89.77%

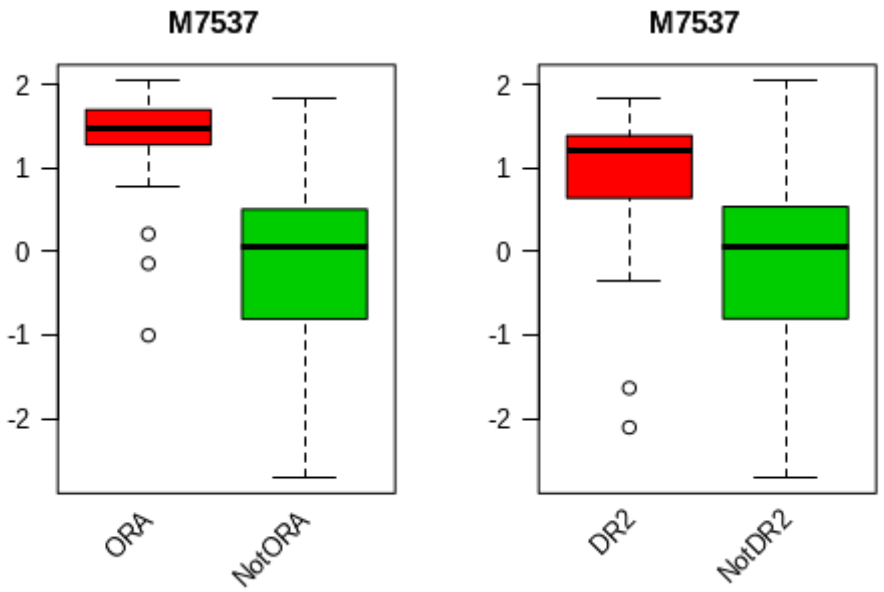


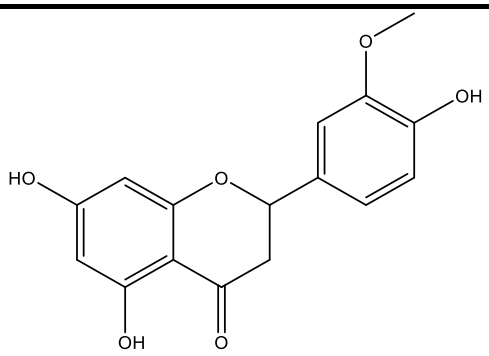
Figure 7.7 – Loading values of mass spectral feature M7537 in ORA and DR2 populations compared to all other samples. This feature was upregulated in two cultivars out of the 11 analysed.

Hesperetine is a flavaone and structural analogue to hesperetin due to the chiral centre between the pyran ring and cyclohexane; therefore, both hesperetine/hesperetin will be discussed (Yáñez *et al.*, 2008). Hesperitin forms from naringenin in the flavonoid biosynthesis pathway (Kanehisa *et al.*, 2017). As with naringenin, hesperetin has previously been shown to provide antioxidative protection following consumption. Indeed, several studies have shown that dietary naringenin and hesperetin also reduce inflammation and reduce the occurrence of coronary heart disease,

cerebrovascular diseases and asthma (Yoshida *et al.*, 2010). Yoshida *et al.* also demonstrated the ability of hesperetin and naringenin to reduce the incidence of adipocyte lipolysis in obese murine adipocytes, reducing the levels of free fatty acids and reducing subsequent insulin resistance and, therefore, type II diabetes.

Based on the available literature, hesperetin has not been previously found in tomatoes, being primarily confined to citrus fruits. It seems that the flavonoid biosynthesis pathway in tomatoes primarily results in the conversion of naringenin to quercetin via kaempferol, with a small amount of naringenin converted to prunin. Although the route of formation of hesperetin from naringenin is present as a KEGG pathway, it seems likely that the enzymes required for the conversion of naringenin to hesperetin are not present/ sufficiently active in tomatoes. As this is the first report in tomatoes, the authenticity of this identification cannot be confirmed without the confirmation against analytical standards.

7.4.1.4 M7522 - Homoeriodictyol

Proposed Molecular Structure	
Name / IUPAC Name	Homoeriodictyol / 5,7-dihydroxy-2-(4-hydroxy-3-methoxyphenyl)-2,3-dihydro-1-benzopyran-4-one
Molecular Formula	$C_{16}H_{14}O_6$
Exact Mass	302.07904
Mass±H	303.08632
Detected Accurate Mass	303.08759
Isotope Score / Fragmentation Score	4.14 / 29.54
Total Explained Intensity	82.31%
SIRIUS Score	88.84%

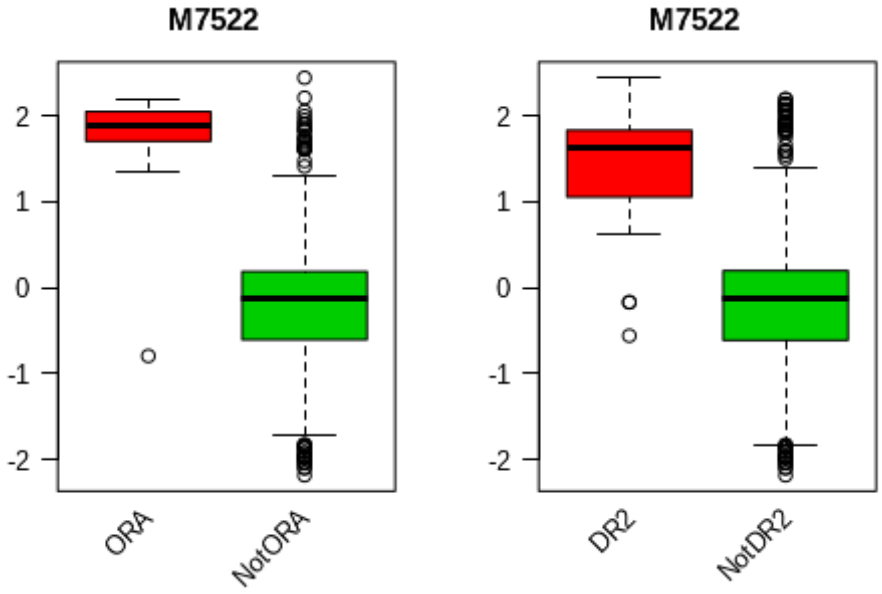
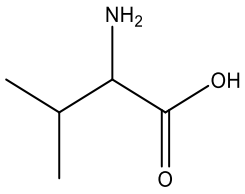


Figure 7.8 – Loading values of mass spectral feature M7537 in ORA and DR2 populations compared to all other samples. This feature was upregulated in two cultivars out of the 11 analysed.

In nature homoeriodictyol is primarily found in *Eriodictyon glutinosum/californicum* a shrub common to North and Central America, also known as yerba santa. Homoeriodictyol is one of the four primary flavanones isolated from yerba santa. It is capable of masking/modifying bitterness and increasing appetite as well as being widely used as a homeopathic remedy for a number of

respiratory ailments (Hochkogler *et al.*, 2017, Ley *et al.*, 2005). Ley *et al.* showed that eriodictyol, homoeriodictyol and its sodium salt were very effective in masking the bitterness of 7 compounds, without providing either a taste or flavour themselves. Hochkogler *et al.* found that homoeriodictyol was capable of enhancing the appetites and food intake of 27 participants when consumed alongside a caloric load of ~75g glucose.

7.4.1.5 M0898 – Valine

Proposed Molecular Structure	
Name / IUPAC Name	Valine /
Molecular Formula	$C_5H_{11}NO_2$
Exact Mass	117.07898
Mass±H	118.08626
Detected Accurate Mass	118.08701
Isotope Score / Fragmentation Score	0.27 / 33.48
Total Explained Intensity	99.45%
SIRIUS Score	70.12%

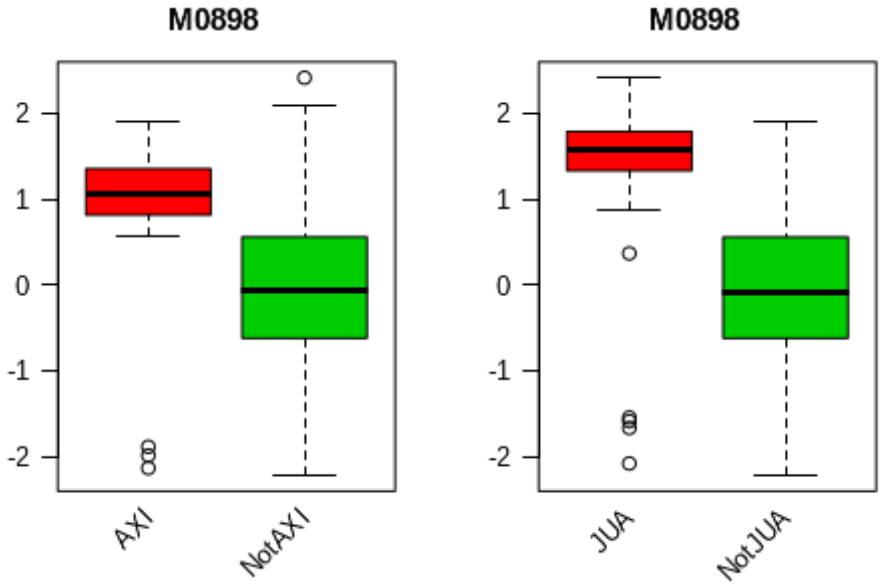


Figure 7.9 – Loading values of mass spectral feature M0898 in AXI and JUA populations compared to all other samples. This feature was upregulated in two cultivars out of the 11 analysed.

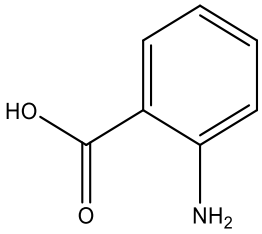
Valine is a branched chain, proteogenic α-amino acid which derived from pyruvate. The routes of formation of the three branched chain amino acids are very comparable, all involving the condensation of a molecule of pyruvate with an acetaldehyde, which is derived from a second pyruvate for valine or α-ketobutyrate in the synthesis of isoleucine (Umbarger, 1978). The intermediate α-ketoisovalerate is the point at which valine and leucine biosynthesis diverge, with

formation of valine through transamination with alanine as the amino donor or conversion to α-Isoproplymalate through the action of Coenzyme A (CoA).

Free valine accounts for a small proportion of the free amino acid content of tomato fruits at various ripening stages. Boggio *et al.* found free valine concentrations of 151, 176, 145 nmol/g in green, yellow and red 'Platense' tomatoes accounting for 1.90%, 1.40% and 0.90% of the total free amino acids respectively (Boggio *et al.*, 2000). Sorrequieta *et al.* observed a similar reduction in free valine in the ripening of 'Micro-Tom' tomatoes, presenting with 2.8, 2.4 and 1.8% of molar content of amino acids at green, yellow and red stages of ripening. Branched chain amino acid transaminases (BCATs), the final enzyme in the biosynthetic routes of the formation of valine, leucine and isoleucine have been previously shown to play a role in the catabolism of the same amino acids. Kochevenko *et al.* demonstrated the ability of the BCATs responsible for the formation of valine, leucine and isoleucine, to catabolise these amino acids, yielding the precursor α-keto acid of each. This process was shown to significantly contribute to fruit respiration in immature/younger fruits, but not in more well-developed tomatoes. In addition, the formation of the α-keto acids of these amino acids was proposed as the precursors to a number of volatiles involved in fresh tomato flavour, including 2- and 3- methylbutanal and methylbutanol, rather than the amino acids themselves (Kochevenko *et al.*, 2012).

The direct influence of valine on the quality aspects of fresh tomatoes is difficult to define. Its role as a proteogenic amino acid solidifies its importance in all manner of enzymatic reactions and biosynthetic processes. Its importance as a free amino acid is less well defined. The volatiles isobutyl acetate, 3-methylbutanal and 3-methylbutanal derive from α-ketoisovalerate, the direct precursor of valine. Of these, 3-methylbutanal and 3-methylbutanol are both present in fresh tomato at levels sufficient to impact flavour and aroma (Buttery *et al.*, 1988, Tandon *et al.*, 2000).

7.4.1.6 M3536 - Anthranilate

Proposed Molecular Structure	
Name / IUPAC Name	Anthranilate / 2-Aminobenzoic acid
Molecular Formula	$C_7H_7NO_2$
Exact Mass	137.04768
Mass±H	138.05496
Detected Accurate Mass	138.05513
Isotope Score / Fragmentation Score	1.98 / 18.25
Total Explained Intensity	100%
SIRIUS Score	72.99%

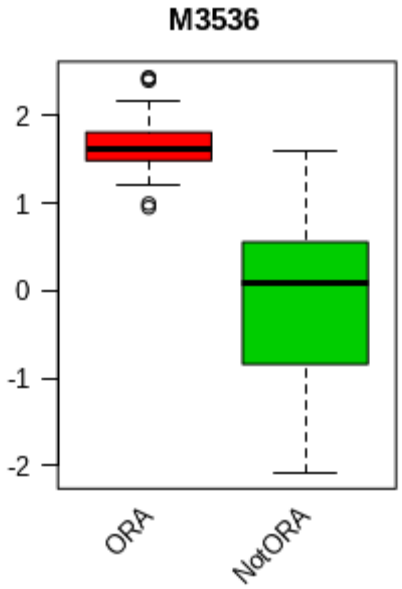


Figure 7.10 – Loading values of mass spectral feature M0898 in ORA populations compared to all other samples. This feature was upregulated only ORA fruits out of the 11 cultivars analysed.

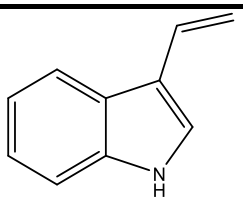
Anthranilate, 2-aminobenzoic acid, is technically an amino acid, presenting both carboxylic acid and amino groups, however it is rarely noted as such. Anthranilate is an important precursor in the biosynthesis pathway of tryptophan (Kanehisa *et al.*, 2017, Tzin and Galili, 2010).

Barsan *et al.* confirmed that the enzymes responsible for the formation of anthranilate and subsequent conversion to tryptophan are located in the chromoplasts of tomato fruits (Barsan *et al.*, 2010). This also suggests that biosynthesis of branched chain amino acids occurs within tomato fruits, rather just through import from vegetative tissues. In addition, the authors recognised that subunits of α- and β-anthranilate synthase were homologues to those present in *Arabidopsis thaliana* where they have been confirmed as key features in the production and regulation of auxins (Barsan *et al.*, 2010). Anthranilate can be shown to influence the overall quality of tomato fruits by multiple means, all stemming from its role as a precursor to tryptophan synthesis. Tryptophan is involved in the biosynthesis of many biologically active compounds shown to be both important to plant health and growth as well as being nutritionally beneficial. This ranges from phytohormones such as auxins (indole-3-acetate, particularly) and serotonin related to proper fruit set and maturation, to the antiparasitic action of camalexin and synthesis of niacin (vitamin B_3) (Hildebrandt *et al.*, 2015).

7.4.2 Undefined Biomarkers

This section includes compounds that do not have well defined or understood roles in fruit or plant health or quality. As such, the influence and importance of these compounds on fresh tomatoes is not well understood, therefore hypotheses about their roles have been proposed, but, until the compound identity is confirmed through analysis of standards, further elucidation is not possible.

7.4.2.1 M3555 - 3-ethenyl-1H-indole

Proposed Molecular Structure	
Name / IUPAC Name	N/A / 3-ethenyl-1H-indole
Molecular Formula	$C_{10}H_9N$
Exact Mass	143.07350
Mass±H	144.08078
Detected Accurate Mass	144.08127
Isotope Score / Fragmentation Score	1.76 / 40.17
Total Explained Intensity	92.83%
SIRIUS Score	73.21%

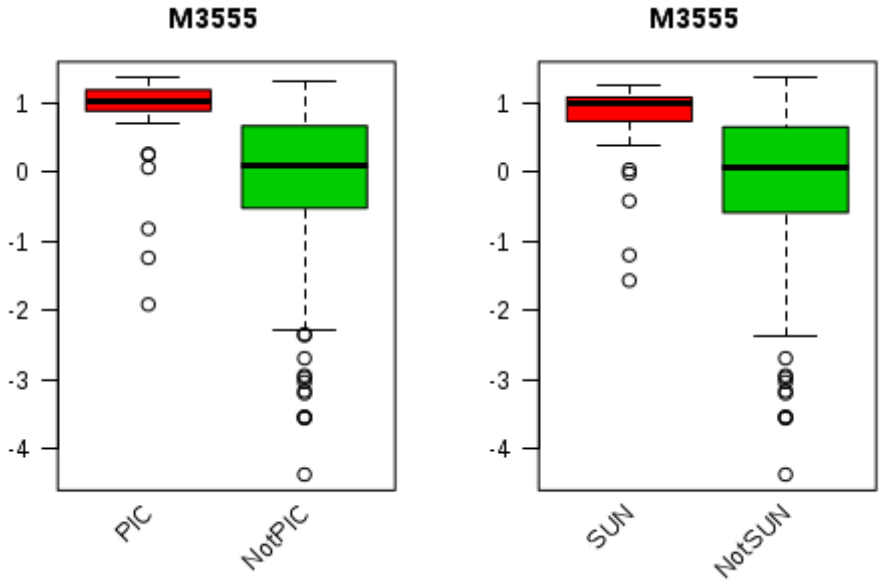


Figure 7.11 – Loading values of mass spectral feature M3555 in PIC and SUN populations compared to all other samples. This feature was upregulated only two cultivars out of the 11 analysed.

As far as can be determined at this point, the biosynthesis of 3-ethenyl-1H-indole has not been reported in plants. Several indole compounds form from tryptophan metabolism and the biosynthesis of indole-3-acetate, one of the most potent auxin compounds in plants. Auxins are important plant growth regulating hormones highly active in fruit set and initial growth of fruits in many plant species, including tomatoes (Serrani *et al.*, 2008, de Jong *et al.*, 2009). Although there does not appear to be a biosynthetic route of formation of 3-ethenyl-1H-indole, it is possible that it derives from indole following tryptophan metabolism. Alternatively, there are a number of structurally similar indole compounds and this match could be inaccurate with the true identity being that of a similar compound.

Additional target features for follow up analyses and continued identification can be seen in **Appendix 5**.

7.5 Conclusions

Using untargeted LC-MS profiling of the polar metabolites in 11 different commercial tomato cultivars, including two harvests of a single cultivar, followed by biomarker isolation using ROC curves, successful categorisation of all 11 experimental classes was achieved. The use of binary experimental classes and ROC analyses was an effective method of data reduction, clearly highlighting those features that were key drivers in the differences between and separation of the experimental classes, which constituted less than 2% of the total detected mass spectral features. Those features were then isolated for further investigation by MS2 analyses and processing through SIRIUS for tentative identification. Of these, good matches were achieved on 7 compounds, 6 of which were directly involved in fruit flavour and quality or plant health and function. Most of the successful MS2 targets are involved in the biosynthesis of tryptophan or utilise it as a precursor, indicating this pathway may be more active in certain cultivars of tomatoes as opposed to other cultivars. It is particularly apparent in cultivars such as Oranjestar, Juanita and DR2, where many of these potential biomarkers were significantly upregulated.

ROC curves were also very efficient methods of identifying the features that were most significantly different in the different seasonal populations of ‘Elegance’ fruits. ROC curves identified 74 mass spectral features that displayed the most significant concentrational change over the two harvest periods, as determined by Wilcoxon-Rank-Sum test. The relevant details of these compounds have been provided, pending further analysis to confirm their identities by MS2. Following identification and characterisation of the most significant differences in composition between harvest seasons, the pathways and related secondary metabolites will be assessed to better understand the effects of the growth and harvest period on the chemical composition of tomato fruits.

7.6 Future Work

This study clearly demonstrates significant differences between the analysed cultivars as well as the ability to correctly classify samples into their respective cultivar group based on a limited number of compounds that are upregulated in specific classes. This approach could be evaluated as new cultivars are introduced, better estimating the ability for large scale profiling as a method of cultivar differentiation and biomarker identification. In addition, one of the most labour and time intensive and inefficient aspects of commercial tomato growing is the development of new cultivars for market. This process often relies on the production of thousands of cross hybridisations with the aim of narrowing down the successful crosses to less than ten potential trial varieties. A profiling metabolomics workflow would provide the ability to identify the shifts in the metabolome following the cross hybridisation of new cultivars, particularly useful when trying to pass on specific characteristics of popular cultivars to modern hybrids. Expanding on this methodology with the inclusion of a targeted metabolomic workflow for semi-quantitative or quantitative analysis (using internal standard spiking) metabolites known to influence quality, would dramatically streamline the breeding process for new, commercial crops. If properly implemented, the trial varieties showing downregulation of important compounds could be eliminated in a more efficient manner than the current process. This current study compares very different cultivars and types of tomatoes, where classification has been possible; therefore, comparing very similar hybrids may require optimisation and fine-tuning of the workflow, to ensure that classification and biomarker determination was as successful.

Further investigation into the remaining biomarkers, including sample pooling and concentration to assist in low abundance compound isolation, is planned for the coming months. Further targets could be isolated at higher concentrations in the concentrated pool samples, than were available in the samples during the original MS2 analyses. The current tentative identifications, provided by SIRIUS, will also be confirmed through side-by-side fragmentation of authentic standards, in addition to those isolated following concentration. As it stands, the pathways most in flux between the current cultivars are the biosynthesis of tryptophan and the phenylpropanoid pathway, both of which were involved in the production of 6 of the 7 isolated biomarker targets. Further investigations into these, possibly using a quantitative trait loci (QTL) genetic approach, coupled with proteomics to better understand the activity and abundance of the enzymes responsible for the formation of these biomarkers could shed a great deal of light on observed differences in their expression in the current cultivars, particularly valuable due to the importance of these compounds both to the plant and consumers.

8 Shifts in the Polar Metabolome of Three Cherry Tomato Cultivars During Fruit Ripening: An Untargeted, On-Truss Study

8.1 Chapter Abstract

The ripening profiles of 3 commercial tomatoes (*Solanum Lycopersicum cv.* 'Piccolo', 'Axiani' and 'Juanita') were analysed by LC-MS metabolomic profiling and subsequent feature identification through MS2 isolation and fragmentation. Replicate trusses, 10, for each cultivar were selected which displayed specific ripening stages at predefined positions per truss, corresponding to 'Table Ripe', 'Red', 'Light Red', 'Orange', 'Breaker/Turning' and ' Mature Green'. Each of the three cultivars displayed similar trends in the ripening profile, as demonstrated through PLS-DA. This relationship was driven, in part, by features that were similarly important in each of the cultivars and, in part, by features that were more cultivar dependant. This was attributed to primary metabolites driving the trend and the more cultivar specific secondary metabolites highlighting the differences between each cultivar. Overlap between ripening stages was more apparent in the three most ripe experimental groups, although differences between them were still easily discernible. The transitional stages of ripening, particularly the 'Breaker/Turning' group showed the most significant biochemical shift. The most important features, as determined by PLS-DA loadings, were taken forward for subsequent MS/MS fragmentation and identification using SIRIUS. Tentative identities of 9 metabolites were achieved, 5 of which played direct roles in fruit quality or flavour. Of these two were amino acids, leucine and arginine, which showed inverse relationships with ripening, with lecine decreasing and arginine increasing during fruit ripening. Caffeic acid, a cell wall constituent, showed an increase which coincided with the increased cell wall loosening in later ripening stages. Also, serotonin, which is important in fruit and flower set and development increased during the ripening of fruits.

8.2 Introduction

The process of ripening is vital for the conversion of relatively unpalatable plant tissues to the fruits widely consumed worldwide, both by humans and other animal species. This dramatic conversion of plant tissue is controlled by significant biochemical shifts, which are, in turn, dictated by enzymes and their encoding genes. In tomatoes as with most fruit, this process can broadly be characterised as retention of water, cellular expansion, cell wall loosening, assimilation of metabolites, conversion of storage macromolecules to nutritionally and organoleptically desirable compounds and the synthesis of coloured pigments (Alexander and Grierson, 2002, Baldwin *et al.*, 1991b, Balibrea *et al.*, 2006, Boggio *et al.*, 2000, Bramley, 2002, Giovannoni, 2001, Oms-Oliu *et al.*, 2011, Raffo *et al.*, 2002, Stern *et al.*, 1994). In terms of fruit quality development, all these aspects play essential roles in final fruit quality, flavour and nutrition. Traditional, targeted approaches to quality and flavour determination are often confined by design, to single compound class determination per analysis. This allows for robust quantification of the most flavour-active and quality determining compounds. However, flavour and quality are typically a cumulative effect of a multitude of different compounds and chemistries, many of which require specialised, focused and individual approaches to quantify. These approaches are laborious and demanding in terms of consumables and access to a variety of analytical platforms. The expansion and solidification of –omics technologies as routes for crop and foodstuff profiling and improvement allows for significant streamlining of developmental workflows. For discriminative studies, untargeted metabolomics is a powerful tool for highlighting the most significantly different metabolites between experimental classes. Further investigation into these compounds can provide metabolite structure, identification and the route of biosynthesis, providing additional information into the biochemical differences between experimental classes. Fully comprehensive profiling of the entirety of an organism's metabolites through a single analytical approach is still unachievable, as there is no extraction methodology or analytical technique with 100% metabolite coverage. Therefore, the chosen extraction and analytical platform directly determines the detectable metabolites. Metabolomic approaches, particularly utilising LC-MS, are able to extract and detect the presence of thousands of compounds in a single analytical run, assisted by soft ionisation techniques which retain single molecular ions per metabolite, avoiding the confusion of coeluting, highly fragmented molecules. However, due to the broad metabolite coverage, untargeted metabolomics is unable to provide quantitative data and, as such, cannot completely replace targeted analysis (Lu *et al.*, 2008). The coupling of untargeted and targeted metabolomics approaches has been shown to be a valuable method for both profiling a significant proportion of the metabolites in addition to simultaneously quantifying a select group of analytes of interest (Cajka and Fiehn, 2016, Rochat, 2016). For the purposes of crop improvement, many flavour-active metabolites or those vital to their biosynthesis can be included as calibration standards, either using external calibration curves, or ideally, authentic,

stable isotopomers as multi-point internal calibration, referred to as isotope dilution mass spectrometry (Fernie and Schauer, 2009, Keurentjes, 2009, Langridge and Fleury, 2011, Tieman *et al.*, 2017). Using a streamlined, omics approach to understanding the biochemical routes of formation of organoleptic and nutritionally desirable compounds is a powerful strategy for crop improvement. Recent works on tomato metabolomics have utilised a wide array of analytical platforms and data processing techniques to demonstrate differences in the metabolome on a cultivar, ripening stage, ancestor species and knockout/ treatment basis, showing the flexibility and applicability of the technology (Carreno-Quintero *et al.*, 2013, de Vos *et al.*, 2011, de Vos *et al.*, 2007, Long *et al.*, 2006, Moco *et al.*, 2007, Semel *et al.*, 2007, Tikunov *et al.*, 2005). Metabolomics is particularly important in the analysis of complex organisms, due to the wide coverage of metabolites provided by efficient extraction and analyses. In the plant kingdom alone, there are thought to be more than 200,000 chemically unique metabolites, spanning the previously analysed plant-based matrices (Wurtzel and Kutchan, 2016). It can be presumed that each of these maintains significant roles in plant health, proliferation and cellular function as it is unlikely that energy would be wasted on the synthesis of compounds with little to no function or tangible benefit to the organism. Therefore, monitoring, characterising and identifying shifts in the complement of metabolites present in developing fruits can help to elicit the routes of formation, related metabolites and processes involved in their biosynthesis. This has been previously demonstrated as a strong complement to novel breeding strategies, particularly those that are genomics led (Fernie and Schauer, 2009, Keurentjes, 2009, Langridge and Fleury, 2011, Tieman *et al.*, 2017).

This chapter will focus on the differentiation of the polar metabolome of 6 distinct ripening stages of three cherry tomato cultivars. Trusses selected for analysis displayed each of the desired ripening stages per truss, enabling direct relationships to be drawn between plants, trusses and fruits. The strongest drivers in the differentiation of ripening stages were subjected to further LC/MS/MS analyses in order to confirm their identities as biomarkers of ripening stages in the studied cultivars. Biomarkers are identified as common across the study and on a per cultivar basis. Following metabolomic profiling by LC-MS, the most significant mass spectral features were isolated and fragmented by LC-MS/MS. Resulting fragmentation spectra were uploaded to SIRIUS (Lehrstuhl Bioinformatik, Jena, Germany) for tentative *in silico* identification using isotopic abundance patterns and fragmentation trees.

8.3 Experimental Design

This study used the same samples that displayed the desired on-truss, ripening profile as was used in Chapter 6. The positions used for each cultivar can be seen in **Table 8.1**, different truss positions were used per cultivar due to the different mean number of fruits set, per truss by each.

Table 8.1 – The truss position of fruits used in this study. Mean fruits per truss vary between cultivars, therefore different positions were selected to spread out the sampled fruit along the trusses. Final position was 12 for PIC, 14-16 for AXI and 14-17 for JUA, but was always Mature Green. Table first presented in Chapter 6.

		Position on Truss		
Sample Number	Ripening Stage	Piccolo (PIC)	Axiani (AXI)	Juanita (JUA)
1	Table Ripe (TR)	1	1	1
2	Red (R)	3	3	4
3	Light Red (LR)	4	5	6
4	Orange (O)	6	8	8
5	Breaker/Turning (BR/T)	8	11	10
6	Mature Green (MG)	Final	Final	Final

8.4 Metabolite Profiling from MS1 Analyses of Ripening Fruits

Although this experiment is based on the progression of fruit ripening, it also has a truss positional element, as well as single trusses making up the 10 biological replicates used in the study. Therefore, the progression of ripeness is inverse to the sample number, with sample number correlating to truss positions, proximal to distal. The ripening profile of each of the cultivars followed a very similar trend between the first three dimensions in the data, but the first 3 components only explained 39.2%, 47.5% and 31.4% of the variation in the data, which implies that there are additional, secondary relationships between the samples, that are less powerful than the metabolite change caused by the ripening profile. The ‘n’ shaped profile that can be seen in **Figure 8.1** is due to the relatedness of samples being displayed as spatial differences in PLS calculations.

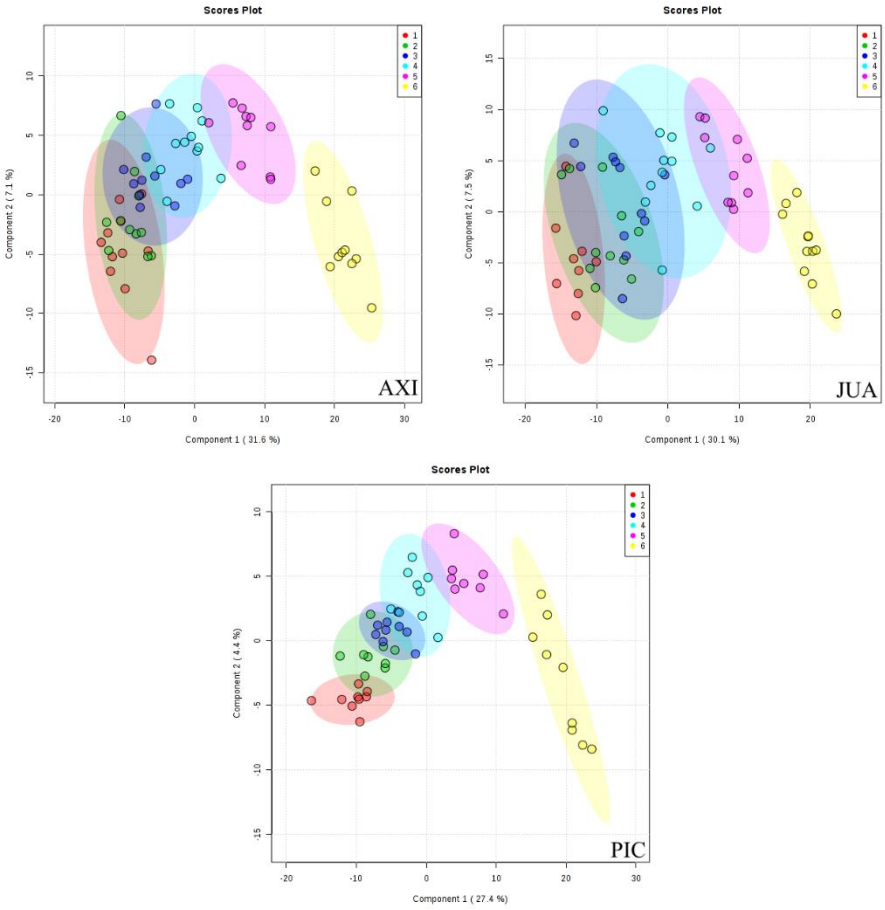


Figure 8.1 – 2D PLS-DA plots of the ripening profiles of 10 trusses of Axiani, Juanita and Piccolo fruits, based on 6 positional ripening stages on truss (as described previously). X axis represents component 1 and Y axis represents component 2 in each case. X=31.6%, 30.1% and 27.4% and Y= 7.1%, 7.5% and 4.4% for AXI, JUA and PIC respectively. Samples correspond to ● Red Ripe, ● Red,● Light Red,● Orange ● Breaker/Turning, ● Mature Green.

Some of these secondary relationships could be driven by the formation of intermediates required for the biosynthesis of specific ripening related metabolites. Production of such intermediates is likely to occur during some of the ripening stage transitions, with very little endogenous intermediates at the onset and culmination of ripening. Therefore, MS features that follow this trajectory during ripening would cause groups 1 and 6 to be more comparable as the more 'stable' states of fruit ripeness, in contrast to the transitional stages where much of the internal biochemistry of the fruits is in flux. The most powerful relationship between samples follows the ripening arc, showing that, although there are many other biochemical shifts in the data, the dominant changes are those that differentiate samples based on ripening progression. Interestingly, the overlap between ripening stages is more pronounced in some cultivars than others, with Juanita showing significant similarity between different ripening stages/fruit positions than Piccolo or Axiani. As ripening stages were classified visually, mirroring the standard practice in commercial settings, this could indicate more significant shifts within the active biochemical pathways in Juanita fruit, independent of carotenoid biosynthesis. In each case, positions 1-3 are much more closely related than the final three positions, showing that many of

the biochemical shifts in the metabolome of tomato fruits occur in the initial, transitional ripening stages. A plateauing of biochemical shifts at the later stages of ripeness is expected, as many of the metabolites in flux at the transitional stages 'breaker/turning' and 'orange', will have specific function in changing the physiology and composition of the fruit.

The primary drivers in this observable relationship for AXI can be seen in **Figure 8.2**, JUA in **Figure 8.3** and PIC in **Figure 8.4**. For the purposes of understanding and visualising the data in the most accessible capacity, only the top 50 most important features in each PLS-DA model have been included. However, many of the other detected features also show similar, but less pronounced concentrational changes over the ripening process in the samples.

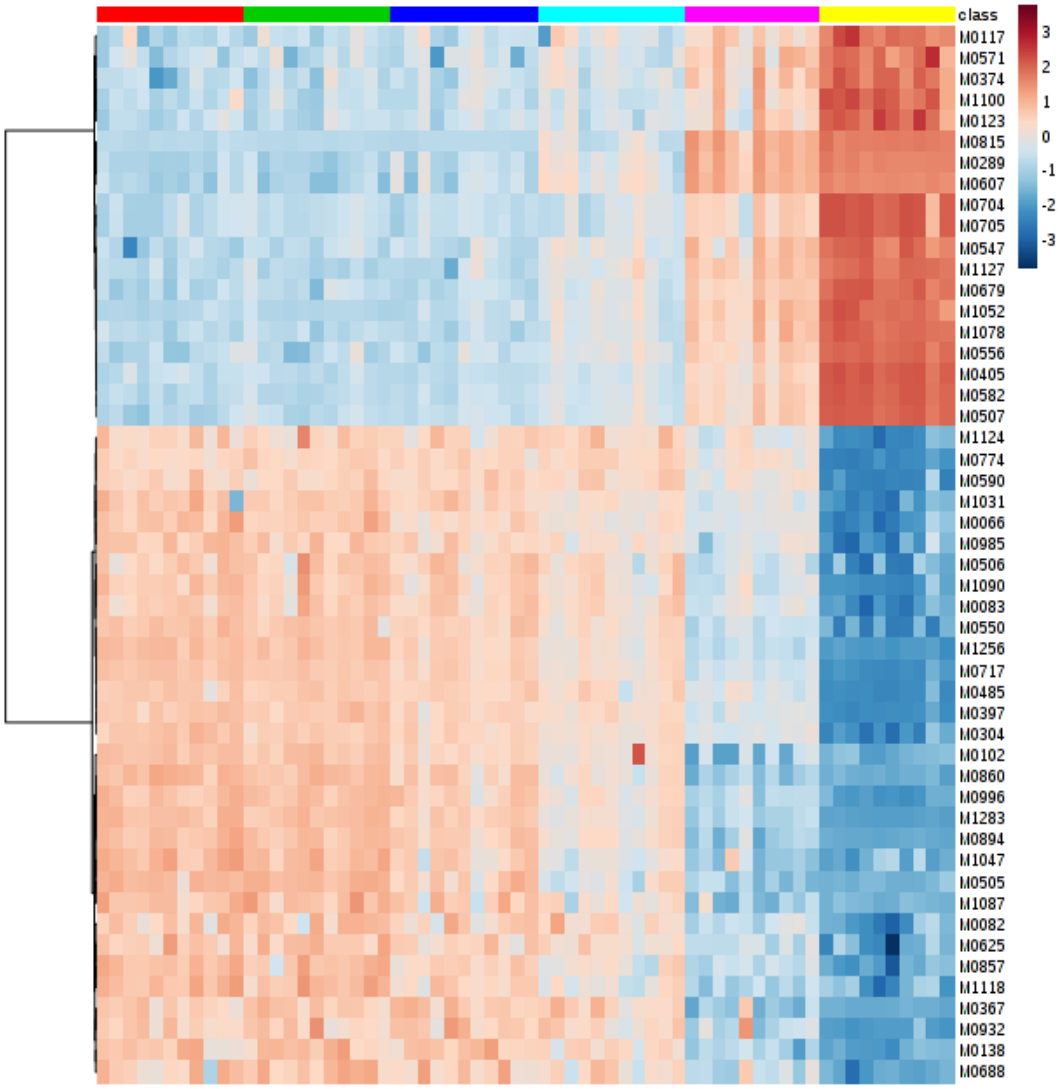


Figure 8.2 – Heatmap of the relative concentrations of the top 25 important features in 65 Axiani samples as determined by PLS-DA. Distance measures are calculated based on absolute Elucidian distance with clustering determined by Ward's method. Graphic generated based on the normalised peak table of 1,329 MS features, with autoscaling of features. Classes correspond to ○ Red Ripe, ○ Red, ○ Light Red, ○ Orange, ○ Breaker/Turning, ○ Mature Green.

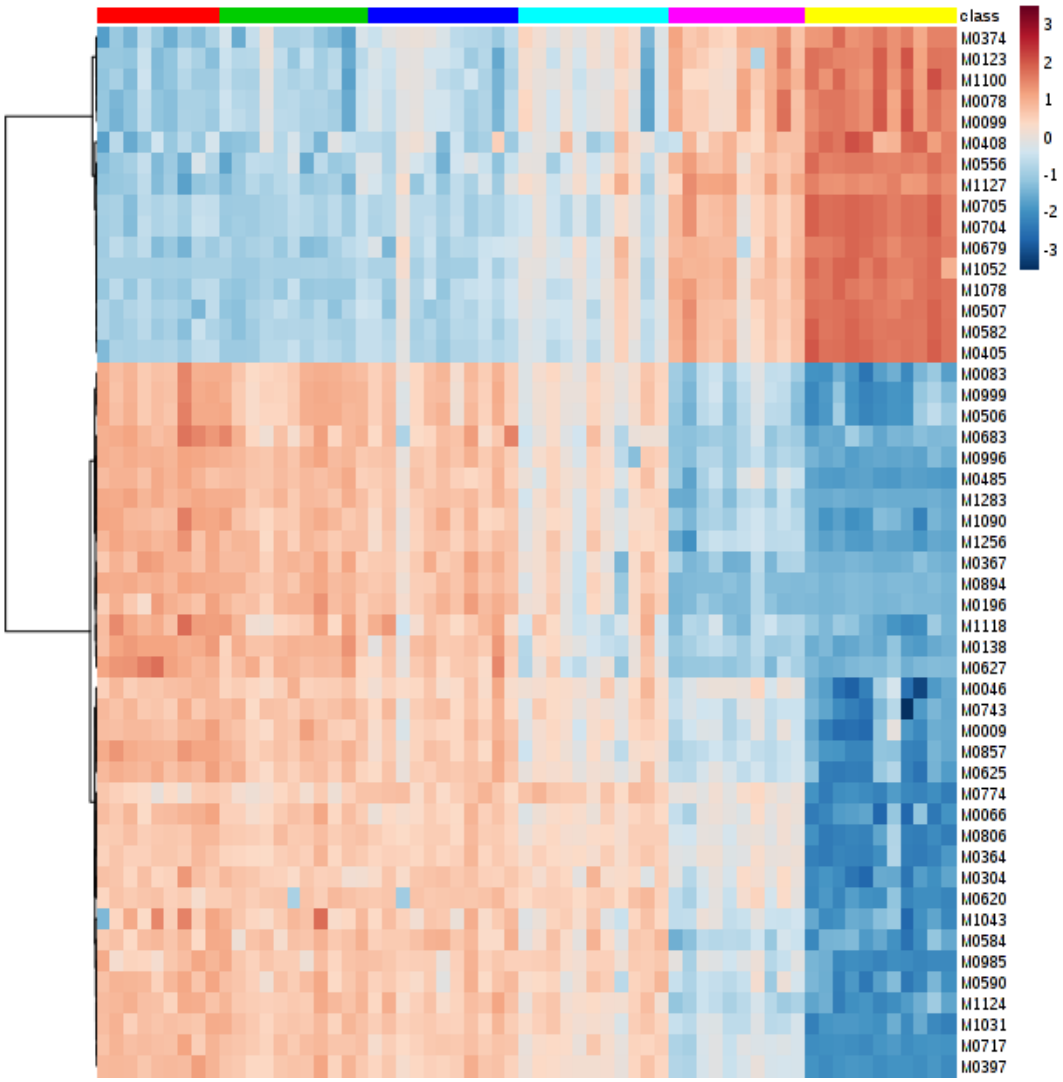


Figure 8.3 – Heatmap of the relative concentrations of the top 25 important features in 65 Juanita samples as determined by PLS-DA. Distance measures are calculated based on absolute Elucidian distance with clustering determined by Ward's method. Graphic generated based on the normalised peak table of 1,329 MS features, with autoscaling of features. Classes correspond to ○ Red Ripe, ○ Red, ○ Light Red, ○ Orange, ○ Breaker/Turning, ○ Mature Green.

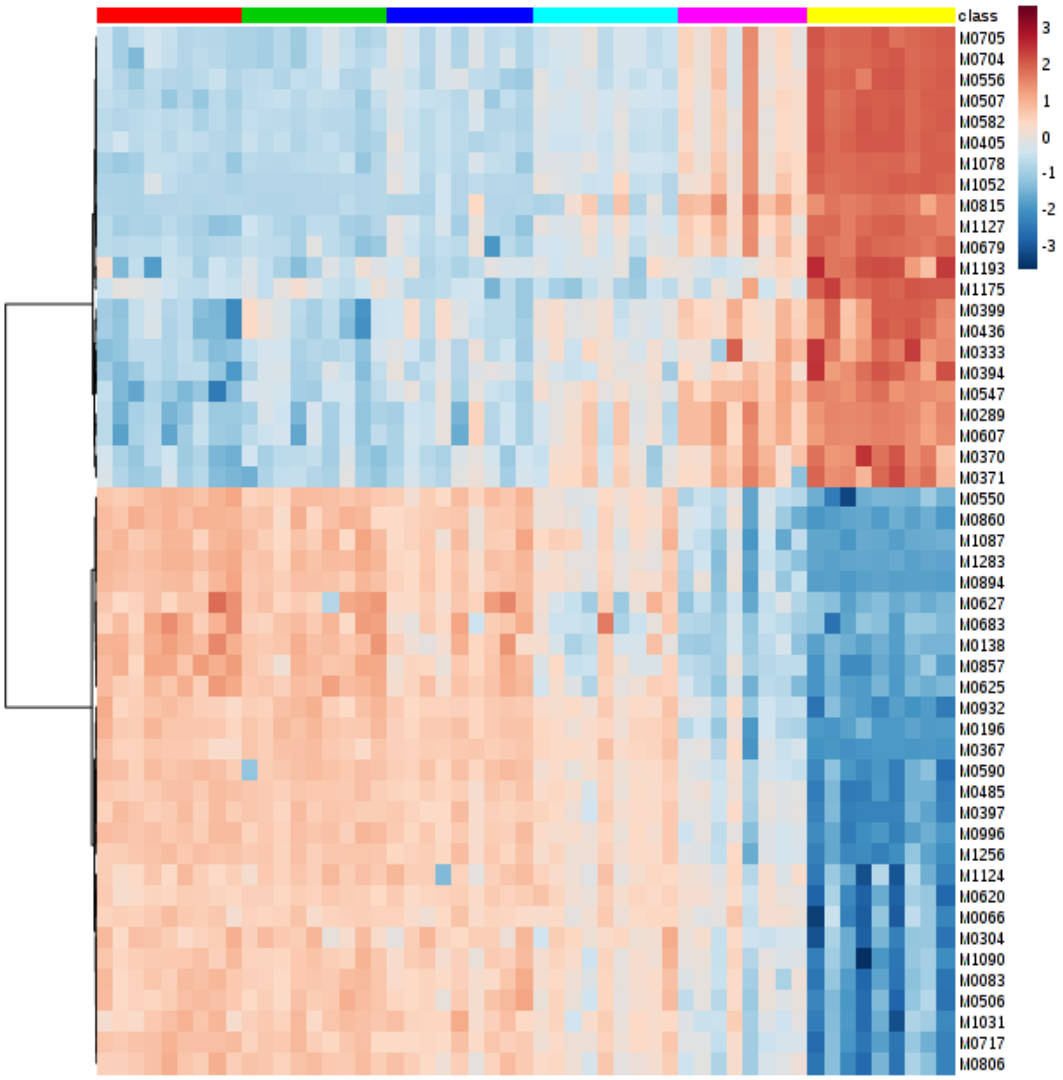


Figure 8.4 – Heatmap of the relative concentrations of the top 25 important features in 65 Piccolo samples as determined by PLS-DA. Distance measures are calculated based on absolute Elucidian distance with clustering determined by Ward's method (Ward Jr, 1963). Graphic generated based on the normalised peak table of 1,329 MS features, with autoscaling of features. Classes correspond to ○ Red Ripe, ○ Red, ○ Light Red, ○ Orange ○ Breaker/Turning, ○ Mature Green.

Figure 8.2, **Figure 8.3** and **Figure 8.4** highlight the most influential mass spectral features in relation to the PLS relationship observable in **Figure 8.1**. The significant overlap of the riper positions, classes 1-3, is echoed in the heatmaps, with very few observable differences in any of the cultivars aside from slight concentrational shifts in certain metabolites. This indicates that, even at the 'Light Red' stage of ripening, many of the processes responsible for the synthesis or catabolism of metabolites during ripening have plateaued or halted. As expected, the point where ripening 'kick starts', between mature green and breaker fruits, is the most dynamic, with strong shifts in the production or catabolism of every metabolite visible on each heatmap. During this time, significant biochemical modification of the fruits occurs at a cellular and process level. Fruits begin catabolising chlorophyll, converting chloroplasts to chromoplasts and synthesising various pigments, primarily carotenoids. Lycopene, has been shown to increase from undetected

levels in mature green fruits to between 50-125 mg/Kg in fully ripened tomatoes, depending on cultivar (Arias *et al.*, 2000a, Brandt *et al.*, 2006, López Camelo and Gómez, 2004). In addition, monosaccharides accumulate from the catabolism of storage polymers and imported sucrose. Change in concentration of other endogenous metabolites ranging from amino acids, volatiles organic acids and antioxidants have been previously reported (Sorrequieta *et al.*, 2010, Baldwin *et al.*, 1991b, Alexander and Grierson, 2002, Kosma *et al.*, 2010). Not all of the changes that occur in the composition of ripening fruits are directly pertinent to quality and flavour. However, many of the unrelated compounds may have similar biosynthetic routes, precursors/intermediates, metabolic functions and defence functionality. Although not directly perceivable during consumption, the importance of these processes and the metabolites for overall fruit viability and continued growth and ripening cannot be overlooked. Therefore, although much of the efforts in this work have gone towards investigating the formation of flavour-active and quality determining biomarkers and metabolites, other compounds that play a more background role in fruit functions are not excluded from identification and characterisation.

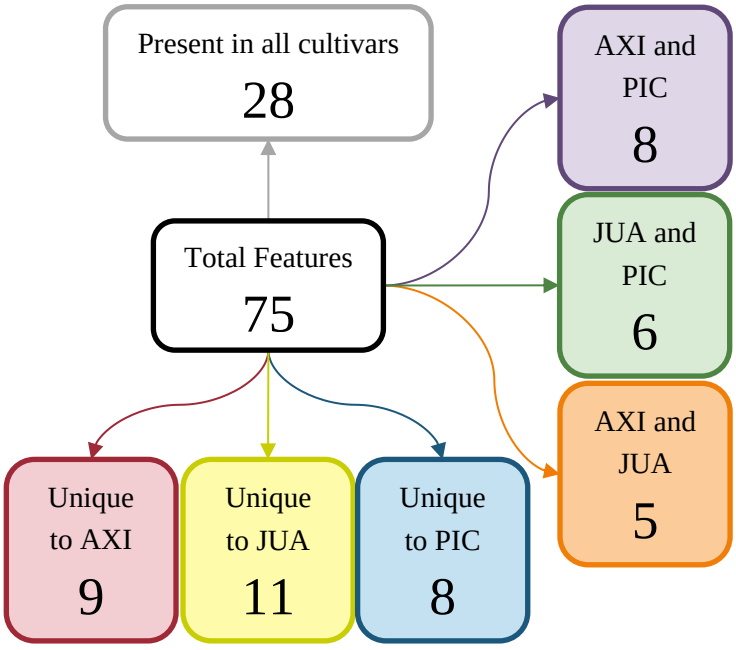


Figure 8.5 – Distribution of features based on calculated importance by PLS-DA. Separated into those that are unique to a single cultivar and those that appear in two or three.

A list was populated from each of the features that occurred in at least one of the heatmaps above, a total of 75 unique features. Of that number, 28 were strongly correlated to the ripening process in each of the three cultivars, either up- or downregulated. In addition, 9, 11 and 8 features were unique to the ripening processes in Axiani, Juanita and Piccolo fruits respectively. The remaining features were absent in the top 50 list of one cultivar, but present for the other two, suggesting similar biosynthetic pathways are downregulated in one cultivar and more active in the others. This is visualised in **Figure 8.5** above. Is not to say that those that are only in one of the top 50

lists are not present in the other cultivars at all, it is just indicative of less significant changes in this feature throughout the ripening process. The differences between the cultivars may be due to the subtle differences in genetics of each cultivar, with certain biosynthesis pathways for non-vital secondary metabolites upregulated in one cultivar and under expressed in others.

8.5 Cultivar and Ripening Biomarker Identification through SIRIUS Matching

All the significant features of the PLS-DA analysis that scored above 1 were viable targets for MS2 isolation, fragmentation and identification and were therefore investigated. However, upon performing MS2 analyses, not all targets were suitable for this methodology, either due to coeluting, interfering masses, decrease in peak area between MS1 and MS2 analyses, inability to find/isolate the molecular ion or a combination of these factors. Therefore, of the 144 features taken forward for MS2 investigation, only 60 were confirmed as being isolated and successfully fragmented. Moreover, some of these targets were of low abundance and therefore the MS2 spectra were too 'noisy' to yield identity matches above 70% in SIRIUS. The following section focuses on those compounds which were correctly isolated and reliably fragmented and produced greater than 70% identity matches in the SIRIUS software. Matches and identifications from the SIRIUS workflow are considered tentative at present, with the analysis of authentic, analytical standards required for confirmation. The remaining mass spectral features are listed, along with their PLS-DA loading scores, to indicate their importance and need for follow up study to confirm their identities as seen in **Appendix 6**.

8.5.1 Quality Related Biomarkers

8.5.1.1 M0379 – Serotonin / 3-(2-aminoethyl)-1H-indol-5-ol

Mass spectral feature M0379 was shown to be present above the peak area cut-off threshold value in 16 of the 18 experimental classes, with only groups 1 and 4 of JUA not presenting sufficient for isolation and identification by MS2.

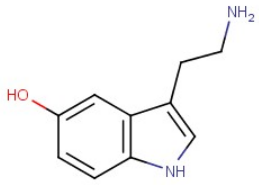
Proposed Molecular Structure	
Name / IUPAC Name	Serotonin / 3-(2-aminoethyl)-1H-indol-5-ol
Molecular Formula	$C_{10}H_{12}N_2O$
Exact Mass	176.09496
Mass±H	177.10370
Detected Accurate Mass	177.09642
Isotope Score / Fragmentation Score	3.47 / 32.35
Total Explained Intensity	92.64%
SIRIUS Score	92.25%

Figure 8.6 below shows the normalised distribution of M0379 across the ripening profile of each cultivar. A general trend of increasing accumulation as fruits reach full ripeness is easily observable. Later stages of ripening present much more comparable concentrations

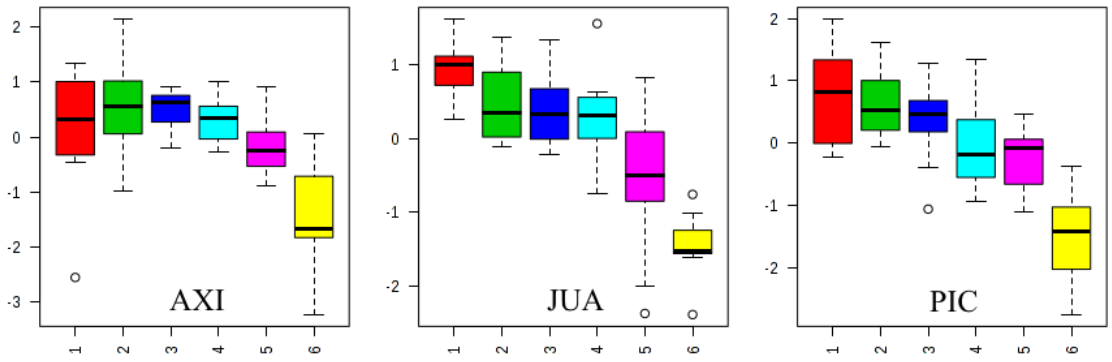


Figure 8.6 – Normalised concentration following PLS-DA analysis for M0379 in each cultivar following data normalisation. Ripening is inverse to group numbers, whereby red ripe (RR) fruits are group 1 and mature green (MG) fruits are group 6. Boxplots represent quartile based distribution of replicates with median samples marked as horizontal, black bars (n=10 replicate samples per group, plus one group pool).

Serotonin, 3-(2-aminoethyl)-1H-indol-5-ol, also commonly referred to as 5-Hydroxytryptamine, is a biochemical signalling and messenger molecule widely found in both plants and animals. Serotonin is derived from tryptophan, via tryptamine, through the sequential action of tryptophan decarboxylase (TDC) and tryptamine 5-hydroxylase (T5H) (Kang *et al.*, 2008, Murch *et al.*, 2000). In animals, serotonin plays vital roles in cognition, mood, cardiovascular health and is the precursor for the formation of melatonin (Frazer and Hensler, 1999, Côté *et al.*, 2004). Serotonin was first identified as a natural plant product by Bowden *et al.* in 1954 during their study of *Mucuna pruriens*, otherwise known as velvet bean (Bowden *et al.*, 1954). Since then, serotonin has been identified in over 42 separate species of plants and is known to play a role in numerous developmental processes and pathogen defence (Kang *et al.*, 2007, Ishihara *et al.*, 2008). The purportedly diverse roles of serotonin in plants range from regulation of vegetative and reproductive tissue growth, plant morphology and reproduction, amongst others (Odjakova and Hadjiivanova, 1997, Murch *et al.*, 2001, Kang *et al.*, 2008). Primarily, serotonin is localised within reproductive tissues, with numerous studies reporting orders of magnitude higher concentrations in fruits and seeds as opposed to vegetative tissues in various plants (Ly *et al.*, 2008, Pilar Nicasio *et al.*, 2005, Giridhar and Ravishankar, 2009, Fellows and Bell, 1971, Akula *et al.*, 2011). However, serotonin is one of several compounds thought to protect against biological attack by insects, pathogens and potentially larger herbivores through skin irritation (Schildknecht, 1981, Ishihara *et al.*, 2008, Huang *et al.*, 2011, Bowden *et al.*, 1954). Serotonin has been previously reported to be present in tomato fruits a number of studies (Udenfriend *et al.*, 1959, Ly *et al.*, 2008, Akula *et al.*, 2011, Feldman and Lee, 1985, Kang *et al.*, 2008). Feldman and Lee reported that tomatoes had a mean serotonin concentration of 3.2 ±0.6 µg/g, considered to be in the 'high' category of plant based dietary sources of serotonin. Additionally, the authors noted that both the seeds and flesh of the fruits contained similar levels, 3.2 and 3.7 µg/g respectively, but that the skin only contained 0.8 µg/g (Feldman and Lee, 1985). This is contrary to the findings in other fruits, specifically banana and pineapple, where the exocarp presented the highest levels of serotonin accumulation, which would agree with its postulated role in dissuading predation by insects and small herbivores (Udenfriend *et al.*, 1959, Akula *et al.*, 2011). Serotonin has been shown to increase from 0.18-3.75 µg/g through the ripening process in tomato fruits, with an eventual decrease to 2.9 µg/g in over-ripe tomatoes (Akula *et al.*, 2011). This contrasts with the noted reduction in serotonin levels in many other fruits and plants throughout maturation and ripening as reviewed and discussed by Akula, Giridhar and Ravishankar (Akula *et al.*, 2011). However, the findings of this study agree with the increase in serotonin concentration in ripening fruits, with development of serotonin throughout ripening shown in **Figure 8.6** above.

8.5.1.2 M0743 – Adenosine, 2-(6-amino-9H-purin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol

Proposed Molecular Structure	
Name / IUPAC Name	Adenosine / 2-(6-amino-9H-purin-9-yl)-5-(hydroxymethyl)oxolane-3,4-diol
Molecular Formula	$C_{10}H_{13}N_5O_4$
Exact Mass	267.09675
Mass±H	268.10529
Detected Accurate Mass	267.09801
Isotope Score / Fragmentation Score	2.88 / 48.74
Total Explained Intensity	100.0%
SIRIUS Score	97.24%

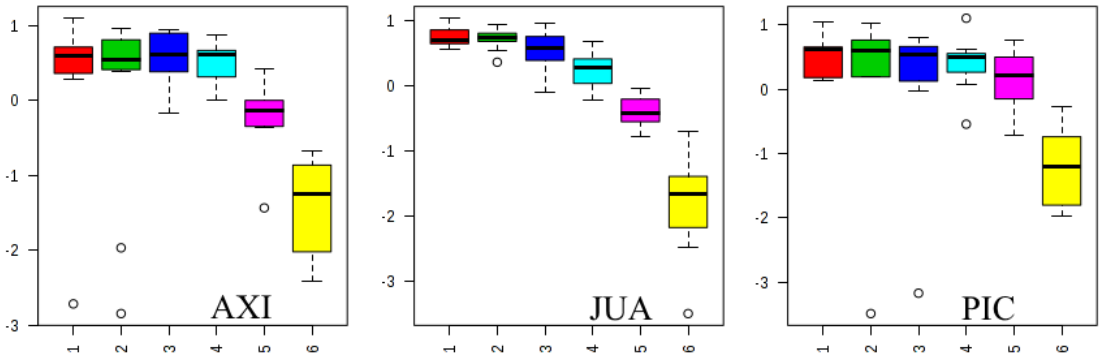


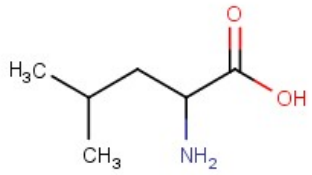
Figure 8.7 – Normalised concentration following PLS-DA analysis for M0743 in each cultivar following data normalisation. Ripening is inverse to group numbers, whereby red ripe (RR) fruits are group 1 and mature green (MG) fruits are group 6. Boxplots represent quartile based distribution of replicates with median samples marked as horizontal, black bars (n=10 replicate samples per group, plus one group pool).

Adenosine is a purine nucleoside prevalent in all branches of life. Nucleotides are a vital class of compound that play roles in both primary and secondary metabolism, energy transfer, enzyme cofactors as well as the synthesis of DNA and expression of genes (Zrenner *et al.*, 2006). There are several routes of formation of nucleosides and nucleotides in plants, this can be broadly separated into *de novo* synthesis or scavenging and repurposing through biochemical processes. Zrenner states that the abundance of scavenging and salvage routes of nucleoside/nucleotide repurposing is probably due to the significantly reduced energy requirements in comparison to *de*

novo synthesis. *De novo* synthesis is a multistep process which occurs in the mitochondria and involves the biosynthesis of nucleosides from smaller precursor compounds such as CO2, amino acids and tetrahydrofolate, which requires 4 instances of ATP derivative dephosphorylation per synthesised nucleotide (Moffatt and Ashihara, 2002, Zrenner *et al.*, 2006). However, salvage pathways use the breakdown of nucleotides, particularly the dephosphorylation of mon-di and triphosphate nucleotides, to regenerate supplies of nucleosides, utilising only a single instance of ATP dephosphorylation.

In plants, adenine and uridine are more abundant than guanine or cytosine nucleotides, in part due to their role in the biosynthesis of other nucleotides and due to the importance of adenine nucleotides as energy transfer molecules through the dephosphorylation of ATP to ADP (Zrenner *et al.*, 2006). One of the most defining roles of adenosine and its related nucleotides in plants, is their role in the synthesis of cytokinin, a vital phytohormone involved in the development and maturation of fruits in many plant species (McAtee *et al.*, 2013, Mariotti *et al.*, 2011, Moffatt and Ashihara, 2002). Cytokinins have previously been shown to be important factors in the proper fruit set and development of tomato fruits by several studies (Srivastava and Handa, 2005, Matsuo *et al.*, 2012). Additionally, Adenosine monophosphate (AMP), derived from adenosine, is the most abundant, flavour-active nucleotide in ripe tomato fruits by a factor of 2-3 (Salles *et al.*, 2003, Oruna-Concha *et al.*, 2007).

8.5.1.3 M0833 – Leucine, 2-amino-4-methylpentanoic acid

Proposed Molecular Structure	
Name / IUPAC Name	Leucine / 2-amino-4-methylpentanoic acid
Molecular Formula	$C_6H_{13}NO_2$
Accurate Mass	131.09463
Mass±H	132.10292
Detected Accurate Mass	131.09564
Isotope Score / Fragmentation Score	1.36 / 14.43
Total Explained Intensity	96.04%
SIRIUS Score	85.21%

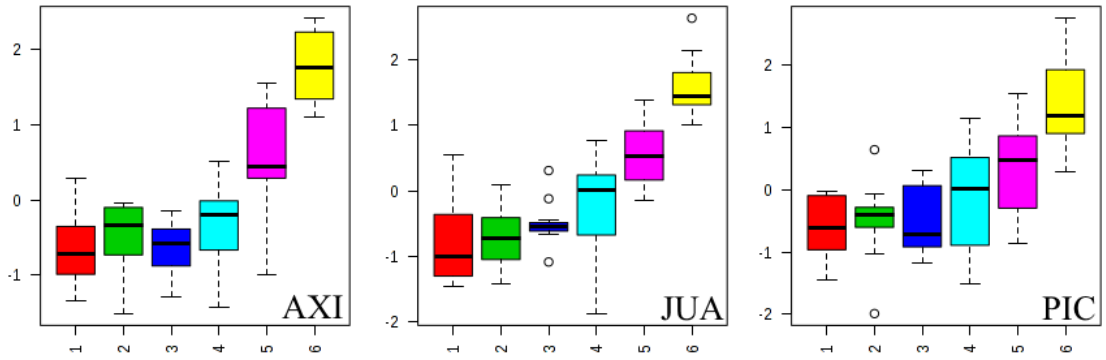
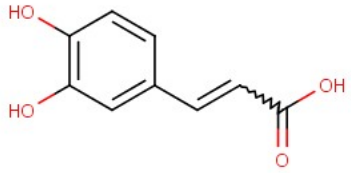


Figure 8.8 – Normalised concentration following PLS-DA analysis for M0833 in each cultivar following data normalisation. Ripening is inverse to group numbers, whereby red ripe (RR) fruits are group 1 and mature green (MG) fruits are group 6. Boxplots represent quartile based distribution of replicates with median samples marked as horizontal, black bars (n=10 replicate samples per group, plus one group pool).

The trapping of inorganic nitrogen as ammonia, nitrate or gaseous N_2 into carbon-based biomolecules is largely accounted for by the *de novo* synthesis of amino acids in higher plants, a vital aspect of plant growth and development (Lam *et al.*, 1996). Leucine is formed from the pyruvate amino acid synthesis pathway, with an amino group introduced from a second, donating molecule of pyruvate (Umbarger, 1978). The formation of alanine, valine and leucine is all directly controlled through the pyruvate pathway, with each amino acid deriving the majority of its carbon atoms from the breakdown of pyruvate. Additionally, there is a degree of overlap between the aspartate derived lysine and isoleucine, both of which consist of carbon liberated from both pyruvate and aspartate (Umbarger, 1978). Leucine is formed from a lateral pathway of α-ketovalerate, whereby, rather than transamination into valine, α-ketovalerate is converted to leucine through a set of sequential enzymatic reactions. An increase in Leucine content between mature green and ripening fruit has been previously shown by Boggio *et al.* where an increase from 175±48, 214±63 to 1.7161±48 nmol/g fresh weight as fruit progressed through green, yellow and red stages respectively (Boggio *et al.*, 2000). The increase in free leucine in ripe fruits is important for overall flavour, as leucine is the precursor to several flavour active volatiles, namely 3-methylbutanal and 3-methylbutanol (Zhang *et al.*, 2015). Additionally, one of the proposed biosynthetic routes of 2-isobutylthiazole utilises leucine or 3-methylbutanal as a precursor (Mathieu *et al.*, 2009). The accumulation of leucine would potentially yield the expected rise in these compounds in ripe fruits when compared to green tomatoes, particularly if their formation is substrate-limited.

8.5.1.4 M1047 – Caffeic acid, 3-(3,4-dihydroxyphenyl)prop-2-enoic acid

Proposed Molecular Structure	
Name / IUPAC Name	Caffeic acid / 3-(3,4-dihydroxyphenyl)prop-2-enoic acid
Molecular Formula	$C_9H_8O_4$
Exact Mass	180.04226
Mass±H	181.05045
Detected Accurate Mass	180.04317
Isotope Score / Fragmentation Score	3.03 / 85.97
Total Explained Intensity	99.53%
SIRIUS Score	86.94%

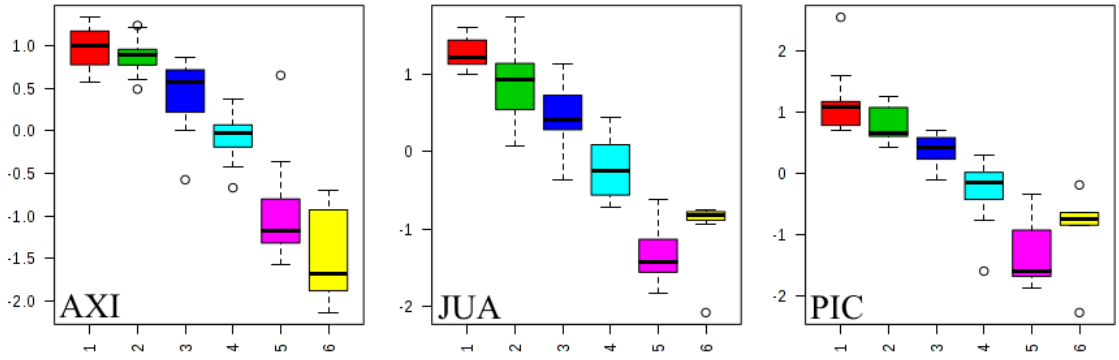


Figure 8.9 – Normalised concentration following PLS-DA analysis for M1047 in each cultivar following data normalisation. Ripening is inverse to group numbers, whereby red ripe (RR) fruits are group 1 and mature green (MG) fruits are group 6. Boxplots represent quartile based distribution of replicates with median samples marked as horizontal, black bars (n=10 replicate samples per group, plus one group pool).

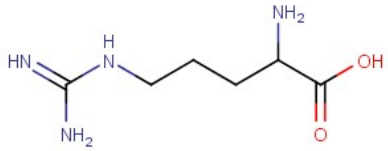
Caffeic acid, otherwise known as 3-(3,4-dihydroxyphenyl)prop-2-enoic acid, is a hydroxycinnamic acid ubiquitous in higher plants. Hydroxycinnamic acids form from the phenylpropanoid pathway, which is also involved in the formation of lignin and suberin, flavonoids, anthocyanins and tannins (Humphreys and Chapple, 2002, Dixon *et al.*, 2002, Schmutz *et al.*, 1993). Caffeic acid and its polymers are intermediates in lignin and suberin biosynthesis and, therefore, are often very abundant in woody tissues or low-moisture content plants (Halpin *et al.*, 1994, Sederoff *et al.*, 1994). In addition, caffeic acid is a cell wall component in many plant tissues, commonly increasing during cell wall loosening (Zimmerlin *et al.*, 1994). The phenylpropanoid pathway involves the deamination of phenylalanine, sequential enzymatic

reactions then synthesis a wide range of compounds including caffeic acid and lignin (Dixon *et al.*, 2002). Compounds derived from this biosynthetic route are extremely varied, with functions such as pigmentation, antioxidants, prooxidants, antibiotics, plant defence and cellular structure (Dixon *et al.*, 2002, Humphreys and Chapple, 2002).

Martínez-Valverde *et al.* previously reported the presence of hydroxycinnamic acids in the ripe fruits of 9 tomato cultivars. The authors showed a significant degree of inter-cultivar variability in the content of caffeic acid, between 1.39±0.34 and 13.00±1.62 for 'Senior' and 'Ramlette' tomatoes respectively (Martínez-Valverde *et al.*, 2002). Gautier *et al.* monitored the concentrations of caffeic acid glucoside and two unidentified caffeic acid derivatives during on-truss ripening of 'Cervil' tomatoes. They identified a non-significant rise in caffeic acid glucoside of 34.9-44.5 mg/Kg fresh weight, between immature green and red ripe fruits. This is contrasts with the previously reported accumulation of caffeic acid glucoside between immature green (1,120 nmol/g dry wt.) and pink fruits (1,720 nmol/g dry wt.) followed by a decrease at full ripeness (540 nmol/g dry wt.) (Fleuriet and Macheix, 1981). More interestingly, Gautier *et al.* noted the unidentified caffeic acid derivatives were undetected in immature fruits, rising to 16.6 and 11.0 mg/Kg by full ripeness (Gautier *et al.*, 2008). This could indicate an increase in caffeic acid throughout the ripening of tomato fruits, or the increased rate of conversion of caffeic acid to related derivatives.

Caffeic acid is not thought to contribute flavour directly, but as a component of lignin production, may be related to the levels of hydroxycinnamic acid and lignin derived volatiles in tomato fruits, such as methyl salicylate, eugenol and benzoic acid, which arise from tissue wounding and catabolism of cell wall constituents (Zhang *et al.*, 2015).

8.5.1.5 M1102 – Arginine, 2-amino-5-carbamimidamidopentanoic acid

Proposed Molecular Structure	
Name / IUPAC Name	Arginine / 2-amino-5-carbamimidamidopentanoic acid
Molecular Formula	$C_6H_{14}N_4O_2$
Exact Mass	174.11168
Mass±H	175.11980
Detected Accurate Mass	174.11252
Isotope Score / Fragmentation Score	0.00 / 37.01
Total Explained Intensity	96.16%
SIRIUS Score	94.91%

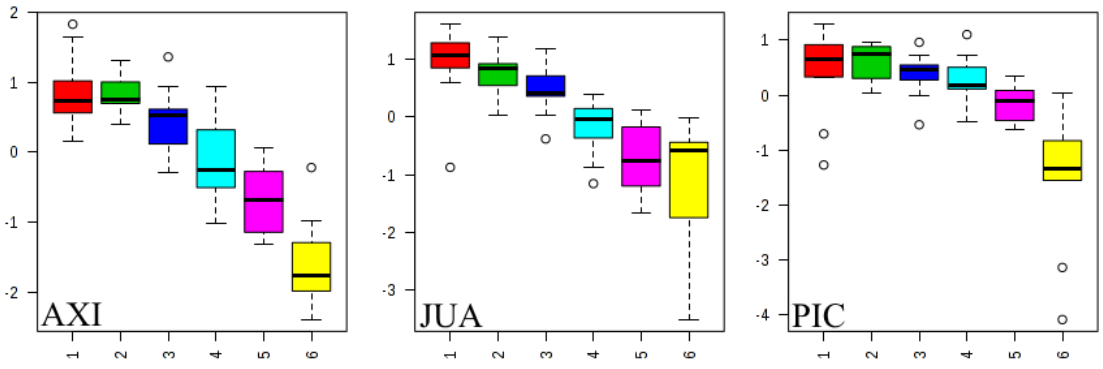


Figure 8.10 – Normalised concentration following PLS-DA analysis for M1102 in each cultivar following data normalisation. Ripening is inverse to group numbers, whereby red ripe (RR) fruits are group 1 and mature green (MG) fruits are group 6. Boxplots represent quartile based distribution of replicates with median samples marked as horizontal, black bars (n=10 replicate samples per group, plus one group pool).

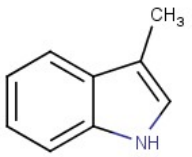
Unlike leucine above, arginine derives its carbon backbone from glutamate, along with proline and glutamine. The biosynthesis of arginine diverts from that of proline following the reduction of the γ-carboxyl of glutamate, when cyclisation is prevented through the transfer of an acetyl group from coenzyme A (CoA) forming N-acetylglutamate, the first intermediate in the biosynthetic route of arginine. Without the transfer of the acetyl group, the reduced glutamate would cyclise for form glutamic-γ-semialdehyde, an intermediate in proline synthesis (Umbarger, 1978). The formation of arginine from glutamate is via ornithine, which is well represented by both Winter *et al.* and Slocum. There are two routes of formation of arginine from glutamate, the cyclic and linear pathways, which have been reported in plants, bacteria and fungi, however animals are not able to progress ornithine or arginine synthesis beyond the formation of N-acetylglutamate (Slocum, 2005, Winter *et al.*, 2015).

Arginine is unique in proteogenic amino acids, in that it contains four nitrogen atoms and the highest nitrogen to carbon ratio (Winter *et al.*, 2015). This makes it well suited to fixing and storage of organic nitrogen in plants. Indeed, arginine has been shown to contribute up to 40% of the total nitrogen present in the storage proteins of the seeds of 379 angiosperm species (Van Etten *et al.*, 1967). In addition, arginine and its precursor ornithine, are precursors to the formation of several polyamine compounds, namely spermine, spermidine and putrescine in plants (Page *et al.*, 2012, Bagni and Tassoni, 2001). Polyamines have been shown to be vital for the proper formation of flowers, fertilisation and particularly essential to proper fruit set and development as well as playing roles in stress responses in higher plants (Majumdar *et al.*, 2013, Pathak *et al.*, 2014, Page *et al.*, 2012).

As with leucine, Boggio *et al.* demonstrated a rise in arginine concentration between green and yellow tomato fruits and a subsequent decrease at red ripe fruits back to concentrations comparable to those at mature green (Boggio *et al.*, 2000). Additionally, the polyamines derived from arginine have been shown to be present at the highest concentration in immature fruits, with a decline throughout fruit maturation and ripening (Dibble *et al.*, 1988). Rastogi, Dulson and Rothstein showed that arginine decarboxylase (ADC), one of the enzymes responsible for the initial conversion of arginine to putrescine, is expressed at greater levels in mature green fruits than any other maturity between immature and red ripe fruits (Rastogi *et al.*, 1993). Based on the findings of Dibble *et al.* this increase in ADC concentration does not yield additional putrescine in tomato fruits, suggesting that there are additional limitations or rate-limiting steps in present, preventing greater accumulation of the polyamide. This may explain the rise in free arginine, as one of the routes of metabolism slows in ripening fruits as opposed to maturing tomatoes.

8.5.2 Undefined Biomarkers

8.5.2.1 M0133 – Scatole, 3-methyl-1H-indole

Proposed Molecular Structure	
Name / IUPAC Name	Scatole / 3-methyl-1H-indole
Molecular Formula	C_9H_9N
Exact Mass	131.07350
Mass±H	132.08134
Detected Accurate Mass	131.07406
Isotope Score / Fragmentation Score	1.01 / 26.92
Total Explained Intensity	60.14%
SIRIUS Score	73.66%

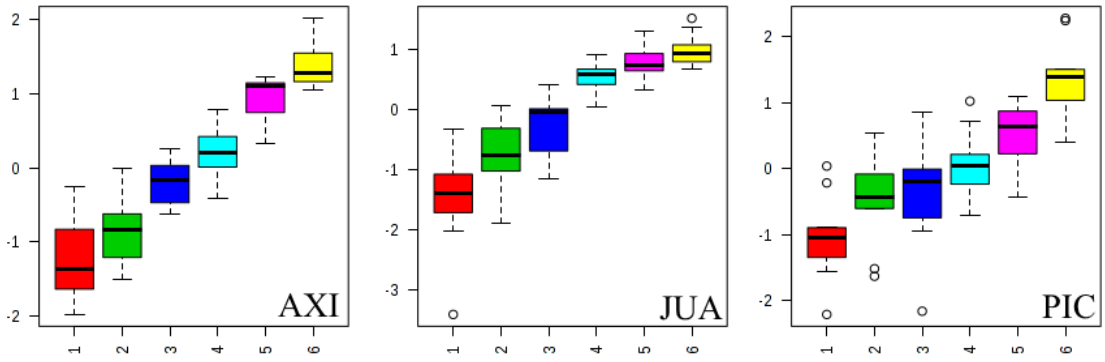


Figure 8.11 – Normalised concentration following PLS-DA analysis for M0133 in each cultivar following data normalisation. Ripening is inverse to group numbers, whereby red ripe (RR) fruits are group 1 and mature green (MG) fruits are group 6. Boxplots represent quartile based distribution of replicates with median samples marked as horizontal, black bars (n=10 replicate samples per group, plus one group pool).

Scatole is a naturally occurring indole, commonly found through digestion and catabolism of tryptophan, and therefore often associated with faeces and waste. This is compounded by the odour of the compound being 'fecal' at high concentrations. However, scatole is also present at lower concentrations in several flowers, and at which point, it presents as a pleasant, floral odour. As a member of the indole family, it is possible that biosynthesis routes for both scatole and other important indoles are formed through related pathways. One of the most biologically important indoles is indole-3-acetic acid, structurally similar to scatole, but with a carboxylic acid group attached to methyl present in scatole (Ljung *et al.*, 2002). Indole-3-acetic acid (IAA) is one of the

most potent and active auxin in many species of plants as well as being synthesised by bacteria in a synergistic plant-microbe relationship (Lambrecht *et al.*, 2000, Spaepen *et al.*, 2007). Like scatole, IAA can be synthesised from tryptophan by plants, although there is an additional tryptophan independent route of formation (Hull *et al.*, 2000).

8.5.2.2 M0370 – (4E)-2-aminooctadec-4-ene-1,3,10-triol

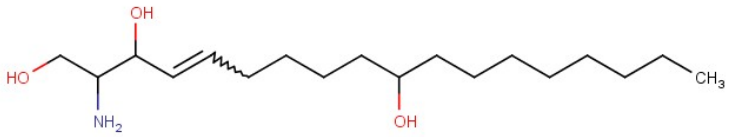
Proposed Molecular Structure	
Name / IUPAC Name	N/A / (4E)-2-aminooctadec-4-ene-1,3,10-triol
Molecular Formula	$C_{18}H_{37}NO_3$
Exact Mass	315.27734
Mass±H	316.28690
Detected Accurate Mass	315.27962
Isotope Score / Fragmentation Tree Score	4.14 / 150.45
Total Explained Intensity	98.12%
SIRIUS Score	73.59%

Figure 8.12 – Normalised concentration following PLS-DA analysis for M0370 in each cultivar following data normalisation. Ripening is inverse to group numbers, whereby red ripe (RR) fruits are group 1 and mature green (MG) fruits are group 6. Boxplots represent quartile based distribution of replicates with median samples marked as horizontal, black bars (n=10 replicate samples per group, plus one group pool).

The PLS-DA analysis revealed a trend of decreasing M0370 concentration in ripening tomato fruits. Comparable concentrations appear to be present in red fruits, positions 1-3, with higher concentrations in increasingly green, less mature fruits. There was little difference in the content of M0370 between cultivars, implying its role in ripening and associated biochemical shifts in the

fruit and its metabolism are common across multiple cultivars. This relationship may be true for most commercial hybrids, but the sample population of three cherry tomatoes is too small to confirm this.

The proposed identification of M0370 has not been previously reported and is not in any of the popular chemical databases, including those utilised for compound matching by SIRIUS. Based on the structure of the compound, it is possibly a derivative of sphingosine, a long chain alcohol, which occurs frequently in nature across plants, animals, bacteria and fungi (Sperling *et al.*, 1998). The reversible conversion of sphingosine to sphingosine-1-phosphate is an important cell signalling process in higher eukaryotes (Spiegel and Milstien, 2003).

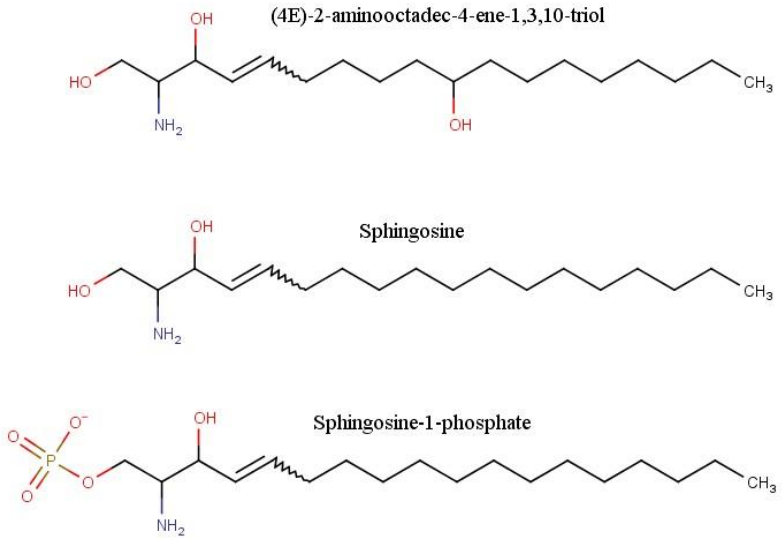
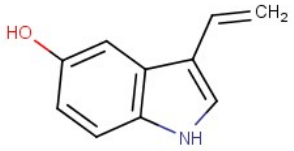


Figure 8.13 - Structural similarities between M0370 (top) and sphingosine and sphingosine-1-phosphate. Key difference is the hydroxylation of M0370 at carbon 10.

Figure 8.13, shows the structural similarities between the proposed identity of M0370 and sphingosine and its derivatives. The main difference is the additional hydroxyl group, which is uncommon in sphingosine derivatives; the main comparison is phytosphingosine, which gains an additional hydroxyl group, but sacrifices the double bond at C4. Based on the information gathered so far, the identity of this compound is still in question. Acquisition of standards of similar compounds may help to elucidate the correct identity or confirm the prediction made by SIRIUS.

8.5.2.3 M0380 – 3-ethenyl-1H-indol-5-ol

Proposed Molecular Structure	
Name / IUPAC Name	N/A / 3-ethenyl-1H-indol-5-ol
Molecular Formula	$C_{10}H_9NO$
Exact Mass	159.06841
Mass±H	160.07610
Detected Accurate Mass	159.06882
Isotope Score / Fragmentation Score	2.47 / 76.73
Total Explained Intensity	94.94%
SIRIUS Score	76.32%

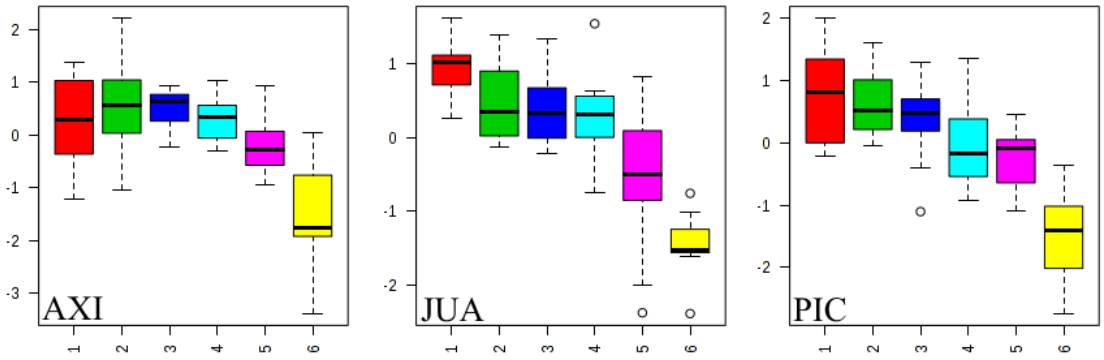
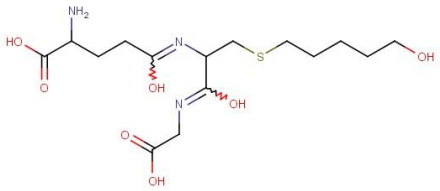


Figure 8.14 – Normalised concentration following PLS-DA analysis for M0380 in each cultivar following data normalisation. Ripening is inverse to group numbers, whereby red ripe (RR) fruits are group 1 and mature green (MG) fruits are group 6. Boxplots represent quartile based distribution of replicates with median samples marked as horizontal, black bars (n=10 replicate samples per group, plus one group pool).

After searching, there are no reports of 3-ethenyl-1H-indol-5-ol, a chemical analog of serotonin, in databases or published works as far as can be determined at this point. Based on the structural similarities and very comparable retention times with serotonin, M0379, it is likely that the biosynthetic routes for this compound are similar. The function and role of 3-ethenyl-1H-indol-5-ol is unclear, but as with other analogs of serotonin, such as Bufotenine, it is possible that it is psychoactive in animals and plays roles in plant growth and development in plants.

8.5.2.4 M0996 – 2-amino-4-([1-(carboxymethyl-C-hydroxycarbonimidoyl)-2-[(5-ydroxypentyl) sulfanyl]ethyl]-C-hydroxycarbonimidoyl)butanoic acid

Proposed Molecular Structure	
Name / IUPAC Name	N/A / 2-amino-4-([1-(carboxymethyl-C-hydroxycarbonimidoyl)-2-[(5-hydroxypentyl)sulfanyl]ethyl]-C-hydroxycarbonimidoyl)butanoic acid
Molecular Formula	$C_{15}H_{27}N_3O_7S$
Exact Mass	393.15697
Mass±H	394.16528
Detected Accurate Mass	393.15800
Isotope Score / Fragmentation Score	2.39 / 50.30
Total Explained Intensity	93.63%
SIRIUS Score	73.49%

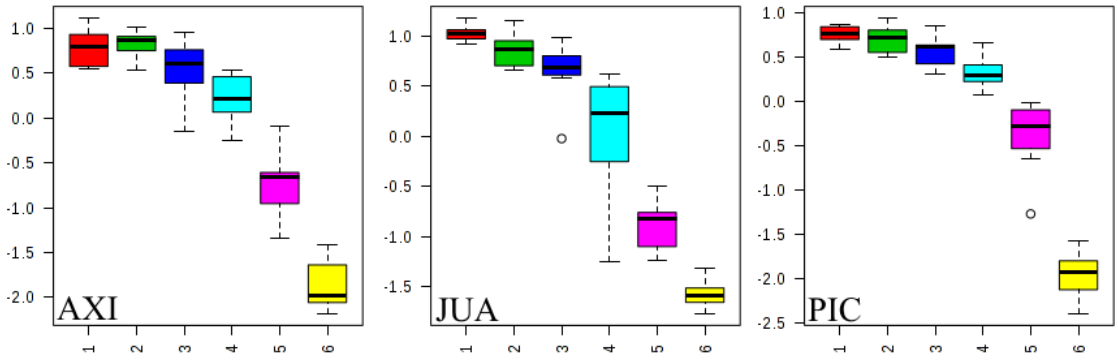


Figure 8.15 – Normalised concentration following PLS-DA analysis for M0996 in each cultivar following data normalisation. Ripening is inverse to group numbers, whereby red ripe (RR) fruits are group 1 and mature green (MG) fruits are group 6. Boxplots represent quartile based distribution of replicates with median samples marked as horizontal, black bars (n=10 replicate samples per group, plus one group pool).

There are no matches as to the identity of M0996 in published works or any of the available databases for metabolite identification. Based on the compound's structure it is possibly a derivative of glutathione, as it shares a strong similarity in terms of structure of the nitrogen and hydroxyl containing portion of the molecule. A possible route of synthesis in tomato may be from the condensation of 1,5-pentandiol to the thiol group of glutathione, with conversion of the amino groups to imidic acids as a preceding or succeeding step. Glutathione has been widely reported

as being present in tomatoes and other plants, as well as in bacteria, animals and fungi (Scheller *et al.*, 1987). The lack of literature surrounding the presence of 1,5-pentandiol in either plants or tomatoes suggests the reaction may be with another, unidentified compound, which would provide the pentanol chain.

8.6 Conclusions

The ripening profiles of three commercial, cherry tomatoes were characterised through LC-MS metabolomic profiling. Significant changes of numerous metabolites were visible in the dataset, with some of the most important metabolites strongly influencing the ripening relationship of all three cultivars. Other features were more cultivar specific, either less important in one of the cultivars and strong drivers in the relationships observed in the others, or only strongly influencing single cultivars and being underrepresented in the others. This indicates that at least some biochemical pathways are up- or downregulated based on the genetics of the individual cultivar. These pathways are likely to be responsible for the formation of metabolites that are either more abundant or absent in the cultivar, therefore strong drivers in separating those fruits from those of other distinct cultivars. This exploratory approach has revealed the ability to monitor the transition of over 1000 distinct mass spectral features over the ripening process of on-truss fruit at different ripening stages. In addition, 144 features that were most important drivers in ripening were taken forward for LC-MS/MS analysis for characterisation and identification. Of these, 73 were isolated and fragmented at multiple energy levels, with 9 scoring sufficiently well in the SIRIUS workflow to be considered tentatively identified. Of those that were identified in this manner, 5 of them were thought to impact fruit quality or flavour, either as flavour precursors, influencing fruit growth and development, roles in cellular structure or as nutritionally important compounds, such as antioxidants.

The remaining 4 compounds were less well defined in the literature and their identities and roles must be elucidated through the analysis of authentic standards before their impact on fruit quality can be proposed.

8.7 Future Work

A number of the aspects of this work could be repeated with the knowledge gained from this initial profiling. In doing so, more of the strongest drivers in ripening, both on a cultivar basis and common across cultivars, could be isolated and characterised. Significant improvements in the quality of the data are now possible as the workflow is well established, enabling rapid transition from initial profiling to target isolation and identification to be achieved in future studies. The reduction of time between the initial profiling and target isolation would dramatically minimise

changes in the samples, ensuring more of the significant features were isolatable. In addition, a pooling and concentration step could be applied to the MS2 workflow by which large pool samples could be made from the individual replicates and then concentrated by a factor of 10 or 20, making the isolation of less abundant metabolites much more achievable. This process would require initial validation and a suitable concentration methodology would need to be selected to minimise the impact of the process on the metabolites present in the pool samples. In addition, MS1 profiling could be repeated on the concentrated pool samples, increasing the overall peak areas and improving the isotopic abundance patterns of MS2 targets which would, in turn, enable more accurate identity prediction with SIRIUS or equivalent workflows.

Following this initial, untargeted metabolomic profiling approach it seems likely that a targeted workflow focused on some of the more important compounds in tomato flavour and quality would provide significant information on the activity of biochemical pathways responsible for quality. This may be a powerful route to replace the traditional targeted approaches that have been employed for the last 50 years, allowing targeting of some of the most important features and semi-quantitative determination of cultivar or treatment-driven effects to be better understood. As demonstrated by multiple research groups in the last 5 years, a combined omics approach is likely to lead to the most comprehensive understanding of the biological processes and metabolomic fluxes in tomato ripening and quality. Pairing genomics, transcriptomic, proteomics and metabolomics together would provide absolute coverage of the system biology of the target material, however, initially metabolomics and proteomics/ transcriptomics may reduce overall investment of time and money while still allowing for the identification of the most important pathways in fruit development and ripening. As a potential route of quality assurance and commercial product improvement, reliance on just two omics approaches would reduce the cost to the industry whilst providing a significant amount of information about the fruits.

9 Overall Conclusions and Future Work

9.1 Concluding Remarks

The primary aim of this study was to investigate the quality of fresh, commercial tomatoes, and those compounds responsible for the desirable organoleptic traits associated with current, successful commercial cultivars with the objective to identify potential new routes for genetic-led, breeding strategies. A multifaceted approach was applied to enable the greatest coverage of fruit biochemistry, relevant to the overall quality and perceived organoleptic character of the studied fruits. This encompassed metabolomic profiling, targeted analysis of taste and flavour-active compounds, sensory profiling, market research and correlation of metadata to crop quality. Moreover, the effect of growth and harvest season, ripening catalysed development of flavour and taste-active compounds and localisation of these compounds in different tissues of tomatoes were also explored better inform future breeding programs of potential targets for improving current cultivars.

Metabolomic profiling was conducted on ten commercially grown tomato cultivars consisting of one beefsteak at two harvests, three salad types, two plum and four cherry type tomatoes, one of which was a non-standard ripening ‘tangerine’ tomato. Using binary classification methods of ‘cultivar’ vs. ‘not cultivar’ allowed for the use of multivariate ROC curves for cluster discrimination. This approach revealed 134 discriminating features that were upregulated in one or more cultivars. With these features alone, samples could be correctly clustered based on cultivars. These discriminating features were taken forward for fragmentation and identification by MS/MS. Of these, 7 compounds were tentatively identified using Sum formula Identification by Ranking Isotope patterns Using mass (SIRIUS) data analysis workflow. The identified compounds were mainly related to tryptophan biosynthesis, with three being flavanones, which derive from the phenylpropanoid pathway and tryptophan biosynthesis, including naringenin, prunin and hesperetin. The cultivars most commonly representing upregulation of these compounds and associated pathways were Oranjestar, Juanita and DR2, the last two of which were closely clustered following PCA, indicating strong similarities in fruit biochemistry, from two distinctly different types of tomato. This further reinforces the power of marrying genomic and metabolomic approaches as useful tools of crop monitoring and improvement and is particularly valuable, in current investigations attempting to reintroduce genetic variation to modern hybrids via crosses with wild-type species.

The quantification of flavour and taste-active metabolites through targeted chemical analyses enabled highly effective classification of each of the studied cultivars based on volatile profile alone, achieving a correct classification rate of 91.7% following cross validation of the model.

Salad type cultivars presented the only difficulty in terms of classification, with much more comparable volatile profiles across the class of tomatoes than cherry or plum types. In addition, the correlation of sensory analysis intensity meta-data against the equivalent calculated intensity based on chemical analysis generated highly correlated linear regression models for sweetness, sourness and umami using related, but not identical samples. This indicates that predictive sensory analysis models for the primary gustatory senses responsible for good tomato taste are likely highly achievable following an expanded and more dedicated experimental design. Previous modelling of analytical and sensory data has attempted to include both olfactory and gustatory values, which is far more complex and susceptible to variance. Avoiding multivariate modelling in favour of simple linear regression may enable trained sensory analysts to become the approved, industry standard for the measurement of these aspects of tomato quality, eliminating the reliance on less reliable simplistic measurements such as total soluble solids or pH. Alternatively, the models also allow for the estimation of each of the gustatory sensations based on targeted analysis of the taste active compounds. Expanding this approach to include olfactory impact of cultivars may be possible after strong predictive capacity is achieved for the gustatory parameters.

Targeted and not targeted approaches employed in the investigations of the evolution of the biochemical traits during ripening in 3 cherry tomato cultivars enabled better understanding of the metabolic shifts particularly at the pivotal 'Breaker/Turning' point of fruit ripeness. Amino acid, sugar, volatiles and organic acids were monitored highlighting the genetic variation at all stages of ripening between cultivars. Metabolomic data revealed 144 discriminant features of which 9 were tentatively identified and included amino acids arginine and leucine, antioxidants caffeic acid and indole derivatives which have been shown to regulate the ripening process. Although only a small sample of the discriminant features were tentatively identified there is scope to expand this work in order to characterise further potential biomarkers of ripening that could be targets for genetic manipulation to enable improved control and/or more homogenous ripening.

This study has demonstrated distinct localisation effects for several compounds important to fruit flavour and taste, indicating the roles of various tissues in the perception and impact of fresh tomato consumption. In addition, the targeted analysis of the endogenous volatile fraction of tomatoes revealed that only 10-20% of the lipid oxidation derived volatiles are present in the intact fruit while more than 80% of the C6 aldehydes form following tissue damage and disruption. Moreover, the presence of 2-isobutylthiazole at significantly higher levels than previously reported in literature was thought to be due to the on-truss storage of samples and prevention of volatile losses on tissue disruption. This finding is highly significant as the volatile has been previously correlated with increased liking in fresh tomato cultivars. This may also

indicate that the truss plays a more active role in tomato quality than previously thought, with particularly high concentrations of this desirable compound being observed.

Market research using open forum, sensory analysis and questionnaires indicated that communication of pertinent research and commercial knowledge about tomato flavour and quality with consumers is potentially not adequate at present. Information about quality aspects such as flavour being typically inversely related to fruit size, post-purchase storage practices, differences in tomato quality relative to price and the benefits of buying high quality, British fruits over prematurely harvested imported fruit are perhaps not effectively disseminated between average UK consumers. One industry lead step to improving public opinion and engagement may focus on advertising in supermarkets indicating some of these facts.

9.2 Future Directions

This research has identified many avenues of interest that would likely lead to better understanding of tomato flavour and quality, new breeding strategies, correlation based models as methods of standard quality assessment and better consumer engagement.

One of the most promising research direction that maybe highly beneficial to the commercial sector is the formation of standardised correlations between instrumental analysis and trained sensory panel intensity data. In this study analytical and sensory data was derived from comparable fruits, harvested from the same plants and growth environments, which enabled the strong correlation between sweetness, sourness and umami intensity values. However, an experimental design focussed on the development of these simple linear regression models using the same fruit for each measurement may further improve the accuracy of the analytical vs sensory intensity models. A method like this would be far more sophisticated and valuable than the current practice of simplistic %brix or pH measurements, potentially becoming the industry standard for new cultivar assessment. The correlation and prediction of volatiles and the resulting organoleptic sensation has been previously demonstrated to be significantly more challenging. However, inclusion of the relevant attributes and analytical determination of these compounds in this experimental design, may enable the evolution of the constructed models to include partial or full flavour inputs as well.

The role of 2-isobutylthiazole as well as a definitive route of formation seems to be the next logical step in understanding this compound, its role in tomato flavour and the mechanism by which it accumulates to such a high degree prior to fruit damage. Based on preliminary analysis of the truss itself, a link between the truss and fruit, either by direct import or adsorption of the compound to the fruit surface following release from the truss seems plausible. Due to its strong

correlation to consumer liking, this would indicate some of the reasons behind on-truss tomatoes being perceived as higher quality. Additionally, if adsorption to the fruit surface is the method by which it accumulates there may be scope to incorporating the compound at predefined concentrations in the edible coatings currently being developed for fresh produce. This may lead to an increase in shelf life and fruit stability from the coating and increase consumer liking due to the 2-isobutylthaizole spike.

The discriminate metabolites of cultivars, seasonality of the 'Elegance' crops and biochemical flux associated with fruit ripening are all potential targets for further investigations. The identification of the compounds by the SIRIUS workflow used in this thesis is only tentative and subject to confirmation using analytical standards. Additionally, there are many features of significance that were not isolatable during this work. Sample concentration and potentially, fractionation, may yield higher abundant targets or eliminate background components to allow for better isolation and identification of these features.

Consumer engagement and a greater degree of interaction between the typical UK consumer and tomato growers may help to educate the public, as well as improving public opinion on tomatoes. A comprehensive, wide ranging, market research survey may be able to expand upon the findings of this thesis. Development of such as survey is challenging, as it needs to glean significant information from the public, whist still being short and non-invasive. However, better understanding of the level of knowledge of the aspects of tomato flavour, purchasing habits of consumers and the areas of most dissatisfaction may help to further inform new research directions.

10 References

Abegaz, E. G., Tandon, K. S., Scott, J. W., Baldwin, E. A. & Shewfelt, R. L. 2004. Partitioning taste from aromatic flavor notes of fresh tomato (Lycopersicon esculentum, Mill) to develop predictive models as a function of volatile and nonvolatile components. *Postharvest Biology and Technology,* 34**,** 227-235.

Akarachantachote, N., Chadcham, S. & Saithanu, K. 2014. Cutoff threshold of variable importance in projection for variable selection. *International Journal of Pure and Applied Mathematics,* 94**,** 307-322.

Akihiro, T., Koike, S., Tani, R., Tominaga, T., Watanabe, S., Iijima, Y., Aoki, K., Shibata, D., Ashihara, H., Matsukura, C., Akama, K., Fujimura, T. & Ezura, H. 2008. Biochemical Mechanism on GABA Accumulation During Fruit Development in Tomato. *Plant and Cell Physiology,* 49**,** 1378-1389.

Akula, R., Giridhar, P. & Ravishankar, G. A. 2011. Phytoserotonin. *Plant Signaling & Behavior,* 6**,** 800-809.

Alexander, L. & Grierson, D. 2002. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. *Journal of Experimental Botany,* 53**,** 2039-2055.

Amemiya, T., Kanayama, Y., Yamaki, S., Yamada, K. & Shiratake, K. 2005. Fruit-specific V-ATPase suppression in antisense-transgenic tomato reduces fruit growth and seed formation. *Planta,* 223**,** 1272.

Andersen, C. M. & Bro, R. 2010. Variable selection in regression—a tutorial. *Journal of Chemometrics,* 24**,** 728-737.

Anthon, G. E., LeStrange, M. & Barrett, D. M. 2011. Changes in pH, acids, sugars and other quality parameters during extended vine holding of ripe processing tomatoes. *Journal of the Science of Food and Agriculture,* 91**,** 1175-1181.

Arias, R., Lee, T.-C., Logendra, L. & Janes, H. 2000a. Correlation of Lycopene Measured by HPLC with the L*, a*, b* Color Readings of a Hydroponic Tomato and the Relationship of Maturity with Color and Lycopene Content. *Journal of Agricultural and Food Chemistry,* 48**,** 1697-1702.

Arias, R., Lee, T. C., Specca, D. & Janes, H. 2000b. Quality Comparison of Hydroponic Tomatoes (Lycopersicon esculentum) Ripened On and Off Vine. *Journal of Food Science,* 65**,** 545-548.

Arvidson, K. & Friberg, U. 1980. Human taste: response and taste bud number in fungiform papillae. *Science,* 209**,** 807.

Auerswald, H., Peters, P., Brückner, B., Krumbein, A. & Kuchenbuch, R. 1999a. Sensory analysis and instrumental measurements of short-term stored tomatoes (Lycopersicon esculentum Mill.). *Postharvest Biology and Technology,* 15**,** 323-334.

Auerswald, H., Schwarz, D., Kornelson, C., Krumbein, A. & Brückner, B. 1999b. Sensory analysis, sugar and acid content of tomato at different EC values of the nutrient solution. *Scientia Horticulturae,* 82**,** 227-242.

Bagni, N. & Tassoni, A. 2001. Biosynthesis, oxidation and conjugation of aliphatic polyamines in higher plants. *Amino acids,* 20**,** 301-317.

Bai, Y. & Lindhout, P. 2007. Domestication and breeding of tomatoes: what have we gained and what can we gain in the future? *Annals of botany,* 100**,** 1085-1094.

Baldwin, E. A., Goodner, K. & Plotto, A. 2008. Interaction of volatiles, sugars, and acids on perception of tomato aroma and flavor descriptors. *Journal of food science,* 73**,** S294-S307.

Baldwin, E. A., Nisperos-Carriedo, M. O., Baker, R. & Scott, J. W. 1991a. Quantitative analysis of flavor parameters in six Florida tomato cultivars (Lycopersicon esculentum Mill). *Journal of Agricultural and Food Chemistry,* 39**,** 1135-1140.

Baldwin, E. A., Nisperos-Carriedo, M. O. & Moshonas, M. G. 1991b. Quantitative Analysis of Flavor and Other Volatiles and for Certain Constituents of Two Tomato Cultivars during Ripening. *Journal of the American Society for Horticultural Science,* 116**,** 265-269.

Baldwin, E. A., Scott, J. W., Einstein, M. A., Malundo, T. M. M., Carr, B. T., Shewfelt, R. L. & Tandon, K. S. 1998. Relationship between Sensory and Instrumental Analysis for Tomato Flavor. *Journal of the American Society for Horticultural Science,* 123**,** 906-915.

Baldwin, E. A., Scott, J. W., Shewmaker, C. K. & Schuch, W. 2000. Flavor trivia and tomato aroma: biochemistry and possible mechanisms for control of important aroma components. *HortScience,* 35**,** 1013-1021.

Balibrea, M. E., Martínez-Andújar, C., Cuartero, J., Bolarín, M. C. & Pérez-Alfocea, F. 2006. The high fruit soluble sugar content in wild Lycopersicon species and their hybrids with cultivars depends on sucrose import during ripening rather than on sucrose metabolism. *Functional Plant Biology,* 33**,** 279-288.

Barsan, C., Sanchez-Bel, P., Rombaldi, C., Egea, I., Rossignol, M., Kuntz, M., Zouine, M., Latché, A., Bouzayen, M. & Pech, J.-C. 2010. Characteristics of the tomato chromoplast revealed by proteomic analysis. *Journal of Experimental Botany,* 61**,** 2413-2431.

Bartoshuk, L. M., Rennert, K., Rodin, J. & Stevens, J. C. 1982. Effects of temperature on the perceived sweetness of sucrose. *Physiol Behav,* 28**,** 905-10.

Batu, A. 2004. Determination of acceptable firmness and colour values of tomatoes. *Journal of Food Engineering,* 61**,** 471-475.

Bauchet, G., Grenier, S., Samson, N., Segura, V., Kende, A., Beekwilder, J., Cankar, K., Gallois, J. L., Gricourt, J. & Bonnet, J. 2017. Identification of major loci and genomic regions controlling acid and volatile content in tomato fruit: implications for flavor improvement. *New Phytologist,* 215**,** 624-641.

Baum, G., Lev-Yadun, S., Fridmann, Y., Arazi, T., Katsnelson, H., Zik, M. & Fromm, H. 1996. Calmodulin binding to glutamate decarboxylase is required for regulation of glutamate and GABA metabolism and normal development in plants. *The EMBO Journal,* 15**,** 2988-2996.

Beckles, D. M. 2012. Factors affecting the postharvest soluble solids and sugar content of tomato (Solanum lycopersicum L.) fruit. *Postharvest Biology and Technology,* 63**,** 129-140.

Belloir, C., Neiers, F. & Briand, L. 2017. Sweeteners and sweetness enhancers. *Curr Opin Clin Nutr Metab Care,* 20**,** 279-285.

Belluscio, L. & Katz, L. C. 2001. Symmetry, Stereotypy, and Topography of Odorant Representations in Mouse Olfactory Bulbs. *The Journal of Neuroscience,* 21**,** 2113.

Bennett, R. N. & Wallsgrove, R. M. 1994. Secondary metabolites in plant defence mechanisms. *New phytologist,* 127**,** 617-633.

Bergougnoux, V. 2014. The history of tomato: from domestication to biopharming. *Biotechnology advances,* 32**,** 170-189.

Bertin, N. 1995. Competition for Assimilates and Fruit Position Affect Fruit Set in Indeterminate Greenhouse Tomato. *Annals of Botany,* 75**,** 55-65.

Bertin, N. & Gary, C. 1990. TOMATO FRUIT SET AND COMPETITION FOR ASSIMILATES, DURING THE EARLY PRODUCTION PERIOD. *Protected Cultivation, XXIII IHC 303***,** 121-126.

Betancourt, L. A., Stevens, M. A. & Kader, A. A. 1977. Accumulation and loss of sugars and reduced ascorbic acid in attached and detached tomato fruits. *J. Am. Soc. Hortic. Sci,* 102**,** 721-723.

Beullens, K., Kirsanov, D., Irudayaraj, J., Rudnitskaya, A., Legin, A., Nicolaï, B. M. & Lammertyn, J. 2006. The electronic tongue and ATR–FTIR for rapid detection of sugars and acids in tomatoes. *Sensors and Actuators B: Chemical,* 116**,** 107-115.

Bezman, Y., Mayer, F., Takeoka, G. R., Buttery, R. G., Ben-Oliel, G., Rabinowitch, H. D. & Naim, M. 2002. Differential Effects of Tomato (Lycopersicon esculentum Mill) Matrix on the Volatility of Important Aroma Compounds†. *Journal of Agricultural and Food Chemistry,* 51**,** 722-726.

Bilbao, M. d. L. M., Andres-Lacueva, C., Jauregui, O. & Lamuela-Raventos, R. M. 2007. Determination of flavonoids in a Citrus fruit extract by LC–DAD and LC–MS. *Food Chemistry,* 101**,** 1742-1747.

Bino, R. J., De Vos, C. H. R., Lieberman, M., Hall, R. D., Bovy, A., Jonker, H. H., Tikunov, Y., Lommen, A., Moco, S. & Levin, I. 2005. The light-hyperresponsive high pigment-2dg mutation of tomato: alterations in the fruit metabolome. *New Phytologist,* 166**,** 427-438.

Birch, L. L. 1998. Development of food acceptance patterns in the first years of life. *Proceedings of the Nutrition Society,* 57**,** 617-624.
Böcker, S., Letzel, M. C., Lipták, Z. & Pervukhin, A. 2009. SIRIUS: decomposing isotope patterns for metabolite identification†. *Bioinformatics,* 25**,** 218-224.
Boggio, S. B., Palatnik, J. F., Heldt, H. W. & Valle, E. M. 2000. Changes in amino acid composition and nitrogen metabolizing enzymes in ripening fruits of Lycopersicon esculentum Mill. *Plant Science,* 159**,** 125-133.
Bonnans, S. & Noble, A. 1993. Effect of sweetener type and of sweetener and acid levels on temporal perception of sweetness, sourness and fruitiness. *Chemical senses,* 18**,** 273-283.
Bouché, N. & Fromm, H. 2004. GABA in plants: just a metabolite? *Trends in Plant Science,* 9**,** 110-115.
Boukobza, F. & Taylor, A. J. 2002. Effect of postharvest treatment on flavour volatiles of tomatoes. *Postharvest Biology and Technology,* 25**,** 321-331.
Bowden, K., Brown, B. G. & Batty, J. E. 1954. 5-Hydroxytryptamine: its Occurrence in Cowhage. *Nature,* 174**,** 925.
Bramley, P. M. 2002. Regulation of carotenoid formation during tomato fruit ripening and development. *Journal of Experimental Botany,* 53**,** 2107-2113.
Brandt, S., Pék, Z., Barna, É., Lugasi, A. & Helyes, L. 2006. Lycopene content and colour of ripening tomatoes as affected by environmental conditions. *Journal of the Science of Food and Agriculture,* 86**,** 568-572.
Breslin, P. & Beauchamp, G. 1997. Salt enhances flavour by suppressing bitterness. *Nature,* 387**,** 563.
Breslin, P. A. & Spector, A. C. 2008. Mammalian taste perception. *Current Biology,* 18**,** R148-R155.
Breslin, Paul A. S. 2013. An Evolutionary Perspective on Food and Human Taste. *Current Biology,* 23**,** R409-R418.
BTGA. 2014. *British Tomato Growers Association* [Online]. Available: http://www.britishtomatoes.co.uk [Accessed 26/11/2014].
Bult, J. H. F., de Wijk, R. A. & Hummel, T. 2007. Investigations on multimodal sensory integration: Texture, taste, and ortho- and retronasal olfactory stimuli in concert. *Neuroscience Letters,* 411**,** 6-10.
Bushdid, C., Magnasco, M. O., Vosshall, L. B. & Keller, A. 2014. Humans can discriminate more than 1 trillion olfactory stimuli. *Science,* 343**,** 1370-1372.
Buttery, R. 1993. Quantitative and sensory aspects of flavor of tomato and other vegetables and fruits. *Flavor science: Sensible principles and techniques***,** 259-286.
Buttery, R. G. & Ling, L. C. Volatile compounds of tomato fruit and plant parts: relationship and biogenesis. ACS symposium series (USA), 1993.
Buttery, R. G., Seifert, R. M., Guadagni, D. G. & Ling, L. C. 1970. Characterization of additional volatile components of tomato. *Journal of Agricultural and Food Chemistry,* 19**,** 524-529.
Buttery, R. G., Teranishi, R. & Ling, L. C. 1987. Fresh tomato aroma volatiles: a quantitative study. *Journal of Agricultural and Food Chemistry,* 35**,** 540-544.
Buttery, R. G., Teranishi, R., Ling, L. C., Flath, R. A. & Stern, D. J. 1988. Quantitative studies on origins of fresh tomato aroma volatiles. *Journal of Agricultural and Food Chemistry,* 36**,** 1247-1250.
Buttery, R. G., Teranishi, R., Ling, L. C. & Turnbaugh, J. G. 1990. Quantitative and sensory studies on tomato paste volatiles. *Journal of Agricultural and Food Chemistry,* 38**,** 336-340.
Cajka, T. & Fiehn, O. 2016. Toward Merging Untargeted and Targeted Methods in Mass Spectrometry-Based Metabolomics and Lipidomics. *Analytical Chemistry,* 88**,** 524-545.
Cantu, D., Vicente, A., Greve, L., Dewey, F., Bennett, A., Labavitch, J. & Powell, A. 2008. The intersection between cell wall disassembly, ripening, and fruit susceptibility to Botrytis cinerea. *Proceedings of the National Academy of Sciences,* 105**,** 859-864.
Carrari, F., Baxter, C., Usadel, B., Urbanczyk-Wochniak, E., Zanor, M.-I., Nunes-Nesi, A., Nikiforova, V., Centero, D., Ratzka, A., Pauly, M., Sweetlove, L. J. & Fernie, A. R. 2006. Integrated Analysis of Metabolite and Transcript Levels Reveals the Metabolic Shifts

That Underlie Tomato Fruit Development and Highlight Regulatory Aspects of Metabolic Network Behavior. *Plant Physiology,* 142**,** 1380-1396.
Carrari, F. & Fernie, A. R. 2006. Metabolic regulation underlying tomato fruit development. *Journal of Experimental Botany,* 57**,** 1883-1897.
Carreno-Quintero, N., Bouwmeester, H. J. & Keurentjes, J. J. B. 2013. Genetic analysis of metabolome–phenotype interactions: from model to crop species. *Trends in Genetics,* 29**,** 41-50.
Catterall, W. A. 2000. From ionic currents to molecular mechanisms: the structure and function of voltage-gated sodium channels. *Neuron,* 26**,** 13-25.
Causse, M., Buret, M., Robini, K. & Verschave, P. 2003. Inheritance of nutritional and sensory quality traits in fresh market tomato and relation to consumer preferences. *Journal of food science,* 68**,** 2342-2350.
Causse, M., Duffe, P., Gomez, M. C., Buret, M., Damidaux, R., Zamir, D., Gur, A., Chevalier, C., Lemaire-Chamley, M. & Rothan, C. 2004. A genetic map of candidate genes and QTLs involved in tomato fruit size and composition. *Journal of Experimental Botany,* 55**,** 1671-1685.
Causse, M., Friguet, C., Coiret, C., Lepicier, M., Navez, B., Lee, M., Holthuysen, N., Sinesio, F., Moneta, E. & Grandillo, S. 2010. Consumer preferences for fresh tomato at the European scale: a common segmentation on taste and firmness. *J Food Sci,* 75**,** S531-41.
Causse, M., Saliba-Colombani, V., Lecomte, L., Duffé, P., Rousselle, P. & Buret, M. 2002. QTL analysis of fruit quality in fresh market tomato: a few chromosome regions control the variation of sensory and instrumental traits. *Journal of Experimental Botany,* 53**,** 2089-2098.
Centeno, D. C., Osorio, S., Nunes-Nesi, A., Bertolo, A. L., Carneiro, R. T., Araújo, W. L., Steinhauser, M.-C., Michalska, J., Rohrmann, J. & Geigenberger, P. 2011. Malate plays a crucial role in starch metabolism, ripening, and soluble solid content of tomato fruit and affects postharvest softening. *The Plant Cell,* 23**,** tpc. 109.072231.
Cevallos-Cevallos, J. M., Reyes-De-Corcuera, J. I., Etxeberria, E., Danyluk, M. D. & Rodrick, G. E. 2009. Metabolomic analysis in food science: a review. *Trends in Food Science & Technology,* 20**,** 557-566.
Chandrashekar, J., Hoon, M. A., Ryba, N. J. P. & Zuker, C. S. 2006. The receptors and cells for mammalian taste. *Nature,* 444**,** 288.
Chaudhari, N. & Roper, S. D. 2010. The cell biology of taste. *The Journal of Cell Biology,* 190**,** 285.
Chen, G., Hackett, R., Walker, D., Taylor, A., Lin, Z. & Grierson, D. 2004. Identification of a specific isoform of tomato lipoxygenase (TomloxC) involved in the generation of fatty acid-derived flavor compounds. *Plant physiology,* 136**,** 2641-2651.
Chew, B. L., Fisk, I. D., Fray, R., Tucker, G. A., Bodi, Z., Ferguson, A., Xia, W. & Seymour, G. B. 2017. The effect of adenosine monophosphate deaminase overexpression on the accumulation of umami-related metabolites in tomatoes. *Plant Cell Reports,* 36**,** 81-87.
Cho, J. H., Lee, D., Park, J. H., Kim, K. & Lee, I. B. 2002. Optimal approach for classification of acute leukemia subtypes based on gene expression data. *Biotechnology progress,* 18**,** 847-854.
Colquhoun, T. A., Schwieterman, M. L., Gilbert, J. L., Jaworski, E. A., Langer, K. M., Jones, C. R., Rushing, G. V., Hunter, T. M., Olmstead, J. & Clark, D. G. 2013. Light modulation of volatile organic compounds from petunia flowers and select fruits. *Postharvest Biology and Technology,* 86**,** 37-44.
Cortina, P. R., Santiago, A. N., Sance, M. M., Peralta, I. E., Carrari, F. & Asis, R. 2018. Neuronal network analyses reveal novel associations between volatile organic compounds and sensory properties of tomato fruits. *Metabolomics,* 14**,** 57.
Côté, F., Fligny, C., Fromes, Y., Mallet, J. & Vodjdani, G. 2004. Recent advances in understanding serotonin regulation of cardiovascular function. *Trends in molecular medicine,* 10**,** 232-238.
Crozier, A., Jaganath, I. B. & Clifford, M. N. 2009. Dietary phenolics: chemistry, bioavailability and effects on health. *Natural product reports,* 26**,** 1001-1043.
Cubero-Leon, E., Peñalver, R. & Maquet, A. 2014. Review on metabolomics for food authentication. *Food Research International,* 60**,** 95-107.

Dai, N., Schaffer, A., Petreikov, M., Shahak, Y., Giller, Y., Ratner, K., Levine, A. & Granot, D. 1999. Overexpression of Arabidopsis hexokinase in tomato plants inhibits growth, reduces photosynthesis, and induces rapid senescence. *The plant cell,* 11**,** 1253-1266.

Damon, S., Hewitt, J., Nieder, M. & Bennett, A. B. 1988. Sink Metabolism in Tomato Fruit: II. Phloem Unloading and Sugar Uptake. *Plant Physiology,* 87**,** 731-736.

Davies, J. N. 1966. Changes in the non-volatile organic acids of tomato fruit during ripening. *Journal of the Science of Food and Agriculture,* 17**,** 396-400.

Davies, J. N., Hobson, G. E. & McGlasson, W. B. 1981. The constituents of tomato fruit — the influence of environment, nutrition, and genotype. *C R C Critical Reviews in Food Science and Nutrition,* 15**,** 205-280.

de Jong, M., Mariani, C. & Vriezen, W. H. 2009. The role of auxin and gibberellin in tomato fruit set. *Journal of Experimental Botany,* 60**,** 1523-1532.

de Koning, A. N. M. 1994. *Development and dry matter distribution in glasshouse tomato : a quantitative approach.* Proefschrift Wageningen, De Koning.

de León-Sánchez, F. D., Pelayo-Zaldívar, C., Rivera-Cabrera, F., Ponce-Valadez, M., Ávila-Alejandre, X., Fernández, F. J., Escalona-Buendía, H. B. & Pérez-Flores, L. J. 2009. Effect of refrigerated storage on aroma and alcohol dehydrogenase activity in tomato fruit. *Postharvest Biology and Technology,* 54**,** 93-100.

de Vos, R. C., Hall, R. D. & Moing, A. 2011. Metabolomics of a model fruit: tomato. *Annual Plant Reviews, Biology of Plant Metabolomics,* 43**,** 109-155.

de Vos, R. C. H., Moco, S., Lommen, A., Keurentjes, J. J. B., Bino, R. J. & Hall, R. D. 2007. Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. *Nat. Protocols,* 2**,** 778-791.

Deewatthanawong, R., Rowell, P. & Watkins, C. B. 2010. γ-Aminobutyric acid (GABA) metabolism in CO2 treated tomatoes. *Postharvest Biology and Technology,* 57**,** 97-105.

Deltsidis, A. I., Sims, C. A. & Brecht, J. K. 2018. Ripening Recovery and Sensory Quality of Pink Tomatoes Stored in Controlled Atmosphere at Chilling or Nonchilling Temperatures to Extend Shelf Life. *HortScience,* 53**,** 1186-1190.

Di Mascio, P., Kaiser, S. & Sies, H. 1989. Lycopene as the most efficient biological carotenoid singlet oxygen quencher. *Archives of Biochemistry and Biophysics,* 274**,** 532-538.

Dibble, A. R., Davies, P. J. & Mutschler, M. A. 1988. Polyamine content of long-keeping alcobaca tomato fruit. *Plant Physiol,* 86**,** 338-40.

DiLeo, M. V., Strahan, G. D., den Bakker, M. & Hoekenga, O. A. 2011. Weighted correlation network analysis (WGCNA) applied to the tomato fruit metabolome. *PLoS One,* 6**,** e26683.

Dixon, R. A., Achnine, L., Kota, P., Liu, C. J., Reddy, M. S. & Wang, L. 2002. The phenylpropanoid pathway and plant defence—a genomics perspective. *Molecular plant pathology,* 3**,** 371-390.

Dudareva, N., Klempien, A., Muhlemann, J. K. & Kaplan, I. 2013. Biosynthesis, function and metabolic engineering of plant volatile organic compounds. *New Phytologist,* 198**,** 16-32.

Dührkop, K., Scheubert, K. & Böcker, S. 2013. Molecular Formula Identification with SIRIUS. *Metabolites,* 3**,** 506.

Dunn, W. B. & Ellis, D. I. 2005. Metabolomics: Current analytical platforms and methodologies. *TrAC Trends in Analytical Chemistry,* 24**,** 285-294.

Elmore, J. S., Koutsidis, G., Dodson, A. T., Mottram, D. S. & Wedzicha, B. L. 2005. Measurement of Acrylamide and Its Precursors in Potato, Wheat, and Rye Model Systems. *Journal of Agricultural and Food Chemistry,* 53**,** 1286-1293.

Etminan, M., Takkouche, B. & Caamaño-Isorna, F. 2004. The Role of Tomato Products and Lycopene in the Prevention of Prostate Cancer: A Meta-Analysis of Observational Studies. *Cancer Epidemiology Biomarkers & Prevention,* 13**,** 340-345.

FAOSTAT. 2017. *Production / Crops / Values* [Online]. Available: http://faostat3.fao.org/browse/Q/*/E [Accessed].

Farag, M. A. & Paré, P. W. 2002. C6-Green leaf volatiles trigger local and systemic VOC emissions in tomato. *Phytochemistry,* 61**,** 545-554.

Farrés, M., Platikanov, S., Tsakovski, S. & Tauler, R. 2015. Comparison of the variable importance in projection (VIP) and of the selectivity ratio (SR) methods for variable selection and interpretation. *Journal of Chemometrics,* 29**,** 528-536.

Feldman, J. M. & Lee, E. M. 1985. Serotonin content of foods: effect on urinary excretion of 5-hydroxyindoleacetic acid. *The American journal of clinical nutrition,* 42**,** 639-643.
Fellows, L. E. & Bell, E. 1971. Indole metabolism in Piptadenia peregrina. *Phytochemistry,* 10**,** 2083-2091.
Fernandez, X., Duñach, E., Fellous, R., Lizzani-Cuvelier, L. & Loiseau, M. 2002. Thiazolidines and their oxidation products as flavouring compounds. *Flavour and fragrance journal,* 17**,** 432-439.
Fernie, A. R. & Schauer, N. 2009. Metabolomics-assisted breeding: a viable option for crop improvement? *Trends in Genetics,* 25**,** 39-48.
Feussner, I. & Wasternack, C. 2002. THE LIPOXYGENASE PATHWAY. *Annual Review of Plant Biology,* 53**,** 275-297.
Fleuriet, A. & Macheix, J.-J. 1981. Quinyl esters and glucose derivatives of hydroxycinnamic acids during growth and ripening of tomato fruit. *Phytochemistry,* 20**,** 667-671.
Frank, R. A. & Archambo, G. 1986. Intensity and hedonic judgments of taste mixtures: an information integration analysis. *Chemical Senses,* 11**,** 427-438.
Frary, A. & Doğanlar, S. 2003. Comparative genetics of crop plant domestication and evolution. *Turkish Journal of Agriculture and Forestry,* 27**,** 59-69.
Fraser, C. M. & Chapple, C. 2011. The Phenylpropanoid Pathway in Arabidopsis. *The Arabidopsis Book / American Society of Plant Biologists,* 9**,** e0152.
Fraser, P. D., Enfissi, E. M. A., Halket, J. M., Truesdale, M. R., Yu, D., Gerrish, C. & Bramley, P. M. 2007. Manipulation of phytoene levels in tomato fruit: effects on isoprenoids, plastids, and intermediary metabolism. *The Plant cell,* 19**,** 3194-3211.
Frazer, A. & Hensler, J. 1999. Serotonin involvement in physiological function and behavior. Lippincott-Raven, Philadelphia, Pa, USA.
Frydman, A., Weisshaus, O., Bar-Peled, M., Huhman, D. V., Sumner, L. W., Marin, F. R., Lewinsohn, E., Fluhr, R., Gressel, J. & Eyal, Y. 2004. Citrus fruit bitter flavors: isolation and functional characterization of the gene Cm1, 2RhaT encoding a 1, 2 rhamnosyltransferase, a key enzyme in the biosynthesis of the bitter flavonoids of citrus. *The Plant Journal,* 40**,** 88-100.
Fulton, T. M., Bucheli, P., Voirol, E., López, J., Pétiard, V. & Tanksley, S. D. 2002. Quantitative trait loci (QTL) affecting sugars, organic acids and other biochemical properties possibly contributing to flavor, identified in four advanced backcross populations of tomato. *Euphytica,* 127**,** 163-177.
Gardner, H. W. 1995. Biological roles and biochemistry of the lipoxygenase pathway. *HortScience,* 30**,** 197-205.
Gautier, H., Diakou-Verdin, V., Bénard, C., Reich, M., Buret, M., Bourgaud, F., Poëssel, J. L., Caris-Veyrat, C. & Génard, M. 2008. How Does Tomato Quality (Sugar, Acid, and Nutritional Quality) Vary with Ripening Stage, Temperature, and Irradiance? *Journal of Agricultural and Food Chemistry,* 56**,** 1241-1250.
Gebregiworgis, T. & Powers, R. 2012. Application of NMR metabolomics to search for human disease biomarkers. *Combinatorial chemistry & high throughput screening,* 15**,** 595-610.
Giovannoni, J. 2001. MOLECULAR BIOLOGY OF FRUIT MATURATION AND RIPENING. *Annual Review of Plant Physiology and Plant Molecular Biology,* 52**,** 725-749.
Giovannoni, J. J. 2004. Genetic Regulation of Fruit Development and Ripening. *The Plant Cell,* 16**,** S170-S180.
Giovannucci, E. 1999. Tomatoes, Tomato-Based Products, Lycopene, and Cancer: Review of the Epidemiologic Literature. *Journal of the National Cancer Institute,* 91**,** 317-331.
Giovannucci, E., Rimm, E. B., Liu, Y., Stampfer, M. J. & Willett, W. C. 2002. A Prospective Study of Tomato Products, Lycopene, and Prostate Cancer Risk. *Journal of the National Cancer Institute,* 94**,** 391-398.
Giridhar, A. R. P. & Ravishankar, G. 2009. Indoleamines and calcium channels influence morphogenesis in in vitro cultures of Mimosa pudica L. *Plant signaling & behavior,* 4**,** 1136-1141.
Goff, S. A. & Klee, H. J. 2006. Plant Volatile Compounds: Sensory Cues for Health and Nutritional Value? *Science,* 311**,** 815-819.

Gómez-Romero, M., Segura-Carretero, A. & Fernández-Gutiérrez, A. 2010. Metabolite profiling and quantification of phenolic compounds in methanol extracts of tomato fruit. *Phytochemistry,* 71**,** 1848-1864.

Gómez, C., Morrow, R. C., Bourget, C. M., Massa, G. D. & Mitchell, C. A. 2013. Comparison of intracanopy light-emitting diode towers and overhead high-pressure sodium lamps for supplemental lighting of greenhouse-grown tomatoes. *HortTechnology,* 23**,** 93-98.

Gómez, P., Ferrer, M. Á., Fernández-Trujillo, J. P., Calderón, A., Artés, F., Egea-Cortines, M. & Weiss, J. 2009. Structural changes, chemical composition and antioxidant activity of cherry tomato fruits (cv. Micro-Tom) stored under optimal and chilling conditions. *Journal of the Science of Food and Agriculture,* 89**,** 1543-1551.

Green, B. G., Lim, J., Osterhoff, F., Blacher, K. & Nachtigal, D. 2010. Taste mixture interactions: Suppression, additivity, and the predominance of sweetness. *Physiology & Behavior,* 101**,** 731-737.

Griffiths, A., Barry, C., Alpuche-Solis, A. G. & Grierson, D. 1999. Ethylene and developmental signals regulate expression of lipoxygenase genes during tomato fruit ripening. *Journal of experimental botany,* 50**,** 793-798.

Guijas, C., Montenegro-Burke, J. R., Domingo-Almenara, X., Palermo, A., Warth, B., Hermann, G., Koellensperger, G., Huan, T., Uritboonthai, W., Aisporna, A. E., Wolan, D. W., Spilker, M. E., Benton, H. P. & Siuzdak, G. 2018. METLIN: A Technology Platform for Identifying Knowns and Unknowns. *Analytical Chemistry,* 90**,** 3156-3164.

Guil-Guerrero, J. L. & Rebolloso-Fuentes, M. M. 2009. Nutrient composition and antioxidant activity of eight tomato (Lycopersicon esculentum) varieties. *Journal of Food Composition and Analysis,* 22**,** 123-129.

Guiné, R. P., Martín-Belloso, O., Mínguez-Mosquera, M. I., Paliyath, G., Pessoa, F. L., Le Quéré, J.-L., Sidhu, J. S., Sinha, N. & Stanfield, P. 2010. *Handbook of fruit and vegetable flavors,* John Wiley and Sons.

Gustafson, F. G. 1934. PRODUCTION OF ALCOHOL AND ACETALDEHYDE BY TOMATOES. *Plant Physiology,* 9**,** 359-367.

Halket, J. M., Waterman, D., Przyborowska, A. M., Patel, R. K., Fraser, P. D. & Bramley, P. M. 2005. Chemical derivatization and mass spectral libraries in metabolic profiling by GC/MS and LC/MS/MS. *J Exp Bot,* 56**,** 219-43.

Hall, R. D. 2006. Plant metabolomics: from holistic hope, to hype, to hot topic. *New Phytologist,* 169**,** 453-468.

Halpin, C., Knight, M. E., Foxon, G. A., Campbell, M. M., Boudet, A. M., Boon, J. J., Chabbert, B., Tollier, M. T. & Schuch, W. 1994. Manipulation of lignin quality by downregulation of cinnamyl alcohol dehydrogenase. *The Plant Journal,* 6**,** 339-350.

Hartwig, P. & McDaniel, M. R. 1995. Flavor characteristics of lactic, malic, citric, and acetic acids at various pH levels. *Journal of Food Science,* 60**,** 384-388.

Hatanaka, A., Kajiwara, T., Matsui, K. & Kitamura, A. 1992. Expression of lipoxygenase and hydroperoxide lyase activities in tomato fruits. *Zeitschrift für Naturforschung C,* 47**,** 369-374.

Heitz, T., Bergey, D. R. & Ryan, C. A. 1997. A gene encoding a chloroplast-targeted lipoxygenase in tomato leaves is transiently induced by wounding, systemin, and methyl jasmonate. *Plant Physiology,* 114**,** 1085-1093.

Hernández Suárez, M., Rodríguez Rodríguez, E. & Díaz Romero, C. 2008a. Analysis of organic acid content in cultivars of tomato harvested in Tenerife. *European Food Research and Technology,* 226**,** 423-435.

Hernández Suárez, M., Rodríguez Rodríguez, E. M. & Díaz Romero, C. 2008b. Chemical composition of tomato (Lycopersicon esculentum) from Tenerife, the Canary Islands. *Food Chemistry,* 106**,** 1046-1056.

Hildebrandt, T. M., Nesi, A. N., Araújo, W. L. & Braun, H.-P. 2015. Amino acid catabolism in plants. *Molecular plant,* 8**,** 1563-1579.

Ho, L. & Hewitt, J. 1986. Fruit development. *The tomato crop.* Springer.

Ho, L. C. 1988. Metabolism and Compartmentation of Imported Sugars in Sink Organs in Relation to Sink Strength. *Annual Review of Plant Physiology and Plant Molecular Biology,* 39**,** 355-378.

Ho, L. C. 1996. The mechanism of assimilate partitioning and carbohydrate compartmentation in fruit in relation to the quality and yield of tomato. *Journal of Experimental Botany,* 47**,** 1239-1243.

Ho, L. C., Sjut, V. & Hoad, G. V. 1982. The effect of assimilate supply on fruit growth and hormone levels in tomato plants. *Plant Growth Regulation,* 1**,** 155-171.

Hobson, G. E. 1987. Low-temperature injury and the storage of ripening tomatoes. *Journal of Horticultural Science,* 62**,** 55-62.

Hochkogler, C. M., Liszt, K., Lieder, B., Stöger, V., Stübler, A., Pignitter, M., Hans, J., Widder, S., Ley, J. P., Krammer, G. E. & Somoza, V. 2017. Appetite-Inducing Effects of Homoeriodictyol: Two Randomized, Cross-Over Interventions. *Molecular Nutrition & Food Research,* 61**,** 1700459.

Hongsoongnern, P. & Chambers IV, E. 2008. A LEXICON FOR GREEN ODOR OR FLAVOR AND CHARACTERISTICS OF CHEMICALS ASSOCIATED WITH GREEN. *Journal of Sensory Studies,* 23**,** 205-221.

Huang, T., Jander, G. & de Vos, M. 2011. Non-protein amino acids in plant defense against insect herbivores: representative cases and opportunities for further functional analysis. *Phytochemistry,* 72**,** 1531-1537.

Hubert, J., Münzbergová, Z. & Santino, A. 2008. Plant volatile aldehydes as natural insecticides against stored-product beetles. *Pest Management Science: formerly Pesticide Science,* 64**,** 57-64.

Hull, A. K., Vij, R. & Celenza, J. L. 2000. Arabidopsis cytochrome P450s that catalyze the first step of tryptophan-dependent indole-3-acetic acid biosynthesis. *Proceedings of the National Academy of Sciences,* 97**,** 2379-2384.

Humphreys, J. M. & Chapple, C. 2002. Rewriting the lignin roadmap. *Current Opinion in Plant Biology,* 5**,** 224-229.

Igamberdiev, A. U. & Eprintsev, A. T. 2016. Organic Acids: The Pools of Fixed Carbon Involved in Redox Regulation and Energy Balance in Higher Plants. *Frontiers in Plant Science,* 7**,** 1042.

Iijima, Y., Nakamura, Y., Ogata, Y., Tanaka, K., Sakurai, N., Suda, K., Suzuki, T., Suzuki, H., Okazaki, K., Kitayama, M., Kanaya, S., Aoki, K. & Shibata, D. 2008. Metabolite annotations based on the integration of mass spectral information. *Plant J,* 54**,** 949-62.

Ikeda, K. 1909. On a new seasoning. *J. Tokyo Chem. Soc.,* 30**,** 820-836.

Ingle, R. A. 2011. Histidine biosynthesis. *The arabidopsis book,* 9**,** e0141-e0141.

Ishihara, A., Hashimoto, Y., Tanaka, C., Dubouzet, J. G., Nakao, T., Matsuda, F., Nishioka, T., Miyagawa, H. & Wakasa, K. 2008. The tryptophan pathway is involved in the defense responses of rice against pathogenic infection via serotonin production. *The Plant Journal,* 54**,** 481-495.

Ji, G., Yang, Z. & You, W. 2011. PLS-based gene selection and identification of tumor-specific genes. *IEEE Transactions on Systems, Man, and Cybernetics, Part C (Applications and Reviews),* 41**,** 830-841.

Johansson, L., Haglund, Å., Berglund, L., Lea, P. & Risvik, E. 1999. Preference for tomatoes, affected by sensory attributes and information about growth conditions. *Food Quality and Preference,* 10**,** 289-298.

Jones, R. & Scott, S. 1983. Improvement of tomato flavor by genetically increasing sugar and acid contents. *Euphytica,* 32**,** 845-855.

Kader, A. A., Morris, L., Stevens, M. & Holton, M. 1978. Composition and Flavour Quality of Fresh Market Tomatoes as Influenced by Some Postharvest Handling Procedures. *Journal of American Horticultural Science,* 103**,** 6-13.1978.

Kader, A. A., Stevens, M. A., Albright-Holton, M., Morris, L. L. & Algazi, M. 1977. Effect of fruit ripeness when picked on flavor and composition in fresh market tomatoes. *Journal of the American Society for Horticultural Science,* 102**,** 724-731.

Kanayama, Y., Granot, D., Dai, N., Petreikov, M., Schaffer, A., Powell, A. & Bennett, A. B. 1998. Tomato fructokinases exhibit differential expression and substrate regulation. *Plant Physiology,* 117**,** 85-90.

Kanehisa, M., Furumichi, M., Tanabe, M., Sato, Y. & Morishima, K. 2017. KEGG: new perspectives on genomes, pathways, diseases and drugs. *Nucleic Acids Res,* 45**,** D353-d361.

Kang, K., Kang, S., Lee, K., Park, M. & Back, K. 2008. Enzymatic features of serotonin biosynthetic enzymes and serotonin biosynthesis in plants. *Plant signaling & behavior,* 3**,** 389-390.

Kang, S., Kang, K., Lee, K. & Back, K. 2007. Characterization of tryptamine 5-hydroxylase and serotonin synthesis in rice plants. *Plant Cell Reports,* 26**,** 2009-2015.

Kazeniac, S. J. & Hall, R. M. 1970. FLAVOR CHEMISTRY OF TOMATO VOLATILES. *Journal of Food Science,* 35**,** 519-530.

Kesselmeier, J. & Staudt, M. 1999. Biogenic volatile organic compounds (VOC): an overview on emission, physiology and ecology. *Journal of atmospheric chemistry,* 33**,** 23-88.

Keurentjes, J. J. B. 2009. Genetical metabolomics: closing in on phenotypes. *Current Opinion in Plant Biology,* 12**,** 223-230.

Kind, T. & Fiehn, O. 2006. Metabolomic database annotations via query of elemental compositions: mass accuracy is insufficient even at less than 1 ppm. *BMC Bioinformatics,* 7**,** 234.

Kind, T. & Fiehn, O. 2010. Advances in structure elucidation of small molecules using mass spectrometry. *Bioanalytical Reviews,* 2**,** 23-60.

Kirsh, V. A., Mayne, S. T., Peters, U., Chatterjee, N., Leitzmann, M. F., Dixon, L. B., Urban, D. A., Crawford, E. D. & Hayes, R. B. 2006. A Prospective Study of Lycopene and Tomato Product Intake and Risk of Prostate Cancer. *Cancer Epidemiology Biomarkers & Prevention,* 15**,** 92-98.

Klee, H. J. 2010. Improving the flavor of fresh fruits: genomics, biochemistry, and biotechnology. *New Phytologist,* 187**,** 44-56.

Klee, H. J. & Giovannoni, J. J. 2011. Genetics and Control of Tomato Fruit Ripening and Quality Attributes. *Annual Review of Genetics,* 45**,** 41-59.

Klieber, A., Ratanachinakorn, B. & Simons, D. 1996. Effects of low oxygen and high carbon dioxide on tomato cultivar 'Bermuda'fruit physiology and composition. *Scientia horticulturae,* 65**,** 251-261.

Knapp, S. & Peralta, I. E. 2016. The Tomato (Solanum lycopersicum L., Solanaceae) and Its Botanical Relatives. *In:* CAUSSE, M., GIOVANNONI, J., BOUZAYEN, M. & ZOUINE, M. (eds.) *The Tomato Genome.* Berlin, Heidelberg: Springer Berlin Heidelberg.

Knee, M. & Finger, F. L. 1992. NADP+-malic enzyme and organic acid levels in developing tomato fruits. *Journal of the American Society for Horticultural Science,* 117**,** 799-801.

Kochevenko, A., Araújo, W. L., Maloney, G. S., Tieman, D. M., Do, P. T., Taylor, M. G., Klee, H. J. & Fernie, A. R. 2012. Catabolism of Branched Chain Amino Acids Supports Respiration but Not Volatile Synthesis in Tomato Fruits. *Molecular Plant,* 5**,** 366-375.

Koike, S., Matsukura, C., Takayama, M., Asamizu, E. & Ezura, H. 2013. Suppression of γ-Aminobutyric Acid (GABA) Transaminases Induces Prominent GABA Accumulation, Dwarfism and Infertility in the Tomato (Solanum lycopersicum L.). *Plant and Cell Physiology,* 54**,** 793-807.

Kortstee, A., Appeldoorn, N., Oortwijn, M. & Visser, R. 2007. Differences in regulation of carbohydrate metabolism during early fruit development between domesticated tomato and two wild relatives. *Planta,* 226**,** 929-939.

Kosma, D. K., Parsons, E. P., Isaacson, T., Lü, S., Rose, J. K. & Jenks, M. A. 2010. Fruit cuticle lipid composition during development in tomato ripening mutants. *Physiologia Plantarum,* 139**,** 107-117.

Kress, M. & Waldmann, R. 2006. Chapter 8 Acid Sensing Ionic Channels. *Current Topics in Membranes.* Academic Press.

Krumbein, A. & Auerswald, H. 1998. Characterization of aroma volatiles in tomatoes by sensory analyses. *Food / Nahrung,* 42**,** 395-399.

Krumbein, A., Peters, P. & Brückner, B. 2004. Flavour compounds and a quantitative descriptive analysis of tomatoes (Lycopersicon esculentum Mill.) of different cultivars in short-term storage. *Postharvest Biology and Technology,* 32**,** 15-28.

Lam, H.-M., Coschigano, K., Oliveira, I., Melo-Oliveira, R. & Coruzzi, G. 1996. The molecular-genetics of nitrogen assimilation into amino acids in higher plants. *Annual review of plant biology,* 47**,** 569-593.

Lambrecht, M., Okon, Y., Broek, A. V. & Vanderleyden, J. 2000. Indole-3-acetic acid: a reciprocal signalling molecule in bacteria–plant interactions. *Trends in microbiology,* 8**,** 298-300.

Langridge, P. & Fleury, D. 2011. Making the most of 'omics' for crop breeding. *Trends in Biotechnology,* 29**,** 33-40.

Lee, J. H., Jayaprakasha, G., Rush, C. M., Crosby, K. M. & Patil, B. S. 2018. Production system influences volatile biomarkers in tomato. *Metabolomics,* 14**,** 99.

Leffingwell. 2018. *Odor Detection Thresholds & References : Odor & Flavor Detection Thresholds in Water (In Parts per Billion)* [Online]. Leffingwell & Associates. Available: http://www.leffingwell.com/odorthre.htm [Accessed 09/2018].

Leflaive, J. & Ten-Hage, L. 2009. Chemical interactions in diatoms: role of polyunsaturated aldehydes and precursors. *New Phytologist,* 184**,** 794-805.

Lewinsohn, E., Sitrit, Y., Bar, E., Azulay, Y., Meir, A., Zamir, D. & Tadmor, Y. 2005. Carotenoid Pigmentation Affects the Volatile Composition of Tomato and Watermelon Fruits, As Revealed by Comparative Genetic Analyses. *Journal of Agricultural and Food Chemistry,* 53**,** 3142-3148.

Ley, J. P., Krammer, G., Reinders, G., Gatfield, I. L. & Bertram, H.-J. 2005. Evaluation of Bitter Masking Flavanones from Herba Santa (Eriodictyon californicum (H. & A.) Torr., Hydrophyllaceae). *Journal of Agricultural and Food Chemistry,* 53**,** 6061-6066.

Li, M., Feng, F. & Cheng, L. 2012. Expression patterns of genes involved in sugar metabolism and accumulation during apple fruit development. *PloS one,* 7**,** e33055.

Li, X., Staszewski, L., Xu, H., Durick, K., Zoller, M. & Adler, E. 2002. Human receptors for sweet and umami taste. *Proc Natl Acad Sci U S A,* 99**,** 4692-6.

Lindemann, B. 1996. Taste reception. *Physiological reviews,* 76**,** 719-766.

Lindemann, B., Ogiwara, Y. & Ninomiya, Y. 2002. The Discovery of Umami. *Chemical Senses,* 27**,** 843-844.

Liu, X., Chang, T., Guo, S., Xu, Z. & Li, J. Effect of different light quality of LED on growth and photosynthetic character in cherry tomato seedling. VI International Symposium on Light in Horticulture 907, 2009. 325-330.

Ljung, K., Hull, A. K., Kowalczyk, M., Marchant, A., Celenza, J., Cohen, J. D. & Sandberg, G. 2002. Biosynthesis, conjugation, catabolism and homeostasis of indole-3-acetic acid in Arabidopsis thaliana. *Plant molecular biology,* 49**,** 249-272.

Long, M., Millar, D. J., Kimura, Y., Donovan, G., Rees, J., Fraser, P. D., Bramley, P. M. & Bolwell, G. P. 2006. Metabolite profiling of carotenoid and phenolic pathways in mutant and transgenic lines of tomato: Identification of a high antioxidant fruit line. *Phytochemistry,* 67**,** 1750-1757.

López Camelo, A. F. & Gómez, P. A. 2004. Comparison of color indexes for tomato ripening. *Horticultura Brasileira,* 22**,** 534-537.

Lu, N., Maruo, T., Johkan, M., Hohjo, M., Tsukagoshi, S., Ito, Y., Ichimura, T. & Shinohara, Y. 2012. Effects of supplemental lighting with light-emitting diodes (LEDs) on tomato yield and quality of single-truss tomato plants grown at high planting density. *Environmental Control in Biology,* 50**,** 63-74.

Lu, W., Bennett, B. D. & Rabinowitz, J. D. 2008. Analytical strategies for LC–MS-based targeted metabolomics. *Journal of Chromatography B,* 871**,** 236-242.

Luengwilai, K. & Beckles, M. D. 2010. Climacteric ethylene is not essential for initiating chilling injury in tomato (Solanum lycopersicum) cv. Ailsa Craig. *Journal of Stored Products and Postharvest Research,* 1**,** 1-8.

Luengwilai, K., Fiehn, O. E. & Beckles, D. M. 2010. Comparison of Leaf and Fruit Metabolism in Two Tomato (Solanum lycopersicum L.) Genotypes Varying in Total Soluble Solids. *Journal of Agricultural and Food Chemistry,* 58**,** 11790-11800.

Lugaz, O., Pillias, A. M., Boireau-Ducept, N. & Faurion, A. 2005. Time–Intensity Evaluation of Acid Taste in Subjects with Saliva High Flow and Low Flow Rates for Acids of Various Chemical Properties. *Chemical Senses,* 30**,** 89-103.

Ly, D., Kang, K., Choi, J.-Y., Ishihara, A., Back, K. & Lee, S.-G. 2008. HPLC analysis of serotonin, tryptamine, tyramine, and the hydroxycinnamic acid amides of serotonin and tyramine in food vegetables. *Journal of medicinal food,* 11**,** 385-389.

Lyons, D. B., Allen, W. E., Goh, T., Tsai, L., Barnea, G. & Lomvardas, S. 2013. An epigenetic trap stabilizes singular olfactory receptor expression. *Cell,* 154**,** 325-336.

Lyons, J. M. 1973. Chilling Injury in Plants. *Annual Review of Plant Physiology,* 24**,** 445-466.

Lytovchenko, A., Eickmeier, I., Pons, C., Osorio, S., Szecowka, M., Lehmberg, K., Arrivault, S., Tohge, T., Pineda, B., Anton, M. T., Hedtke, B., Lu, Y., Fisahn, J., Bock, R., Stitt, M., Grimm, B., Granell, A. & Fernie, A. R. 2011. Tomato Fruit Photosynthesis Is Seemingly Unimportant in Primary Metabolism and Ripening But Plays a Considerable Role in Seed Development. *Plant Physiology,* 157**,** 1650-1663.

Majumdar, R., Shao, L., Minocha, R., Long, S. & Minocha, S. C. 2013. Ornithine: The Overlooked Molecule in the Regulation of Polyamine Metabolism3. *Plant and Cell Physiology,* 54**,** 990-1004.

Makhlouf, G. & Blum, A. 1972. Kinetics of the taste response to chemical stimulation: a theory of acid taste in man. *Gastroenterology,* 63**,** 67-75.

Malundo, T. M. M., Shewfelt, R. L. & Scott, J. W. 1995. Flavor quality of fresh tomato (Lycopersicon esculentum Mill.) as affected by sugar and acid levels. *Postharvest Biology and Technology,* 6**,** 103-110.

Marano, M. & Carrillo, N. 1991. Chromoplast formation during tomato fruit ripening. No evidence for plastid DNA methylation. *Plant Molecular Biology,* 16**,** 11-19.

Mariotti, L., Picciarelli, P., Lombardi, L. & Ceccarelli, N. 2011. Fruit-set and Early Fruit Growth in Tomato are Associated with Increases in Indoleacetic Acid, Cytokinin, and Bioactive Gibberellin Contents. *Journal of Plant Growth Regulation,* 30**,** 405.

Martínez-Valverde, I., Periago, M. J., Provan, G. & Chesson, A. 2002. Phenolic compounds, lycopene and antioxidant activity in commercial varieties of tomato (Lycopersicum esculentum). *Journal of the Science of Food and Agriculture,* 82**,** 323-330.

Mathieu, S., Cin, V. D., Fei, Z., Li, H., Bliss, P., Taylor, M. G., Klee, H. J. & Tieman, D. M. 2009. Flavour compounds in tomato fruits: identification of loci and potential pathways affecting volatile composition. *Journal of Experimental Botany,* 60**,** 325-337.

Matsui, K., Sugimoto, K., Mano, J. i., Ozawa, R. & Takabayashi, J. 2012. Differential Metabolisms of Green Leaf Volatiles in Injured and Intact Parts of a Wounded Leaf Meet Distinct Ecophysiological Requirements. *PLoS ONE,* 7**,** e36433.

Matsuo, S., Kikuchi, K., Fukuda, M., Honda, I. & Imanishi, S. 2012. Roles and regulation of cytokinins in tomato fruit development. *Journal of Experimental Botany,* 63**,** 5569-5579.

Mau, J. 2005. The umami taste of edible and medicinal mushrooms. *International Journal of Medicinal Mushrooms,* 7**,** 119.

Maul, F., Sargent, S., Sims, C., Baldwin, E., Balaban, M. & Huber, D. 2000. Tomato flavor and aroma quality as affected by storage temperature. *JOURNAL OF FOOD SCIENCE-CHICAGO-,* 65**,** 1228-1237.

Mayer, F., Takeoka, G. R., Buttery, R. G., Whitehand, L. C., Naim, M. & Rabinowitch, H. D. 2008. Studies on the Aroma of Five Fresh Tomato Cultivars and the Precursors of cis- and trans-4,5-Epoxy-(E)-2-Decenals and Methional. *Journal of Agricultural and Food Chemistry,* 56**,** 3749-3757.

McAtee, P., Karim, S., Schaffer, R. & David, K. 2013. A dynamic interplay between phytohormones is required for fruit development, maturation, and ripening. *Frontiers in Plant Science,* 4**,** 79.

Mcbride, R. L. & Finlay, D. C. 1990. Perceptual integration of tertiary taste mixtures. *Perception & psychophysics,* 48**,** 326-330.

McCaughey, S. A. 2008. The taste of sugars. *Neuroscience & Biobehavioral Reviews,* 32**,** 1024-1043.

McLaughlin, S. & Margolskee, R. F. 1994. The Sense of Taste. *American Scientist,* 82**,** 538-545.

Ménard, C., Dorais, M., Hovi, T. & Gosselin, A. Developmental and physiological responses of tomato and cucumber to additional blue light. V International Symposium on Artificial Lighting in Horticulture 711, 2005. 291-296.

Mencarelli, F. & Saltveit, M. 1988. Ripening of mature-green tomato fruit slices. *J. Amer. Soc. Hort. Sci,* 113**,** 742-745.

Menu, T., Saglio, P., Granot, D., Dai, N., Raymond, P. & Ricard, B. 2004. High hexokinase activity in tomato fruit perturbs carbon and energy metabolism and reduces fruit and seed size. *Plant, Cell & Environment,* 27**,** 89-98.

Michaeli, S. & Fromm, H. 2015. Closing the Loop on the GABA Shunt in Plants: Are GABA metabolism and signaling entwined? *Frontiers in Plant Science,* 6**,** 419.
Miki, N. & Akatsu, K. 1972. Antioxidants in Tomatoes. *NIPPON SHOKUHIN KOGYO GAKKAISHI,* 19**,** 365-370.
Miller, I. J. & Reedy, F. E. 1990. Variations in human taste bud density and taste intensity perception. *Physiology & Behavior,* 47**,** 1213-1219.
Miller, J. & Tanksley, S. 1990. RFLP analysis of phylogenetic relationships and genetic variation in the genus Lycopersicon. *Theoretical and applied genetics,* 80**,** 437-448.
MMR_Research 2018. Unpublished Sensory Profiling Data. MMR Research Worldwide, 104-110 Crowmarsh Battle Barns, Preston Crowmarsh, OX10 6SL, UK.
Moco, S., Bino, R. J., Vorst, O., Verhoeven, H. A., de Groot, J., van Beek, T. A., Vervoort, J. & de Vos, C. H. R. 2006. A Liquid Chromatography-Mass Spectrometry-Based Metabolome Database for Tomato. *Plant Physiology,* 141**,** 1205-1218.
Moco, S., Capanoglu, E., Tikunov, Y., Bino, R. J., Boyacioglu, D., Hall, R. D., Vervoort, J. & De Vos, R. C. H. 2007. Tissue specialization at the metabolite level is perceived during the development of tomato fruit. *Journal of Experimental Botany,* 58**,** 4131-4146.
Moco, S., Forshed, J., De Vos, R. C., Bino, R. J. & Vervoort, J. 2008. Intra-and inter-metabolite correlation spectroscopy of tomato metabolomics data obtained by liquid chromatography-mass spectrometry and nuclear magnetic resonance. *Metabolomics,* 4**,** 202-215.
Moffatt, B. A. & Ashihara, H. 2002. Purine and Pyrimidine Nucleotide Synthesis and Metabolism. *The Arabidopsis Book / American Society of Plant Biologists,* 1**,** e0018.
Mombaerts, P., Wang, F., Dulac, C., Chao, S. K., Nemes, A., Mendelsohn, M., Edmondson, J. & Axel, R. 1996. Visualizing an olfactory sensory map. *Cell,* 87**,** 675-686.
Moretti, C. L., Baldwin, E. A., Sargent, S. A. & Huber, D. J. 2002. Internal Bruising Alters Aroma Volatile Profiles in Tomato Fruit Tissues. *HortScience,* 37**,** 378-382.
Mori, K., Nagao, H. & Yoshihara, Y. 1999. The olfactory bulb: coding and processing of odor molecule information. *Science,* 286**,** 711-715.
Moskowitz, H. R. 1974. Sourness of acid mixtures. *Journal of Experimental Psychology,* 102**,** 640.
Muralla, R., Sweeney, C., Stepansky, A., Leustek, T. & Meinke, D. 2007. Genetic dissection of histidine biosynthesis in Arabidopsis. *Plant Physiol,* 144**,** 890-903.
Murch, S., KrishnaRaj, S. & Saxena, P. 2000. Tryptophan is a precursor for melatonin and serotonin biosynthesis in in vitro regenerated St. John's wort (Hypericum perforatum L. cv. Anthos) plants. *Plant Cell Reports,* 19**,** 698-704.
Murch, S. J., Campbell, S. S. & Saxena, P. K. 2001. The role of serotonin and melatonin in plant morphogenesis: regulation of auxin-induced root organogenesis in in vitro-cultured explants of St. John's wort (Hypericum perforatum L.). *In Vitro Cellular & Developmental Biology-Plant,* 37**,** 786-793.
NCBI. 2018. *Genome Information by Organism* [Online]. National Center for Biotechnology Information. Available: https://www.ncbi.nlm.nih.gov/genome/browse/#!/eukaryotes/plants [Accessed 29/01/2018].
Ninomiya, K. 2002. Umami: a universal taste. *Food reviews international,* 18**,** 23-38.
Noble, A. C., Philbrick, K. C. & Boulton, R. B. 1986. COMPARISON OF SOURNESS OF ORGANIC ACID ANIONS AT EQUAL pH AND EQUAL TITRATABLE ACIDITY. *Journal of Sensory Studies,* 1**,** 1-8.
Odjakova, M. & Hadjiivanova, C. 1997. Animal neurotransmitter substances in plants. *Bulg J Plant Physiol,* 23**,** 94-102.
Ogiso, K., Shimizu, Y., Watanabe, K. & Tonosaki, K. 2000. Possible involvement of undissociated acid molecules in the acid response of the chorda tympani nerve of the rat. *Journal of neurophysiology,* 83**,** 2776-2779.
Olle, M. & Viršile, A. 2013. The effects of light-emitting diode lighting on greenhouse plant growth and quality. *Agricultural and food science,* 22**,** 223-234.
Oms-Oliu, G., Hertog, M. L. A. T. M., Van de Poel, B., Ampofo-Asiama, J., Geeraerd, A. H. & Nicolaï, B. M. 2011. Metabolic characterization of tomato fruit during preharvest

development, ripening, and postharvest shelf-life. *Postharvest Biology and Technology,* 62**,** 7-16.
Oruna-Concha, M.-J., Methven, L., Blumenthal, H., Young, C. & Mottram, D. S. 2007. Differences in Glutamic Acid and 5'-Ribonucleotide Contents between Flesh and Pulp of Tomatoes and the Relationship with Umami Taste. *Journal of Agricultural and Food Chemistry,* 55**,** 5776-5780.
Osorio, S., Ruan, Y.-L. & Fernie, A. R. 2014. An update on source-to-sink carbon partitioning in tomato. *Frontiers in Plant Science,* 5**,** 516.
Ough, C. S. 1963. Sensory Examination of Four Organic Acids Added to Wine. *Journal of Food Science,* 28**,** 101-106.
Page, A. F., Minocha, R. & Minocha, S. C. 2012. Living with high putrescine: expression of ornithine and arginine biosynthetic pathway genes in high and low putrescine producing poplar cells. *Amino Acids,* 42**,** 295-308.
Palanivelu, R., Brass, L., Edlund, A. F. & Preuss, D. 2003. Pollen Tube Growth and Guidance Is Regulated by POP2, an Arabidopsis Gene that Controls GABA Levels. *Cell,* 114**,** 47-59.
Pangborn, R. M. 1961. Taste interrelationships. II. Suprathreshold solutions of sucrose and citric acid. *Journal of Food Science,* 26**,** 648-655.
Pangborn, R. M. 1963. Relative Taste Intensities of Selected Sugars and Organic Acids a. *Journal of Food Science,* 28**,** 726-733.
Passam, H. C., Karapanos, I. C., Bebeli, P. J. & Savvas, D. 2007. A review of recent research on tomato nutrition, breeding and post-harvest technology with reference to fruit quality. *The European Journal of Plant Science and Biotechnology,* 1**,** 1-21.
Pathak, M. R., Teixeira da Silva, J. A. & Wani, S. H. 2014. Polyamines in response to abiotic stress tolerance through transgenic approaches. *GM Crops Food,* 5**,** 87-96.
Pauling, L., Robinson, A. B., Teranishi, R. & Cary, P. 1971. Quantitative analysis of urine vapor and breath by gas-liquid partition chromatography. *Proceedings of the National Academy of Sciences,* 68**,** 2374-2376.
Pelosi, P. 1994. Odorant-Binding Proteins. *Critical Reviews in Biochemistry and Molecular Biology,* 29**,** 199-228.
Pérez-Enciso, M. & Tenenhaus, M. 2003. Prediction of clinical outcome with microarray data: a partial least squares discriminant analysis (PLS-DA) approach. *Human genetics,* 112**,** 581-592.
Perez-Fons, L., Wells, T., Corol, D. I., Ward, J. L., Gerrish, C., Beale, M. H., Seymour, G. B., Bramley, P. M. & Fraser, P. D. 2014. A genome-wide metabolomic resource for tomato fruit from Solanum pennellii. *Sci. Rep.,* 4**,** 3859.
Petreikov, M., Yeselson, L., Shen, S., Levin, I., Schaffer, A. A., Efrati, A. & Bar, M. 2009. Carbohydrate balance and accumulation during development of near-isogenic tomato lines differing in the AGPase-L1 allele. *Journal of the American Society for Horticultural Science,* 134**,** 134-140.
Petro-Turza, M. 1986. Flavor of tomato and tomato products. *Food Reviews International,* 2**,** 309-351.
Pilar Nicasio, M. D., Villarreal, M. L., Gillet, F., Bensaddek, L. & Fliniaux, M.-A. 2005. Variation in the accumulation levels of N, N-dimethyltryptamine in micro-propagated trees and in in vitro cultures of Mimosa tenuiflora. *Natural product research,* 19**,** 61-67.
Piombino, P., Sinesio, F., Moneta, E., Cammareri, M., Genovese, A., Lisanti, M. T., Mogno, M. R., Peparaio, M., Termolino, P., Moio, L. & Grandillo, S. 2013. Investigating physicochemical, volatile and sensory parameters playing a positive or a negative role on tomato liking. *Food Research International,* 50**,** 409-419.
Ponce-Valadez, M., Escalona-Buendía, H. B., Villa-Hernández, J. M., de León-Sánchez, F. D., Rivera-Cabrera, F., Alia-Tejacal, I. & Pérez-Flores, L. J. 2016. Effect of refrigerated storage (12.5°C) on tomato (Solanum lycopersicum) fruit flavor: A biochemical and sensory analysis. *Postharvest Biology and Technology,* 111**,** 6-14.
Prescott, J. 1999. Flavour as a psychological construct: implications for perceiving and measuring the sensory qualities of foods. *Food Quality and Preference,* 10**,** 349-356.
Quinet, M., Dubois, C., Goffin, M.-C., Chao, J., Dielen, V., Batoko, H., Boutry, M. & Kinet, J.-M. 2006. Characterization of tomato (Solanum lycopersicum L.) mutants affected in their

flowering time and in the morphogenesis of their reproductive structure. *Journal of Experimental Botany,* 57**,** 1381-1390.

Radzevičius, A., Karklelienė, R., Viškelis, P., Bobinas, Č., Bobinaitė, R. & Sakalauskienė, S. 2009. Tomato (Lycopersicon esculentum Mill.) fruit quality and physiological parameters at different ripening stages of Lithuanian cultivars. *Agronomy research,* 7**,** 712-718.

Raffo, A., Leonardi, C., Fogliano, V., Ambrosino, P., Salucci, M., Gennaro, L., Bugianesi, R., Giuffrida, F. & Quaglia, G. 2002. Nutritional Value of Cherry Tomatoes (Lycopersicon esculentum Cv. Naomi F1) Harvested at Different Ripening Stages. *Journal of Agricultural and Food Chemistry,* 50**,** 6550-6556.

Rambla, J. L., Tikunov, Y. M., Monforte, A. J., Bovy, A. G. & Granell, A. 2014. The expanded tomato fruit volatile landscape. *Journal of Experimental Botany,* 65**,** 4613-4623.

Ramesh, S. A., Tyerman, S. D., Xu, B., Bose, J., Kaur, S., Conn, V., Domingos, P., Ullah, S., Wege, S., Shabala, S., Feijo, J. A., Ryan, P. R. & Gilliham, M. 2015. GABA signalling modulates plant growth by directly regulating the activity of plant-specific anion transporters. *Nat Commun,* 6**,** 7879.

Rastogi, R., Dulson, J. & Rothstein, S. J. 1993. Cloning of tomato (Lycopersicon esculentum Mill.) arginine decarboxylase gene and its expression during fruit ripening. *Plant Physiology,* 103**,** 829-834.

Rawsthorne, S. 2002. Carbon flux and fatty acid synthesis in plants. *Progress in Lipid Research,* 41**,** 182-196.

Reed, D. R. & Knaapila, A. 2010. Genetics of Taste and Smell: Poisons and Pleasures. *Progress in Molecular Biology and Translational Science,* 94**,** 213-240.

Renault, H., El Amrani, A., Berger, A., Mouille, G., Soubigou-Taconnat, L., Bouchereau, A. & Deleu, C. 2013. γ-Aminobutyric acid transaminase deficiency impairs central carbon metabolism and leads to cell wall defects during salt stress in Arabidopsis roots. *Plant, Cell & Environment,* 36**,** 1009-1018.

Riley, J. C. M. & Thompson, J. E. 1998. Ripening-induced acceleration of volatile aldehyde generation following tissue disruption in tomato fruit. *Physiologia Plantarum,* 104**,** 571-576.

Riley, J. C. M., Willemot, C. & Thompson, J. E. 1996. Lipoxygenase and hydroperoxide lyase activities in ripening tomato fruit. *Postharvest Biology and Technology,* 7**,** 97-107.

Robinson, N. L., Hewitt, J. D. & Bennett, A. B. 1988. Sink Metabolism in Tomato Fruit: I. Developmental Changes in Carbohydrate Metabolizing Enzymes. *Plant Physiology,* 87**,** 727-730.

Rochat, B. 2016. From targeted quantification to untargeted metabolomics: Why LC-high-resolution-MS will become a key instrument in clinical labs. *TrAC Trends in Analytical Chemistry,* 84**,** 151-164.

Roessner-Tunali, U., Hegemann, B., Lytovchenko, A., Carrari, F., Bruedigam, C., Granot, D. & Fernie, A. R. 2003. Metabolic profiling of transgenic tomato plants overexpressing hexokinase reveals that the influence of hexose phosphorylation diminishes during fruit development. *Plant Physiology,* 133**,** 84-99.

Rosales, M. A., Cervilla, L. M., Sánchez-Rodríguez, E., Rubio-Wilhelmi, M. d. M., Blasco, B., Ríos, J. J., Soriano, T., Castilla, N., Romero, L. & Ruiz, J. M. 2011. The effect of environmental conditions on nutritional quality of cherry tomato fruits: evaluation of two experimental Mediterranean greenhouses. *Journal of the Science of Food and Agriculture,* 91**,** 152-162.

Rubico, S. M. & McDaniel, M. R. 1992. Sensory evaluation of acids by free-choice profiling. *Chemical Senses,* 17**,** 273-289.

Ruiz, J. J., Alonso, A., García-Martínez, S., Valero, M., Blasco, P. & Ruiz-Bevia, F. 2005. Quantitative analysis of flavour volatiles detects differences among closely related traditional cultivars of tomato. *Journal of the Science of Food and Agriculture,* 85**,** 54-60.

Sainsbury's. 2016. *Sainsbury's : Groceries* [Online]. Available: http://www.sainsburys.co.uk/shop/gb/groceries [Accessed 05/01/2016].

Saladié, M., Matas, A. J., Isaacson, T., Jenks, M. A., Goodwin, S. M., Niklas, K. J., Xiaolin, R., Labavitch, J. M., Shackel, K. A., Fernie, A. R., Lytovchenko, A., Neill, M. A., Watkins,

C. B. & Rose, J. K. C. 2007. A Reevaluation of the Key Factors That Influence Tomato Fruit Softening and Integrity. *Plant Physiology,* 144**,** 1012.

Salch, Y. P., Grove, M. J., Takamura, H. & Gardner, H. W. 1995. Characterization of a C-5,13-Cleaving Enzyme of 13(S)-Hydroperoxide of Linolenic Acid by Soybean Seed. *Plant Physiology,* 108**,** 1211-1218.

Saliba-Colombani, V., Causse, M., Gervais, L. & Philouze, J. 2000. Efficiency of RFLP, RAPD, and AFLP markers for the construction of an intraspecific map of the tomato genome. *Genome,* 43**,** 29-40.

Salles, C., Nicklaus, S. & Septier, C. 2003. Determination and gustatory properties of taste-active compounds in tomato juice. *Food chemistry,* 81**,** 395-402.

Sankaran, S., Khot, L. R. & Panigrahi, S. 2012. Biology and applications of olfactory sensing system: A review. *Sensors and Actuators B: Chemical,* 171-172**,** 1-17.

Schaffer, A. A. & Petreikov, M. 1997. Sucrose-to-Starch Metabolism in Tomato Fruit Undergoing Transient Starch Accumulation. *Plant Physiology,* 113**,** 739-746.

Scheller, H. V., Huang, B., Hatch, E. & Goldsbrough, P. B. 1987. Phytochelatin Synthesis and Glutathione Levels in Response to Heavy Metals in Tomato Cells. *Plant Physiology,* 85**,** 1031-1035.

Schifferstein, H. N. & Frijters, J. E. 1991. The effectiveness of different sweeteners in suppressing citric acid sourness. *Perception & psychophysics,* 49**,** 1-9.

Schildknecht, H. 1981. Irritant and defense substances of higher plants—a chemical herbarium. *Angewandte Chemie International Edition in English,* 20**,** 164-184.

Schilmiller, A. L., Schauvinhold, I., Larson, M., Xu, R., Charbonneau, A. L., Schmidt, A., Wilkerson, C., Last, R. L. & Pichersky, E. 2009. Monoterpenes in the glandular trichomes of tomato are synthesized from a neryl diphosphate precursor rather than geranyl diphosphate. *Proceedings of the National Academy of Sciences,* 106**,** 10865-10870.

Schmutz, A., Jenny, T., Amrhein, N. & Ryser, U. 1993. Caffeic acid and glycerol are constituents of the suberin layers in green cotton fibres. *Planta,* 189**,** 453.

Schouten, R. E., Woltering, E. J. & Tijskens, L. M. M. 2016. Sugar and acid interconversion in tomato fruits based on biopsy sampling of locule gel and pericarp tissue. *Postharvest Biology and Technology,* 111**,** 83-92.

Schutte, L. & Teranishi, R. 1974. Precursors of sulfur-containing flavor compounds. *Critical Reviews in Food Science & Nutrition,* 4**,** 457-505.

Sederoff, R., Campbell, M., O'Malley, D. & Whetten, R. 1994. Genetic regulation of lignin biosynthesis and the potential modification of wood by genetic engineering in loblolly pine. *Genetic Engineering of Plant Secondary Metabolism.* Springer.

Selli, S., Kelebek, H., Ayseli, M. T. & Tokbas, H. 2014. Characterization of the most aroma-active compounds in cherry tomato by application of the aroma extract dilution analysis. *Food Chemistry,* 165**,** 540-546.

Semel, Y., Schauer, N., Roessner, U., Zamir, D. & Fernie, A. R. 2007. Metabolite analysis for the comparison of irrigated and non-irrigated field grown tomato of varying genotype. *Metabolomics,* 3**,** 289-295.

Serrani, J. C., Ruiz-Rivero, O., Fos, M. & García-Martínez, J. L. 2008. Auxin-induced fruit-set in tomato is mediated in part by gibberellins. *The Plant Journal,* 56**,** 922-934.

Shammai, A., Petreikov, M., Yeselson, Y., Faigenboim, A., Moy-Komemi, M., Cohen, S., Cohen, D., Besaulov, E., Efrati, A., Houminer, N., Bar, M., Ast, T., Schuldiner, M., Klemens, P. A. W., Neuhaus, E., Baxter, C. J., Rickett, D., Bonnet, J., White, R., Giovannoni, J. J., Levin, I. & Schaffer, A. 2018. Natural genetic variation for expression of a SWEET transporter among wild species of Solanum lycopersicum (tomato) determines the hexose composition of ripening tomato fruit. *The Plant Journal,* 96**,** 343-357.

Shelp, B. J., Walton, C. S., Snedden, W. A., Tuin, L. G., Oresnik, I. J. & Layzell, D. B. 1995. Gaba shunt in developing soybean seeds is associated with hypoxia. *Physiologia Plantarum,* 94**,** 219-228.

Shen, J., Tieman, D., Jones, J. B., Taylor, M. G., Schmelz, E., Huffaker, A., Bies, D., Chen, K. & Klee, H. J. 2014. A 13-lipoxygenase, TomloxC, is essential for synthesis of C5 flavour volatiles in tomato. *Journal of Experimental Botany,* 65**,** 419-428.

Simkin, A. J., Schwartz, S. H., Auldridge, M., Taylor, M. G. & Klee, H. J. 2004. The tomato carotenoid cleavage dioxygenase 1 genes contribute to the formation of the flavor volatiles β-ionone, pseudoionone, and geranylacetone. *The Plant Journal,* 40**,** 882-892.

Slimestad, R. & Verheul, M. 2009. Review of flavonoids and other phenolics from fruits of different tomato (Lycopersicon esculentum Mill.) cultivars. *Journal of the Science of Food and Agriculture,* 89**,** 1255-1270.

Slocum, R. D. 2005. Genes, enzymes and regulation of arginine biosynthesis in plants. *Plant Physiology and Biochemistry,* 43**,** 729-745.

Song, J., Fan, L. & Beaudry, R. M. 1998. Application of Solid Phase Microextraction and Gas Chromatography/Time-of-Flight Mass Spectrometry for Rapid Analysis of Flavor Volatiles in Tomato and Strawberry Fruits. *Journal of Agricultural and Food Chemistry,* 46**,** 3721-3726.

Sorrequieta, A., Ferraro, G., Boggio, S. & Valle, E. 2010. Free amino acid production during tomato fruit ripening: a focus on l-glutamate. *Amino Acids,* 38**,** 1523-1532.

Spaepen, S., Vanderleyden, J. & Remans, R. 2007. Indole-3-acetic acid in microbial and microorganism-plant signaling. *FEMS microbiology reviews,* 31**,** 425-448.

Speirs, J., Lee, E., Holt, K., Yong-Duk, K., Scott, N. S., Loveys, B. & Schuch, W. 1998. Genetic manipulation of alcohol dehydrogenase levels in ripening tomato fruit affects the balance of some flavor aldehydes and alcohols. *Plant Physiology,* 117**,** 1047-1058.

Sperling, P., Zähringer, U. & Heinz, E. 1998. A Sphingolipid Desaturase from Higher Plants IDENTIFICATION OF A NEW CYTOCHROME b 5FUSION PROTEIN. *Journal of Biological Chemistry,* 273**,** 28590-28596.

Spiegel, S. & Milstien, S. 2003. Sphingosine-1-phosphate: an enigmatic signalling lipid. *Nature reviews Molecular cell biology,* 4**,** 397.

Srivastava, A. & Handa, A. K. 2005. Hormonal regulation of tomato fruit development: a molecular perspective. *Journal of plant growth regulation,* 24**,** 67-82.

Stahl, W. & Sies, H. 2003. Antioxidant activity of carotenoids. *Molecular Aspects of Medicine,* 24**,** 345-351.

Stern, D. J., Buttery, R. G., Teranishi, R., Ling, L., Scott, K. & Cantwell, M. 1994. Effect of storage and ripening on fresh tomato quality, Part I. *Food Chemistry,* 49**,** 225-231.

Stevens, M. A., Kader, A. A. & Albright, M. 1979. Potential for increasing tomato flavor via increased sugar and acid content. *Journal of the American Society for Horticultural Science,* 104**,** 40-42.

Stevens, S. S. & Davis, H. 1938. *Hearing: Its psychology and physiology,* American Institute of Physics for the Acoustical Society of America New York.

Stevenson, R. J., Prescott, J. & Boakes, R. A. 1999. Confusing Tastes and Smells: How Odours can Influence the Perception of Sweet and Sour Tastes. *Chemical Senses,* 24**,** 627-635.

Stevenson, R. J. & Tomiczek, C. 2007. Olfactory-induced synesthesias: A review and model. *Psychological Bulletin,* 133**,** 294.

Takayama, M. & Ezura, H. 2015. How and why does tomato accumulate a large amount of GABA in the fruit? *Frontiers in Plant Science,* 6**,** 612.

Tandon, K., Baldwin, E., Scott, J. & Shewfelt, R. 2003. Linking sensory descriptors to volatile and nonvolatile components of fresh tomato flavor. *Journal of food science,* 68**,** 2366-2371.

Tandon, K. S., Baldwin, E. A. & Shewfelt, R. L. 2000. Aroma perception of individual volatile compounds in fresh tomatoes (Lycopersicon esculentum, Mill.) as affected by the medium of evaluation. *Postharvest Biology and Technology,* 20**,** 261-268.

Tanksley, S. D. & McCouch, S. R. 1997. Seed banks and molecular maps: unlocking genetic potential from the wild. *Science,* 277**,** 1063-1066.

Tegeder, M. 2014. Transporters involved in source to sink partitioning of amino acids and ureides: opportunities for crop improvement. *Journal of Experimental Botany,* 65**,** 1865-1878.

TESCO. 2016. *TESCO : Groceries* [Online]. Available: http://www.tesco.com/groceries/ [Accessed 05/01/2016].

Thanet Earth. 2013. *Thanet Earth Marketing* [Online]. Available: http://www.thanetearth.com/ [Accessed 20/01/2015 2015].

The Tomato Genome Consortium 2012. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature,* 485**,** 635-641.

Thorne, S. N. & Efiuvwevwere, B. J. O. 1988. Changes in organic acids in chilled tomato fruit (Lycopersicon esculentum Mill). *Journal of the Science of Food and Agriculture,* 44**,** 309-319.

Tieman, D., Bliss, P., McIntyre, Lauren M., Blandon-Ubeda, A., Bies, D., Odabasi, Asli Z., Rodríguez, Gustavo R., van der Knaap, E., Taylor, Mark G., Goulet, C., Mageroy, Melissa H., Snyder, Derek J., Colquhoun, T., Moskowitz, H., Clark, David G., Sims, C., Bartoshuk, L. & Klee, Harry J. 2012. The Chemical Interactions Underlying Tomato Flavor Preferences. *Current Biology,* 22**,** 1035-1039.

Tieman, D., Taylor, M., Schauer, N., Fernie, A. R., Hanson, A. D. & Klee, H. J. 2006a. Tomato aromatic amino acid decarboxylases participate in synthesis of the flavor volatiles 2-phenylethanol and 2-phenylacetaldehyde. *Proceedings of the National Academy of Sciences,* 103**,** 8287-8292.

Tieman, D., Zhu, G., Resende, M. F., Lin, T., Nguyen, C., Bies, D., Rambla, J. L., Beltran, K. S. O., Taylor, M. & Zhang, B. 2017. A chemical genetic roadmap to improved tomato flavor. *Science,* 355**,** 391-394.

Tieman, D. M., Zeigler, M., Schmelz, E. A., Taylor, M. G., Bliss, P., Kirst, M. & Klee, H. J. 2006b. Identification of loci affecting flavour volatile emissions in tomato fruits. *Journal of Experimental Botany,* 57**,** 887-896.

Tikunov, Y., Lommen, A., de Vos, C. H. R., Verhoeven, H. A., Bino, R. J., Hall, R. D. & Bovy, A. G. 2005. A Novel Approach for Nontargeted Data Analysis for Metabolomics. Large-Scale Profiling of Tomato Fruit Volatiles. *Plant Physiology,* 139**,** 1125-1137.

Tkalcic, M. & Tasic, J. F. Colour spaces: perceptual, historical and applicational background. Eurocon, 2003. 304-308.

Tohge, T., Alseekh, S. & Fernie, A. R. 2014. On the regulation and function of secondary metabolism during fruit development and ripening. *Journal of Experimental Botany,* 65**,** 4599-611.

Tomchik, S. M., Berg, S., Kim, J. W., Chaudhari, N. & Roper, S. D. 2007. Breadth of Tuning and Taste Coding in Mammalian Taste Buds. *The Journal of Neuroscience,* 27**,** 10840.

Tzin, V. & Galili, G. 2010. The Biosynthetic Pathways for Shikimate and Aromatic Amino Acids in Arabidopsis thaliana. *The Arabidopsis Book / American Society of Plant Biologists,* 8**,** e0132.

Udenfriend, S., Lovenberg, W. & Sjoerdsma, A. 1959. Physiologically active amines in common fruits and vegetables. *Archives of Biochemistry and Biophysics,* 85**,** 487-490.

Ugawa, T., Konosu, S. & Kurihara, K. 1992. Enhancing effects of NaCl and Na phosphate on human gustatory responses to amino acids. *Chemical Senses,* 17**,** 811-815.

Umbarger, H. E. 1978. Amino Acid Biosynthesis and its Regulation. *Annual Review of Biochemistry,* 47**,** 533-606.

Utto, W., Mawson, A. J. & Bronlund, J. E. 2008. Hexanal reduces infection of tomatoes by Botrytis cinerea whilst maintaining quality. *Postharvest Biology and Technology,* 47**,** 434-437.

Valle, E. M., Boggio, S. B. & Heldt, H. W. 1998. Free amino acid composition of phloem sap and growing fruit of Lycopersicon esculentum. *Plant and Cell Physiology,* 39**,** 458-461.

Vallverdú-Queralt, A., Jáuregui, O., Medina-Remón, A., Andrés-Lacueva, C. & Lamuela-Raventós, R. M. 2010. Improved characterization of tomato polyphenols using liquid chromatography/electrospray ionization linear ion trap quadrupole Orbitrap mass spectrometry and liquid chromatography/electrospray ionization tandem mass spectrometry. *Rapid Communications in Mass Spectrometry,* 24**,** 2986-2992.

Van Etten, C. H., Kwolek, W. F., Peters, J. E. & Barclay, A. S. 1967. Plant seeds as protein sources of food or feed. Evaluation based on amino acid composition of 379 species. *Journal of Agricultural and Food Chemistry,* 15**,** 1077-1089.

Verhagen, J. V. & Engelen, L. 2006. The neurocognitive bases of human multimodal food perception: sensory integration. *Neuroscience & biobehavioral reviews,* 30**,** 613-650.

Vermeir, S., Beullens, K., Mészáros, P., Polshin, E., Nicolaï, B. M. & Lammertyn, J. 2009. Sequential injection ATR-FTIR spectroscopy for taste analysis in tomato. *Sensors and Actuators B: Chemical,* 137**,** 715-721.

Voet, D. & Voet, J. G. 2004. *Biochemistry.*

Vogel, J. T., Tieman, D. M., Sims, C. A., Odabasi, A. Z., Clark, D. G. & Klee, H. J. 2010. Carotenoid content impacts flavor acceptability in tomato (Solanum lycopersicum). *Journal of the Science of Food and Agriculture,* 90**,** 2233-2240.

Waitrose. 2016. *Waitrose : Groceries* [Online]. Available: http://www.waitrose.com/shop/Browse/Groceries [Accessed 05/01/2016].

Walker, A., Ho, L. & Baker, D. 1978. Carbon Translocation in the Tomato: Pathways of Carbon Metabolism in the Fruit–. *Annals of Botany,* 42**,** 901-909.

Walker, A. J. & Ho, L. C. 1977. Carbon Translocation in the Tomato: Carbon Import and Fruit Growth. *Annals of Botany,* 41**,** 813-823.

Wang, D. & Seymour, G. B. 2017. Tomato flavor: lost and found? *Molecular plant,* 10**,** 782-784.

Wang, F., Sanz, A., Brenner, M. L. & Smith, A. 1993. Sucrose Synthase, Starch Accumulation, and Tomato Fruit Sink Strength. *Plant Physiology,* 101**,** 321-327.

Ward Jr, J. H. 1963. Hierarchical grouping to optimize an objective function. *Journal of the American statistical association,* 58**,** 236-244.

Weckwerth, W. 2003. Metabolomics in systems biology. *Annual review of plant biology,* 54**,** 669-689.

Weimer, C. & Slupsky, C. 2013. *Metabolomics in Food and Nutrition,* Woodhead Publishing.

Winkel, B. S. 2006. The biosynthesis of flavonoids. *The science of flavonoids.* Springer.

Winter, G., Todd, C. D., Trovato, M., Forlani, G. & Funck, D. 2015. Physiological implications of arginine metabolism in plants. *Frontiers in Plant Science,* 6**,** 534.

Wishart, D. S. 2008. Metabolomics: applications to food science and nutrition research. *Trends in Food Science & Technology,* 19**,** 482-493.

Wishart, D. S., Tzur, D., Knox, C., Eisner, R., Guo, A. C., Young, N., Cheng, D., Jewell, K., Arndt, D., Sawhney, S., Fung, C., Nikolai, L., Lewis, M., Coutouly, M. A., Forsythe, I., Tang, P., Shrivastava, S., Jeroncic, K., Stothard, P., Amegbey, G., Block, D., Hau, D. D., Wagner, J., Miniaci, J., Clements, M., Gebremedhin, M., Guo, N., Zhang, Y., Duggan, G. E., Macinnis, G. D., Weljie, A. M., Dowlatabadi, R., Bamforth, F., Clive, D., Greiner, R., Li, L., Marrie, T., Sykes, B. D., Vogel, H. J. & Querengesser, L. 2007. HMDB: the Human Metabolome Database. *Nucleic Acids Res,* 35**,** D521-6.

Wu, Q., Tao, X., Ai, X., Luo, Z., Mao, L., Ying, T. & Li, L. 2018. Contribution of abscisic acid to aromatic volatiles in cherry tomato (Solanum lycopersicum L.) fruit during postharvest ripening. *Plant Physiology and Biochemistry,* 130**,** 205-214.

Wurtzel, E. T. & Kutchan, T. M. 2016. Plant metabolism, the diverse chemistry set of the future. *Science,* 353**,** 1232-1236.

Yamaguchi, S. 1967. The Synergistic Taste Effect of Monosodium Glutamate and Disodium 5′-Inosinate. *Journal of Food Science,* 32**,** 473-478.

Yamaguchi, S. & Ninomiya, K. 2000. Umami and Food Palatability. *The Journal of Nutrition,* 130**,** 921S-926S.

Yamaguchi, S., Yoshikawa, T., Ikeda, S. & Ninomiya, T. 1970. Studies on the Taste of Some Sweet Substances. *Agricultural and Biological Chemistry,* 34**,** 181-197.

Yamaguchi, S., Yoshikawa, T., Ikeda, S. & Ninomiya, T. 1971. Measurement of the relative taste intensity of some L-α-amino acids and 5′-nucleotides. *Journal of Food Science,* 36**,** 846-849.

Yáñez, J. A., Remsberg, C. M., Miranda, N. D., Vega-Villa, K. R., Andrews, P. K. & Davies, N. M. 2008. Pharmacokinetics of selected chiral flavonoids: hesperetin, naringenin and eriodictyol in rats and their content in fruit juices. *Biopharmaceutics & drug disposition,* 29**,** 63-82.

Yao, L. H., Jiang, Y., Shi, J., Tomas-Barberan, F., Datta, N., Singanusong, R. & Chen, S. 2004. Flavonoids in food and their health benefits. *Plant foods for human nutrition,* 59**,** 113-122.

Yelle, S., Hewitt, J. D., Robinson, N. L., Damon, S. & Bennett, A. B. 1988. Sink Metabolism in Tomato Fruit: III. Analysis of Carbohydrate Assimilation in a Wild Species. *Plant Physiology,* 87**,** 737-740.

Yoshida, H., Takamura, N., Shuto, T., Ogata, K., Tokunaga, J., Kawai, K. & Kai, H. 2010. The citrus flavonoids hesperetin and naringenin block the lipolytic actions of TNF-α in mouse adipocytes. *Biochemical and Biophysical Research Communications,* 394**,** 728-732.

Zhang, F., Klebansky, B., Fine, R. M., Xu, H., Pronin, A., Liu, H., Tachdjian, C. & Li, X. 2008. Molecular mechanism for the umami taste synergism. *Proceedings of the National Academy of Sciences of the United States of America,* 105**,** 20930-20934.

Zhang, J., Zhao, J., Xu, Y., Liang, J., Chang, P., Yan, F., Li, M., Liang, Y. & Zou, Z. 2015. Genome-Wide Association Mapping for Tomato Volatiles Positively Contributing to Tomato Flavor. *Frontiers in Plant Science,* 6**,** 1042.

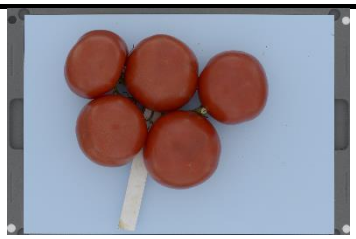
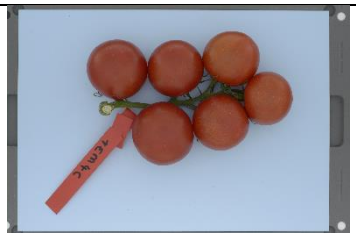
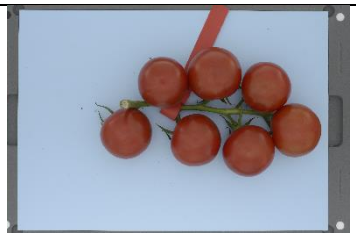
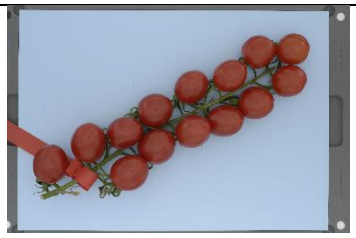
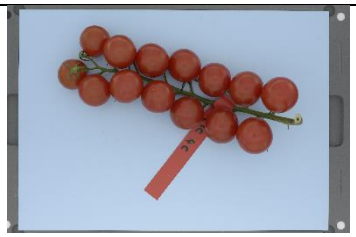
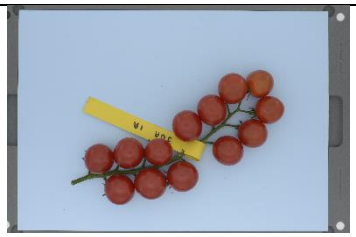
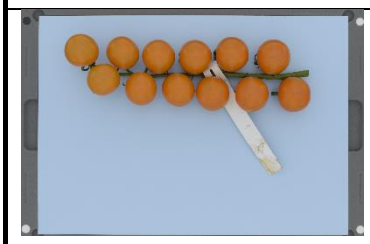
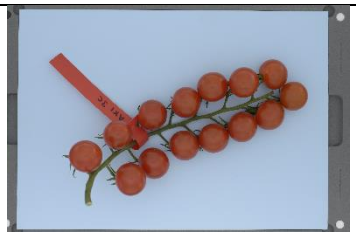
Zimmerlin, A., Wojtaszek, P. & Bolwell, G. P. 1994. Synthesis of dehydrogenation polymers of ferulic acid with high specificity by a purified cell-wall peroxidase from French bean (Phaseolus vulgaris L.). *Biochemical Journal,* 299**,** 747-753.

Zrenner, R., Stitt, M., Sonnewald, U. & Boldt, R. 2006. PYRIMIDINE AND PURINE BIOSYNTHESIS AND DEGRADATION IN PLANTS. *Annual Review of Plant Biology,* 57**,** 805-836.

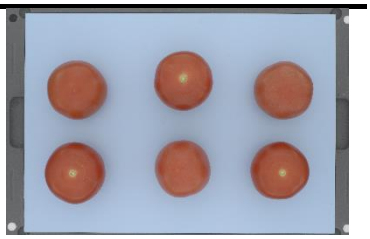
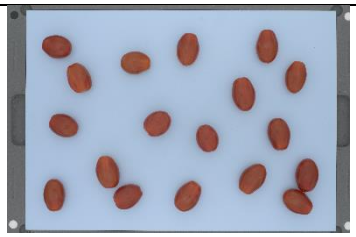
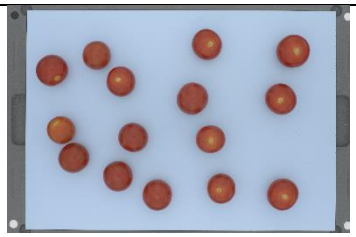
Zuorro, A., Lavecchia, R., Medici, F. & Piga, L. 2013. Enzyme-Assisted Production of Tomato Seed Oil Enriched with Lycopene from Tomato Pomace. *Food and Bioprocess Technology,* 6**,** 3499-3509.

Appendices

Appendix 1 – Images of cultivars used in Chapter 3, 4, 6, 7 and 8.

Tomato Type	Winter	Summer
Beefsteak		
	ELE - Elegance	EL2 – Elegance2
Salad		
	TEM - Temptation	CAM – Campari
		
		194 – P194
Plum		
	SUN - Sunstream	DR2 - DR28090TC
Cherry		
	PIC - Piccolo	JUA – Juanita
		
	ORA – Orange Cherry	AXI – Axiani

Appendix 2 - Images of cultivars used in Chapter 5.

Tomato Type	Cultivars
Salad	
	Valkiria
Baby Plum	
	Angelle
Cherry	
	Genio

Appendix 3 – Questionnaire used for sensory analysis in 2017 and 2018.

Thank you for your participation

QUESTIONAIRE[1]

Participant Information

This first section collects some personal information to use for statistical analysis of the data. This information and any responses on this questionnaire are completely disassociated from your name and only linked by panellist number. If you would like to withdraw from the study at any point please notify the researcher.

What is your Panellist Number: ……..

Gender: ……..

Do you smoke? ……..

Age	Mark with an X
18 OR YOUNGER	
19-25	
26-35	
36-45	
46-55	
56 - 65	
66 OR OLDER	

Please select a first tomato sample to try and continue on with the rest of the questionnaire, thank you for your support and participation.

Thank you for your participation

QUESTIONAIRE[1]

Which tomato sample is		**this?**	**Panellist No.:**	

APPEARANCE: Please observe the sample.

Q1. How would you rate your overall liking of the APPEARANCE of the sample?

CIRCLE ONE CODE ONLY

Don't like it at all.	1	2	3	4	5	6	7	8	9	10	Like it a lot.

Q2. Which of the following phrases best describes your opinion of the COLOUR of the tomato?

Much too Dark	5	
Slightly too Dark	4	
Exactly Right	3	
Slightly too Light	2	
Much too Light	1	

Q3. Which of the following phrases best describes your opinion of the SHININESS of the tomato?

Much too Shiny	5	
Slightly too Shiny	4	
Exactly Right	3	

Slightly too Matt	2	
Much too Matt	1	

Q4. Looking at the following list, which of these descriptors would you say apply to the appearance of the tomato? Tick as many as apply.

Wrinkled		Split	
Shiny		Fresh	
Firm		Soft	
Even distribution of colour		Green	
Watery		Dented	
Dry		Mottled	
Juicy		Other:	

FLAVOUR: Please taste the sample by biting off a small piece, slowly chewing and spreading throughout your mouth.

Q5. How would you rate your overall liking of the FLAVOUR of the sample?

CIRCLE ONE CODE ONLY

Don't like it at all.	1	2	3	4	5	6	7	8	9	10	Like it a lot.

Q6. Looking at the following list, which of these descriptors would you say apply to the flavour of the tomato? Tick as many as apply.

Lingering		Tangy	
Over-ripe		Under-ripe	
Fruity		Sour	
Bland		Musty	
Citrus		Salty	
Bitter		Grass-like	
Earthy		Other:	

MOUTHFEEL/TEXTURE: After eating the tomato, please try to recall the texture or feeling of the tomato during chewing.

Q7. How would you rate your overall liking of the TEXTURE/MOUTHFEEL of the sample?

CIRCLE ONE CODE ONLY

Don't like it at all.	1	2	3	4	5	6	7	8	9	10	Like it a lot.

Q8. Which of the following phrases best describes your opinion of the FIRMNESS of the FLESH of the tomato?

Much too firm	5	
Slightly too firm	4	

Exactly Right	3	
Slightly too soft	2	
Much too soft	1	

Q9. Which of the following phrases best describes your opinion of the JUICINESS of the tomato?

Much too juicy	5	
Slightly too juicy	4	
Exactly Right	3	
Not quite juicy enough	2	
Not at all juicy enough	1	

OVERALL ACCEPTABILITY

Q24. How would you rate the OVERALL ACCEPTABILITY of the sample?

CIRCLE ONE CODE ONLY

Don't like it at all.	1	2	3	4	5	6	7	8	9	10	Like it a lot.

This concludes your evaluation of this sample.

This **concludes** your sensory sampling. If you could please turn over the page and specify your overall most preferred sample by providing the sample code in the box provided.

Appendix 4 – The 74 mass spectral features that were significantly different between the populations of ELE (winter) and EL2 (summer) fruits. The molecular weight, protonated molecular ion in positive mode (M±H) and observed retention time is provided for further.

Mass Spectral Feature	Mean Peak Areas		p Value	Molecular Weight	M±H	RT (min)
	ELE	EL2				
M3090	3,077,542	1,383,205	$1.83E^{-20}$	253.18061	254.18789	3.41
M3099	249,571	113,721	$1.83E^{-20}$	345.12605	346.13333	3.41
M3414	1,836,923	1,075,542	$4.98E^{-17}$	180.04330	181.05058	3.66
M5315	1,146,067	593,951	$3.39E^{-16}$	1,309.60002	1,310.60730	4.65
M4686	478,039	334,277	$9.90E^{-16}$	386.19621	387.20349	4.26
M1897	724,743	436,304	$1.82E^{-15}$	187.08561	188.09289	1.29
M1898	684,508	397,410	$9.90E^{-15}$	169.07508	170.08236	1.29
M2057	373,660	629,155	$9.90E^{-15}$	175.08560	176.09288	1.42
M6232	150,302	80,250	$1.41E^{-14}$	572.19112	573.19840	5.07
M0803	328,280	530,614	$2.22E^{-13}$	174.11286	175.12014	0.82
M2078	2,094,287	1,474,519	$4.66E^{-13}$	120.05848	121.06576	1.44
M1414	562,455	360,749	$9.50E^{-13}$	120.05846	121.06574	0.93
M3153	165,382	135,384	$1.25E^{-12}$	412.13640	413.14368	3.47
M4697	279,084	188,164	$1.89E^{-12}$	408.17816	409.18544	4.27
M0599	1,472,763	1,157,004	$3.68E^{-12}$	211.84569	212.85297	0.79
M2895	129,050	84,433	$1.31E^{-11}$	468.14108	469.14836	3.27
M1875	11,955,038	17,390,070	$1.48E^{-11}$	129.04349	130.05077	1.29
M3756	693,618	476,643	$1.48E^{-11}$	437.22879	438.23607	3.81
M1212	319,833	219,658	$2.40E^{-11}$	290.10233	291.10961	0.92
M4397	1,335,347	978,390	$7.69E^{-11}$	388.17639	389.18367	4.10
M5143	123,144	186,362	$1.08E^{-10}$	632.13949	633.14677	4.57
M1273	22,369,076	16,618,247	$1.20E^{-10}$	267.09863	268.10591	0.92
M1306	2,379,155	1,861,615	$1.50E^{-10}$	262.08161	263.08889	0.92
M4393	1,027,298	783,020	$7.32E^{-10}$	410.15771	411.16499	4.10
M1999	53,126,650	42,090,215	$8.10E^{-10}$	267.09865	268.10593	1.35
M1475	15,145,583	20,829,775	$1.34E^{-09}$	129.04350	130.05078	0.95
M2069	558,180	400,614	$2.40E^{-09}$	137.08496	138.09224	1.43
M0698	483,910	600,912	$3.52E^{-09}$	208.95082	209.95810	0.81
M5543	135,833	252,375	$6.15E^{-09}$	429.24969	430.25697	4.75
M2837	1,384,219	1,946,218	$7.39E^{-09}$	104.06392	105.07120	3.18
M3352	105,065	150,736	$7.40E^{-09}$	318.14606	319.15334	3.61
M0589	499,485	403,394	$2.15E^{-08}$	379.76923	380.77651	0.79
M4075	205,379	142,163	$2.34E^{-08}$	393.23824	394.24552	3.97
M1205	271,457	176,620	$2.46E^{-08}$	331.12809	332.13537	0.92
M0594	2,472,983	1,886,526	$2.78E^{-08}$	173.88984	174.89712	0.79
M4377	254,878	191,872	$3.59E^{-08}$	405.20273	406.21001	4.10
M4503	371,278	565,717	$3.59E^{-08}$	468.16381	469.17109	4.17
M6180	3,979,466	5,463,145	$1.04E^{-07}$	267.19672	268.20400	5.06
M7905	1,486,093	1,899,854	$1.22E^{-07}$	317.29461	318.30189	8.59
M4693	504,182	364,405	$2.12E^{-07}$	224.14227	225.14955	4.26
M1304	6,304,574	5,352,779	$2.29E^{-07}$	283.09380	284.10108	0.92
M1846	2,363,858	1,875,461	$3.35E^{-07}$	262.08148	263.08876	1.29

M7307	173,183	128,618	$4.19E^{-07}$	470.21576	471.22304	5.99
M6941	239,525	173,541	$5.24E^{-07}$	700.27589	701.28317	5.58
M4105	184,291	238,748	$9.35E^{-07}$	419.18215	420.18943	3.98
M1876	326,118	237,043	$1.43E^{-06}$	255.12343	256.13071	1.29
M1498	7,058,922	5,728,906	$2.31E^{-06}$	176.09609	177.10337	0.96
M4612	235,728	196,438	$3.45E^{-06}$	188.12125	189.12853	4.22
M7812	1,371,143	1,126,764	$3.45E^{-06}$	315.27895	316.28623	8.28
M0596	448,629	370,165	$5.12E^{-06}$	295.80797	296.81525	0.79
M1705	1,916,817	2,349,034	$1.49E^{-05}$	176.03341	177.04069	1.25
M0637	126,823	157,497	$1.78E^{-05}$	292.91308	293.92036	0.80
M4376	280,519	208,324	$1.78E^{-05}$	609.39210	610.39938	4.10
M1308	1,955,593	1,703,865	$2.26E^{-05}$	151.05060	152.05788	0.92
M2052	12,251,696	10,929,949	0.000115	220.08510	221.09238	1.42
M0697	1,094,521	1,257,071	0.000128	180.95556	181.96284	0.81
M0696	998,756	1,128,897	0.000184	221.98258	222.98986	0.81
M0287	2,631,626	3,108,530	0.000352	155.07053	156.07781	0.74
M1844	13,547,802	12,288,000	0.000624	307.08512	308.09240	1.29
M2388	2,027,559	2,379,218	0.000685	160.03848	161.04576	2.17
M3295	244,864	217,055	0.000786	400.10886	401.11614	3.58
M1704	354,932	414,119	0.001078	140.01222	141.01950	1.25
M0373	246,956	283,537	0.001126	189.15771	190.16499	0.77
M2062	86,360	101,090	0.001596	312.17027	313.17755	1.43
M3481	123,195	146,732	0.002236	297.20680	298.21408	3.70
M0470	191,928	214,963	0.002747	335.94367	336.95095	0.78
M5549	86,357	100,832	0.002747	206.13203	207.13931	4.75
M2159	244,777	214,590	0.003101	214.01003	215.01731	1.55
M0891	4,307,580	4,718,608	0.005564	119.05925	120.06653	0.84
M0895	152,386,096	163,319,437	0.005778	147.05406	148.06134	0.84
M8462	214,906	276,025	0.008352	453.28872	454.29600	10.09
M0905	120,594,426	129,312,706	0.016127	130.02724	131.03452	0.84
M5171	716,036	645,813	0.016127	206.13192	207.13920	4.58
M0988	1,635,973	1,470,805	0.027777	288.08680	289.09408	0.87

Appendix 5 – Discriminant features of the 10 tomato cultivars used in this study. Features presented are common to all cultivars, or biomarkers of a single cultivar, as determined through binary classification using multivariate ROC curves. Supplementary Data for Chapter 4 and 7.

MSfeature	Most Abundant Cultivar	Monoisotopic Mass	M+H	RT
M0796	PIC	295.06062	296.06790	0.82
M0898	JUA	117.08020	118.08748	0.84
M1048	TEM	521.14469	522.15197	0.88
M1346	PIC	274.11834	275.12562	0.92
M1345	PIC	256.10811	257.11539	0.92
M1871	ELE	250.06377	251.07105	1.29
M1870	PIC	256.10719	257.11447	1.29
M2263	DR2	161.05263	162.05991	1.88
M2263	EL2	161.05263	162.05991	1.88
M2447	PIC	205.07783	206.08511	2.38
M2509	ORA	433.11734	434.12462	2.63
M2525	SUN	227.02607	228.03335	2.65
M2536	PIC	327.13379	328.14107	2.68
M2552	SUN	261.15927	262.16655	2.72
M2554	SUN	198.09041	199.09769	2.72
M2553	DR2	233.12724	234.13452	2.72
M2553	SUN	233.12724	234.13452	2.72
M2640	TEM	378.15258	379.15986	2.89
M2661	PIC	308.13905	309.14633	2.93
M2708	JUA	244.18068	245.18796	3.00
M2730	PIC	250.13350	251.14078	3.03
M2739	ORA	299.10205	300.10933	3.05
M2741	ORA	461.15722	462.16450	3.05
M2804	ORA	602.18782	603.19510	3.14
M2817	PIC	500.15645	501.16373	3.16
M2855	PIC	308.13947	309.14675	3.21
M2863	ORA	360.10551	361.11279	3.22
M2871	PIC	422.17905	423.18633	3.23
M2883	SUN	457.15356	458.16084	3.24
M2882	SUN	457.15567	458.16295	3.24
M2882	TEM	457.15567	458.16295	3.24
M2888	ORA	247.17971	248.18699	3.26
M2888	SUN	247.17971	248.18699	3.26
M2941	DR2	175.06842	176.07570	3.30
M2942	TEM	266.16482	267.17210	3.30
M3077	TEM	238.14393	239.15121	3.40
M3155	JUA	254.18445	255.19173	3.47
M3172	PIC	466.20612	467.21340	3.48
M3242	ORA	182.09273	183.10001	3.54
M3312	PIC	206.14337	207.15065	3.59
M3312	SUN	206.14337	207.15065	3.59
M3314	PIC	189.11719	190.12447	3.59

M3372	PIC	311.25980	312.26708	3.63
M3438	ORA	376.10253	377.10981	3.67
M3441	ORA	203.07755	204.08483	3.68
M3446	ORA	406.15414	407.16142	3.68
M3446	TEM	406.15414	407.16142	3.68
M3463	JUA	284.08913	285.09641	3.69
M3495	TEM	304.15219	305.15947	3.70
M3504	TEM	299.19602	300.20330	3.70
M3516	ORA	504.24995	505.25723	3.71
M3517	ORA	731.26693	732.27421	3.71
M3522	PIC	592.26619	593.27347	3.71
M3537	ORA	138.05217	139.05945	3.71
M3536	ORA	137.04862	138.05590	3.71
M3555	ORA	143.07481	144.08209	3.72
M3558	ORA	653.20040	654.20768	3.72
M3560	ORA	653.19697	654.20425	3.72
M3577	ORA	162.03265	163.03993	3.73
M3597	ORA	1116.20996	1117.21724	3.74
M3624	ORA	213.11636	214.12364	3.75
M3658	CAM	258.19654	259.20382	3.77
M3658	JUA	258.19654	259.20382	3.77
M3664	PIC	526.28307	527.29035	3.77
M3710	JUA	226.16992	227.17720	3.79
M3719	JUA	271.22744	272.23472	3.79
M3729	JUA	452.33843	453.34571	3.79
M3831	JUA	216.14933	217.15661	3.85
M3839	JUA	473.25624	474.26352	3.86
M3837	PIC	526.28321	527.29049	3.86
M3858	TEM	555.14504	556.15232	3.86
M3928	JUA	152.12155	153.12883	3.92
M3930	JUA	372.17779	373.18507	3.92
M3947	DR2	862.34155	863.34883	3.93
M3963	SUN	434.17738	435.18466	3.93
M3967	ORA	175.10471	176.11199	3.93
M4029	SUN	440.13907	441.14635	3.95
M4029	TEM	440.13907	441.14635	3.95
M4045	DR2	456.22502	457.23230	3.96
M4045	JUA	456.22502	457.23230	3.96
M4053	194	440.13616	441.14344	3.96
M4103	PIC	257.18811	258.19539	3.98
M4170	DR2	158.09520	159.10248	4.00
M4185	ORA	405.15889	406.16617	4.01
M4223	AXI	496.30871	497.31599	4.04
M4283	TEM	375.19092	376.19820	4.06
M4295	TEM	179.08286	180.09014	4.07
M4308	DR2	281.15018	282.15746	4.08
M4305	TEM	830.31548	831.32276	4.08
M4315	DR2	566.19377	567.20105	4.08

M4315	TEM	566.19377	567.20105	4.08
M4318	TEM	264.12354	265.13082	4.08
M4326	TEM	550.22827	551.23555	4.08
M4354	DR2	1529.66873	1530.67601	4.09
M4354	TEM	1529.66873	1530.67601	4.09
M4450	PIC	320.18735	321.19463	4.13
M4486	CAM	306.07363	307.08091	4.16
M4488	CAM	590.16520	591.17248	4.16
M4488	SUN	590.16520	591.17248	4.16
M4504	ORA	446.18235	447.18963	4.17
M4504	SUN	446.18235	447.18963	4.17
M4506	SUN	469.16777	470.17505	4.17
M4517	SUN	484.12928	485.13656	4.17
M4559	JUA	490.20678	491.21406	4.19
M4577	JUA	458.10344	459.11072	4.20
M4594	TEM	170.09318	171.10046	4.21
M4631	194	540.14939	541.15667	4.23
M4665	TEM	361.21203	362.21931	4.24
M4682	SUN	463.20789	464.21517	4.25
M4684	SUN	468.16202	469.16930	4.25
M4722	PIC	609.39337	610.40065	4.28
M4741	SUN	231.18537	232.19265	4.30
M4868	DR2	393.09040	394.09768	4.37
M4868	TEM	393.09040	394.09768	4.37
M4966	DR2	272.07091	273.07819	4.43
M4974	ELE	694.36252	695.36980	4.44
M4985	ORA	610.15636	611.16364	4.45
M5016	194	520.18068	521.18796	4.48
M5016	DR2	520.18068	521.18796	4.48
M5017	194	410.10944	411.11672	4.48
M5016	SUN	520.18068	521.18796	4.48
M5016	TEM	520.18068	521.18796	4.48
M5018	SUN	410.10764	411.11492	4.48
M5021	SUN	515.22433	516.23161	4.48
M5024	SUN	520.17809	521.18537	4.48
M5043	ORA	303.11595	304.12323	4.50
M5136	ORA	261.11695	262.12423	4.57
M5137	ORA	278.14367	279.15095	4.57
M5183	SUN	410.10918	411.11646	4.58
M5183	TEM	410.10918	411.11646	4.58
M5188	194	554.16529	555.17257	4.58
M5188	SUN	554.16529	555.17257	4.58
M5193	SUN	410.10714	411.11442	4.58
M5228	JUA	406.15468	407.16196	4.61
M5280	ELE	348.25960	349.26688	4.64
M5357	JUA	620.17495	621.18223	4.66
M5364	JUA	598.19433	599.20161	4.66
M5367	ELE	580.18413	581.19141	4.66

M5367	JUA	580.18413	581.19141	4.66
M5407	CAM	484.36579	485.37307	4.68
M5407	JUA	484.36579	485.37307	4.68
M5557	JUA	450.11963	451.12691	4.75
M5557	TEM	450.11963	451.12691	4.75
M5562	ORA	575.38606	576.39334	4.76
M5579	PIC	150.10558	151.11286	4.76
M5650	194	431.34216	432.34944	4.79
M5739	194	591.37983	592.38711	4.82
M5740	194	591.38267	592.38995	4.82
M5740	CAM	591.38267	592.38995	4.82
M5759	PIC	662.18999	663.19727	4.83
M5781	JUA	343.23734	344.24462	4.84
M5795	CAM	424.34278	425.35006	4.85
M5795	CAM	424.34278	425.35006	4.85
M5799	JUA	942.62648	943.63376	4.85
M5798	JUA	424.34485	425.35213	4.85
M5795	JUA	424.34278	425.35006	4.85
M5809	CAM	324.08677	325.09405	4.86
M5809	PIC	324.08677	325.09405	4.86
M5813	PIC	180.04347	181.05075	4.86
M5829	PIC	162.03255	163.03983	4.86
M5832	JUA	594.16293	595.17021	4.87
M5837	AXI	384.14143	385.14871	4.87
M5847	CAM	294.09699	295.10427	4.88
M5872	CAM	1067.57960	1068.58688	4.88
M6033	194	1066.57115	1067.57843	4.97
M6051	TEM	488.13575	489.14303	4.98
M6081	JUA	339.14675	340.15403	5.00
M6085	DR2	678.29448	679.30176	5.00
M6085	JUA	678.29448	679.30176	5.00
M6210	SUN	318.13225	319.13953	5.06
M6250	PIC	470.30617	471.31345	5.08
M6266	PIC	341.12689	342.13417	5.09
M6276	CAM	396.14305	397.15033	5.10
M6274	PIC	341.12887	342.13615	5.10
M6283	ORA	414.15506	415.16234	5.10
M6288	ORA	426.18954	427.19682	5.10
M6290	TEM	1148.85728	1149.86456	5.10
M6362	PIC	516.13010	517.13738	5.15
M6427	PIC	341.12813	342.13541	5.18
M6433	JUA	272.07080	273.07808	5.19
M6435	JUA	434.12266	435.12994	5.19
M6438	JUA	434.12485	435.13213	5.19
M6502	ORA	387.15583	388.16311	5.25
M6500	ORA	392.11118	393.11846	5.25
M6528	DR2	304.10000	305.10728	5.26
M6591	AXI	410.10929	411.11657	5.32

M6599	TEM	813.57486	814.58214	5.32
M6633	AXI	287.63329	288.64057	5.34
M6633	PIC	287.63329	288.64057	5.34
M6636	PIC	575.26766	576.27494	5.34
M6640	SUN	1375.03001	1376.03729	5.34
M6668	JUA	237.18601	238.19329	5.35
M6768	JUA	408.22651	409.23379	5.43
M6772	JUA	386.24332	387.25060	5.44
M6805	194	678.29357	679.30085	5.48
M6816	ORA	426.18939	427.19667	5.49
M6835	DR2	272.07054	273.07782	5.50
M6857	JUA	516.13027	517.13755	5.52
M6857	PIC	516.13027	517.13755	5.52
M6868	JUA	520.12619	521.13347	5.53
M6900	PIC	500.13363	501.14091	5.55
M6903	SUN	1357.01958	1358.02686	5.55
M6930	CAM	299.29575	300.30303	5.57
M6930	ORA	299.29575	300.30303	5.57
M6964	TEM	162.12692	163.13420	5.60
M7121	CAM	612.41500	613.42228	5.77
M7121	JUA	612.41500	613.42228	5.77
M7181	PIC	466.18501	467.19229	5.83
M7186	PIC	461.22753	462.23481	5.83
M7190	ORA	294.09788	295.10516	5.83
M7190	PIC	294.09788	295.10516	5.83
M7240	TEM	184.07604	185.08332	5.88
M7238	TEM	182.07187	183.07915	5.88
M7237	TEM	154.04078	155.04806	5.88
M7258	TEM	204.05437	205.06165	5.88
M7308	JUA	465.26013	466.26741	5.99
M7330	ELE	593.39793	594.40521	6.07
M7368	SUN	596.15820	597.16548	6.18
M7405	ORA	482.21661	483.22389	6.26
M7404	ORA	442.22347	443.23075	6.26
M7406	ORA	442.22122	443.22850	6.26
M7522	ORA	302.08094	303.08822	6.69
M7537	DR2	302.08142	303.08870	6.81
M7537	ORA	302.08142	303.08870	6.81
M7537	PIC	302.08142	303.08870	6.81
M7585	AXI	624.25137	625.25865	7.12
M7610	DR2	201.08382	202.09110	7.33
M7610	TEM	201.08382	202.09110	7.33
M7652	TEM	273.26931	274.27659	7.65
M7657	SUN	273.26800	274.27528	7.65
M7679	TEM	289.26328	290.27056	7.74
M7759	JUA	392.30659	393.31387	8.07
M7777	JUA	264.09958	265.10686	8.12
M7800	JUA	264.09916	265.10644	8.21

M7901	JUA	208.05389	209.06117	8.59
M8007	PIC	246.11230	247.11958	8.98
M8042	AXI	452.16296	453.17024	9.04
M8292	ELE	293.23775	294.24503	9.74
M8335	AXI	315.22014	316.22742	9.84
M8699	AXI	320.25368	321.26096	10.61
M8793	AXI	335.22499	336.23227	10.81
M8882	AXI	260.21674	261.22402	11.03
M9158	AXI	460.39445	461.40173	11.72
M9160	AXI	442.38305	443.39033	11.72
M9157	PIC	500.38717	501.39445	11.72
M9160	PIC	442.38305	443.39033	11.72
M9159	PIC	478.40468	479.41196	11.72
M9161	PIC	478.40676	479.41404	11.72
M9194	TEM	354.27944	355.28672	11.77

Appendix 6 – Discriminant features and potential biomarkers of cherry tomato ripening, as determined by the analyses of Axiani, Juanita and Piccolo tomatoes throughout ripening. Supporting data for Chapter 8

MSFeature	Present in AXI	JUA	PIC	Molecular Weight	M+H	RT [min]
M1248	Yes	Yes	Yes	335.94092	336.9482	0.798
M1162	Yes	Yes	Yes	208.94915	209.95643	0.827
M0257	Yes			180.95472	181.962	0.828
M0571	Yes		Yes	180.95415	181.96143	0.83
M0662	Yes			221.98127	222.98855	0.831
M0083	Yes	Yes	Yes	222.96457	223.97185	0.837
M1102	Yes			174.11178	175.11906	0.847
M1031	Yes	Yes	Yes	348.06501	349.07229	0.914
M0595	Yes	Yes	Yes	143.05815	144.06543	0.915
M1326	Yes	Yes	Yes	156.00571	157.01299	0.929
M0207	Yes	Yes	Yes	169.07411	170.08139	0.93
M1100	Yes			182.07733	183.08461	0.938
M0590			Yes	249.03078	250.03806	1.35
M0688	Yes	Yes	Yes	307.08368	308.09096	1.355
M0102	Yes		Yes	187.04817	188.05545	1.391
M0078	Yes	Yes		118.04198	119.04926	1.434
M0099	Yes			135.06845	136.07573	1.434
M0743	Yes	Yes	Yes	267.09691	268.10419	1.436
M0009	Yes	Yes	Yes	284.09511	285.10239	1.52
M0833	Yes	Yes		131.09479	132.10207	1.553
M0717	Yes	Yes	Yes	187.04809	188.05537	1.572
M0342	Yes	Yes	Yes	167.08081	168.08809	1.618
M0379	Yes	Yes	Yes	176.09512	177.1024	2.245
M0380	Yes	Yes	Yes	159.0685	160.07578	2.247
M0884	Yes		Yes	341.1327	342.13998	2.334
M0250	Yes	Yes	Yes	381.1629	382.17018	3.09
M0367			Yes	175.06662	176.0739	3.18
M0960	Yes			145.11042	146.1177	3.191
M0660	Yes	Yes	Yes	342.09427	343.10155	3.392
M0894			Yes	394.15929	395.16657	3.408
M0996			Yes	393.15641	394.16369	3.409
M1134	Yes		Yes	528.29396	529.30124	3.5
M0917	Yes		Yes	264.14758	265.15486	3.507
M0169	Yes	Yes		143.07374	144.08102	3.533
M0550	Yes		Yes	101.0845	102.09178	3.581
M0357	Yes		Yes	400.10642	401.1137	3.617
M1047			Yes	180.0424	181.04968	3.646
M0082	Yes		Yes	265.13921	266.14649	3.663
M0604	Yes		Yes	206.14161	207.14889	3.715
M1192		Yes		264.14852	265.1558	3.716
M1193		Yes	Yes	264.14761	265.15489	3.719
M1303	Yes	Yes	Yes	143.07352	144.0808	3.877
M0133	Yes		Yes	131.07373	132.08101	3.879

M1185	Yes			486.20059	487.20787	3.896
M0110	Yes		Yes	208.10991	209.11719	4.07
M0927	Yes		Yes	190.09934	191.10662	4.071
M0066	Yes	Yes	Yes	294.09506	295.10234	4.379
M0685	Yes	Yes	Yes	814.45247	815.45975	4.379
M0633	Yes	Yes	Yes	206.09421	207.10149	4.41
M0627	Yes			201.11548	202.12276	4.553
M0017	Yes	Yes	Yes	206.13075	207.13803	4.638
M1265	Yes	Yes	Yes	472.19426	473.20154	4.638
M0726	Yes		Yes	609.38748	610.39476	4.875
M1120			Yes	447.33463	448.34191	4.876
M1175	Yes		Yes	593.39181	594.39909	4.93
M0046	Yes	Yes	Yes	291.09383	292.10111	5.067
M0088	Yes	Yes	Yes	290.0903	291.09758	5.067
M0485	Yes		Yes	208.10971	209.11699	5.137
M0436	Yes	Yes	Yes	635.40295	636.41023	5.165
M0911	Yes			162.03146	163.03874	5.258
M0364	Yes		Yes	516.12663	517.13391	5.259
M0394	Yes	Yes		331.27194	332.27922	5.424
M0068	Yes			575.26327	576.27055	5.435
M0679		Yes	Yes	575.38196	576.38924	5.541
M0556	Yes	Yes	Yes	473.34642	474.3537	5.568
M0582	Yes	Yes	Yes	636.4053	637.41258	5.597
M0705		Yes	Yes	473.35155	474.35883	5.613
M0749	Yes		Yes	901.50358	902.51086	5.735
M0774			Yes	676.27304	677.28032	5.785
M0117		Yes		387.16772	388.175	6.27
M0408			Yes	576.36642	577.3737	7.306
M0370	Yes	Yes	Yes	315.27705	316.28433	8.371
M0371	Yes	Yes	Yes	315.2779	316.28518	8.371
M1103	Yes			264.91658	265.92386	0.828
M1104		Yes		305.94221	306.94949	0.829
M1105	Yes	Yes		305.94318	306.95046	0.829
M0663	Yes		Yes	221.9806	222.98788	0.833
M0999	Yes		Yes	260.9205	261.92778	0.833
M0506	Yes		Yes	222.96541	223.97269	0.835
M1124			Yes	296.8968	297.90408	0.835
M1090	Yes		Yes	185.0091	186.01638	0.846
M0952			Yes	101.04811	102.05539	0.879
M0616	Yes	Yes		133.07404	134.08132	0.899
M1243	Yes	Yes	Yes	153.07892	154.0862	0.928
M0572	Yes	Yes	Yes	128.01098	129.01826	0.93
M0374		Yes		371.10437	372.11165	0.935
M0626	Yes	Yes		83.03728	84.04456	0.935
M0433	Yes		Yes	146.02187	147.02915	1.228
M1043			Yes	336.06874	337.07602	1.344
M0505	Yes			256.10552	257.1128	1.353
M1231	Yes	Yes	Yes	104.01132	105.0186	1.354

M1283			Yes	88.03514	89.04242	1.395
M0123	Yes	Yes		164.0475	165.05478	1.434
M0490	Yes	Yes		85.08949	86.09677	1.543
M0304	Yes	Yes		318.14258	319.14986	1.56
M0860	Yes		Yes	225.00373	226.01101	1.568
M1256	Yes	Yes	Yes	188.05127	189.05855	1.568
M0349		Yes		181.07414	182.08142	1.654
M0932			Yes	161.05115	162.05843	1.971
M1241	Yes		Yes	188.03204	189.03932	2.376
M0213	Yes			176.09519	177.10247	2.46
M1118	Yes		Yes	87.10521	88.11249	2.543
M0847	Yes		Yes	184.15778	185.16506	2.835
M0639	Yes		Yes	382.16631	383.17359	3.089
M0683	Yes			114.0684	115.07568	3.241
M0641	Yes		Yes	404.16768	405.17496	3.43
M1123	Yes	Yes	Yes	130.04187	131.04915	3.664
M0592	Yes		Yes	189.11498	190.12226	3.715
M0625			Yes	270.10992	271.1172	3.775
M0052	Yes	Yes	Yes	448.13671	449.14399	3.835
M1010	Yes		Yes	114.04677	115.05405	3.877
M0289	Yes			570.37461	571.38189	3.961
M0607	Yes			285.18697	286.19425	3.964
M0815	Yes			276.68321	277.69049	3.974
M0196			Yes	189.08224	190.08952	4.021
M0584	Yes	Yes	Yes	174.08966	175.09694	4.12
M1087			Yes	178.06296	179.07024	4.132
M0857	Yes		Yes	446.17879	447.18607	4.236
M0138			Yes	338.0997	339.10698	4.308
M0186			Yes	815.45616	816.46344	4.343
M0412	Yes		Yes	295.09855	296.10583	4.379
M0300	Yes	Yes	Yes	416.16819	417.17547	4.485
M0985			Yes	532.25231	533.25959	4.614
M0007	Yes	Yes	Yes	224.14114	225.14842	4.638
M0806			Yes	832.46477	833.47205	4.694
M0795	Yes			281.09022	282.0975	4.789
M0703	Yes		Yes	432.34294	433.35022	4.835
M0500	Yes	Yes	Yes	450.35301	451.36029	4.838
M1125			Yes	447.33589	448.34317	4.886
M0715	Yes	Yes	Yes	594.1585	595.16578	4.955
M0652	Yes	Yes	Yes	144.04226	145.04954	5.081
M0545	Yes	Yes	Yes	325.10869	326.11597	5.086
M0547			Yes	577.39721	578.40449	5.124
M0333			Yes	295.0991	296.10638	5.165
M0399	Yes	Yes	Yes	653.41251	654.41979	5.165
M0084			Yes	589.39768	590.40496	5.303
M0620			Yes	208.10981	209.11709	5.306
M1127		Yes	Yes	471.33464	472.34192	5.508
M1052			Yes	767.44596	768.45324	5.577

M1078			Yes	398.72719	399.73447	5.578
M0507	Yes	Yes	Yes	635.40242	636.4097	5.584
M0704		Yes	Yes	473.35018	474.35746	5.584
M0405	Yes	Yes	Yes	474.3532	475.36048	5.598
M0397			Yes	516.12675	517.13403	5.964