Tomato quality:
from the field to the consumer

Interactions between genotype, cultivation and postharvest conditions

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To the people
that like to throw a monkey wrench in our works

Contents

Chapter 1

General introduction

Consumers have become more aware of fruit and vegetable quality (Batt, 2006; Hewett, 2006) and, therefore, they demand a higher quality level of the products they buy. Quality of a fresh product includes attributes and properties like such as colour, texture, flavour and health promoting compounds, and possible damage or defects (Abbott, 1999). Although all these intrinsic characteristics are involved in the definition of quality, for many years most breeding efforts have been mainly directed to improve and maintain the external quality. Selection for yield, fruit size, colour, and shelf life traits might have had unintended negative consequences on fruit quality (Goff and Klee, 2006). Indeed, as reported from a survey of US Department of Agriculture (USDA), the nutrient content of intensively bred crops, selected mainly for rapid growth and increased yield, has drastically dropped in the period between 1950 and 1999 (Davis et al., 2004). Tomato breeding is a vivid example of how selection for certain traits has had indirect negative consequences on consumer's perceived quality. For instance, most modern tomato cultivars contain a mutation that inactivates the UNIFORM transcription factor resulting in more visually appealing and more uniformly ripen fruits. However, these fruits have lower level of carotenoids, and significantly fewer soluble solids (Powell et al., 2012).

Health organizations typically recommend that at least five portions of fruit and vegetables are to be consumed every day as part of a balanced diet; conversely many consumers generally do not regularly eat this quantity of fresh produce even if the average consumer has now access to a broad range of fresh fruit products throughout all the year (Butelli et al., 2008). The high cost of purchasing these products and the inadequate quality are the major deterrent; furthermore more than 20% of the edible fruit purchases are discarded before being eaten (FAO, global food losses and food waste 2011). The highly perishable nature of fruit products often accounts for this postharvest waste owing to their short shelf life.

More effort and attention have to be devoted to improve and optimize quality upon delivery to the consumer. Quality has to be maintained or even enhanced during storage and marketing and it has to be considered as a central trait in fruit chain management. In order to understand quality of agricultural products and to satisfy consumer's demands, the generation of quality during the growing and storage period has to be studied, unravelled, and modelled. The subjects covered in this thesis focus on pre- and post-harvest factors affecting the consumer's perceived quality of tomato (*Solanum lycopersicum, L.*).

1. What is quality?

Several definitions of quality have been proposed over the years:

"Quality is the composite of those characteristics that differentiate individual units of a product, and have significance in determining the degree of acceptability of that unit by the buyer." (Kramer and Twigg, 1983)

"Perceived quality is an idiosyncratic value judgement with respect to the fitness for consumption which is based upon the conscious and/or unconscious processing of quality cues in relation to relevant quality attributes within the context of significant personal and situational variables." (Steenkamp, 1990)

"The term quality implies the degree of excellence of a product or its suitability for a particular use."(Abbott, 1999)

Implicit in all three definitions is that quality is the result of the interaction between the consumer and the product. Quality is a human construct comprising many attributes. In working with quality of agricultural produce, researchers are implicitly and most often unknowingly involved in the psychology of man, and his ever-changing behaviour, his ever-changing desires and the ever-changing fulfilment of these desires. It is generally accepted that the quality of perishable products depends mainly on three factors: the product, the user, and the market situation (Figure 1). Shewfelt (1999) points out that quality is often defined from either a product orientation or a consumer orientation. Quality is assigned to a commodity by the consumer (or user), based on the perceived properties of a particular sample. Consumers perceive those properties (e.g. sugar and acid content) and convert them into attributes (e.g. sweetness and sourness). The value of a particular product, assigned by the user, is also based on the market situation (assigned value) and on the social circumstances of the evaluator and the intended use for the commodity (assigned usability). Consumers ultimately use all three assigned notions to decide whether to accept a product or not (Tijskens and Schouten, 2009). It is therefore difficult to define what quality is, and how to control it. Decomposing the effects of these factors on quality leads to a distinction between the assigned quality and the acceptability of a product. Assigned quality is the quality notion a consumer has of a product and results from evaluating that product with respect to his criteria. Acceptability defines whether the consumer in that particular situation is willing to buy that product. Of course, one must always remember that there is more than one customer in the marketing chain. The next person or institution in the following chain can be considered a customer by the previous one: from grower to the distributor as well as from the wholesaler to the ultimate consumer who actually eats the product. Each passes judgment, and each has its own set of quality or acceptability criteria, often influenced by personal expectations and preferences.

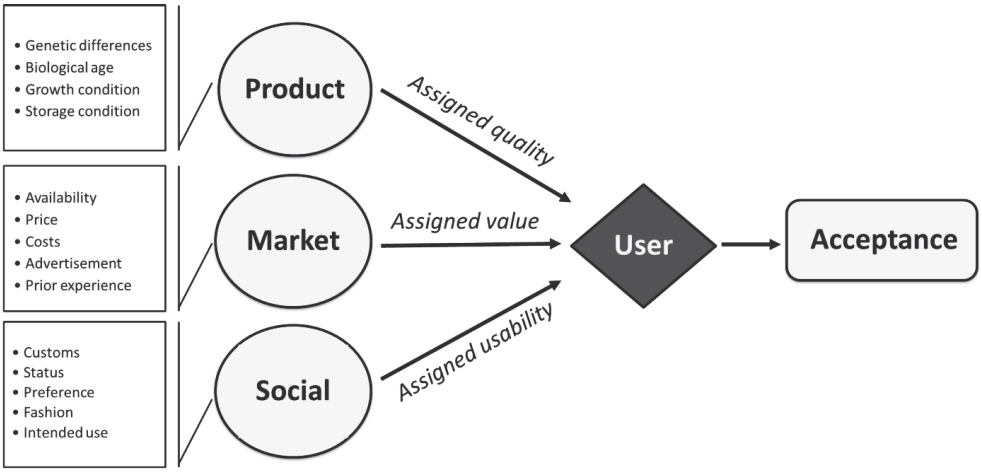

Figure 1. Schematic representation of quality and acceptance. Adapted from Tijskens and Schouten (2009).

People use all of their senses to evaluate quality: sight, smell, taste, touch, and even hearing. The consumer integrates all of those sensory inputs (appearance, aroma, favour, hand-feel, mouth-feel, and chewing sounds) into a final judgment of the acceptability of that fruit or vegetable. There are two problems with using consumers to provide evaluations rather than appreciations. Firstly it is an unnatural task for consumers. People are naturally able to say how much they like a particular attribute but intensive training (as a product expert, as a member of a sensory analytical panel) is required before they are reproducibly able to score the intensity of a particular attribute. Secondly the relationship between the intensity of an attribute and the appreciation is often non-linear.

Instrumental measurements are preferred over sensory evaluations for many research and commercial applications because instruments reduce variations among individuals and can provide a common language among researchers, industry, and consumers. However, the relationship of the instrumental measurement to sensory attributes (e.g. descriptive analysis) and the relationship of those sensory attributes to consumer acceptability must be considered (Shewfelt, 1999). Instruments may be designed to imitate human testing methods or may be statistically related to human perceptions and judgments to predict quality categories. There is an increasing use of non-invasive spectroscopic and other multi-parameter instruments, indicative of an increasing recognition that a given quality attribute or cue is generally a function of several product properties.

The traditional way of thinking about quality and of developing empirical models and data analysis, has to expand to include all available knowledge and information. Traditional models, mainly statistical or empirical models, are no longer sufficiently reliable to predict quality. It becomes progressively clear that a systematic approach, based on the combination of the increased technical and technological capabilities and new mathematical advance modelling analysis, is vitally important to study fruit and vegetable quality. We have to include as much as possible of the available knowledge, both in the pre-harvest (i.e. fruit and vegetable

production) and in the postharvest phase (i.e. distribution, processing, sales, and service) even if the communication between the two areas is often problematic due to differing viewpoints on quality and importance (Tijskens and van Kooten, 2006).

2. Fruit quality attributes

The attributes that combine into quality vary with context. The choice of what to measure, how to measure it, and what values are acceptable are determined by the person requiring the measurement, with consideration of the intended use of the product and of the measurement, available technology, economics and cultural background. The expected quality of a product is generally based on consumer evaluation of quality cues. A quality cue is an attribute of the food product, which can be perceived before purchase and consumption. The individual, based on his personal experience or on information from acquaintances or the media, believes the cue to be highly correlated with product quality. The quality attributes can be divided into experience attributes which are determined before and during usage (flavour, ease of preparation) and credence attributes which are based on beliefs (nutritional value, production methods, food safety) (Tijskens et al., 2001).External quality attributes related to the appearance, for the majority of fruits and vegetables, are:

- firmness
- colour
- absence of defects
- shrivelling (water loss)

Lately, internal quality attributes, related to flavour perception and health, gain more importance for the consumer and hence for the whole chain. These attributes comprise:

- taste (sweet, sour)
- aroma and flavour
- juiciness
- crispness
- absence of fibrousness
- health promoting compounds (vitamins, lycopene, glucosinolates, etc)

2.1. Appearance and colour

Appearance is the key factor for consumers. Fruits and vegetables are expected to have near perfect visual appearance. Main components of visual quality include colour and colour uniformity, glossiness, and absence of defects in shape or skin and lack of disease. Appearance is used throughout the fruit production chain as the primary means of judging the quality of individual units of product (Kays, 1999). The appearance of products is commonly evaluated by considering their size, shape, form, colour, freshness condition, and finally the absence of visual defects (Costa et al., 2011). Particularly, colour is one of the most important food quality attributes affecting consumer acceptance, the taste, and flavour perception (Costa et al., 2011; Grossman and Wisenblit, 1999; Crisosto et al., 2003; Leon et al., 2006; Nisha et al., 2011; Bayarri et al., 2001; Francis, 1995). Colour is considered an important grading factor for most food products since it can provide basic quality information for human perception and has close

association with quality factors such as freshness, maturity, desirability, and food safety (McCaig, 2002). The perception of colour is a complex phenomenon that depends on the composition of the object, on its illumination environment, on the characteristics of the perceiving eye and brain, and on the angles of illumination and viewing.

Many fruits and vegetables undergo colour changes as part of the ripening and senescence process. Unripe fruit is usually green and in many types of fruit, such as apple, peach and grape the green colour becomes lighter during ripening due to breakdown of chlorophyll. This may reveal underlying or new synthetized yellow or red pigments. Peel and pulp often undergo different colour changes, as in apple, melon or banana. In some cases, fruit colour is a strong indicator of eating quality and shelf-life, for example, in tomato. Many pre- and post-harvest factors, such as light, mineral nutrients or abiotic stress, can affect fruit colour independently of other ripeness characteristics.

Tomato colour

The colour and appearance of tomato are the first quality attributes to stimulate a consumer to purchase and consume them. Tomatoes are known for their vibrant red colour which indicates not only maturity and freshness, and therefore level of desired flavour, but also relative content of the beneficial antioxidant metabolites, mainly lycopene.

Colour transformation, together with volatile production and texture decay, is one of the most significant and evident changes of tomato fruits during ripening. Chlorophyll and carotenoids are the major metabolites responsible for the colour of tomatoes. In the early stages of development the chlorophyll imparts a green colour while when the tomato starts the ripening process, the chlorophyll is degraded and carotenoids are synthesized. As most of the pigments that accounts for the perceived tomato colour are found in the pericarp, efforts have been undertaken to link colour measurements with pigment content (Arias et al., 2000). Colour assessment has generally been done based on measurements expressed in the Lab or in the RGB colour space.

Colour assessment is not only important for the determination of the consumer's perceived quality but also for the defining the optimal harvesting time. Timing of harvest depends upon end market requirements and local practices. Picking can start at the mature-green stage, but this can result in fruit, once ripened, being orange-red and having poor flavour. Thus, the 'breaker' or 'turning' stage of fruit development – when colour is turning from green to tannish-yellow - is when most fresh fruit is picked, particularly if it is to be shipped considerable distances.

Colour data, as well as to define tomato quality, are also intensively used to understand the physiological process behind ripening by using mathematical modelling (Tijskens and Evelo, 1994; Hertog et al., 2004; Schouten et al., 2007; Pinheiro et al., 2013). Most approaches are very similar and describe tomato colour development as one process where a green pigment complex is converted into a red pigment complex by an enzyme that is exponentially increasing resulting in a description of the colour transformation as a logistic curve. These kinetic models incorporate the effect of temperature and also describe how the frequently large differences in developmental age at harvest affect the colour formation during storage. This type of process-oriented modelling was also applied by Schouten et al. (2007) in order to define the acceptance period (acceptability) of tomato batches, of different ripening stages, by combining modelling

of colour and texture during storage. This model takes into account that tomatoes can first be unacceptable due to being unripe, then be acceptable, and then be unacceptable again, due to being over-ripe.

2.2. Texture

A general accepted definition of texture states that "texture is the sensory and functional manifestation of the structural, mechanical and surface properties of foods detected through the senses of vision, hearing, touch and kinaesthetic" (Szczesniak, 2002). Texture not only drives consumer preference, but also has a significant impact on shelf life, acceptability, and transportability. Texture, as well as the other principal quality factors (Bourne, 2002), has been recognized as a multi-factorial trait, being composed of several sub-traits grouped in two main categories, such as mechanical (firmness, hardness, stiffness, and elasticity) and acoustic (crispness and crunchiness) components, (Szczesniak, 2002; Costa et al. 2012). These two categories are largely distinguished by their physical nature. Textural quality and related sensory attributes, such as juiciness, turgidity, and crispness do influence human perception of flavour and future research should contribute to improved understanding of the physical and chemical changes that contribute to desirable texture and flavour of fruits and vegetables.

Tomato texture

Texture is one of the critical components for the consumer's perception of tomato fruit quality (Causse et al., 2003; Chaib et al., 2007; Sinesio et al., 2010). Many traits are involved in fruit texture, mainly sensory attributes such as flesh firmness, mealiness, meltiness, juiciness, and crispness. Understanding the key factors that influence texture and ripening-related softening of tomato fruit has been a priority from a horticultural and commercial perspective. Major changes in texture, occurring during fruit ripening, considerably influence post-harvest performance, i.e. transportation, storage, shelf life and pathogen resistance (Brummell and Harpster, 2001). So far, however, unravelling the complex nature of texture has proved to be far more challenging than initially expected, principally because texture is influenced by many factors, including cell wall structure, cuticle properties, cellular turgor and fruit morphology (Vicente et al., 2007). The tomato fruit cell wall is probably the best studied with respect to changes during ripening (Brummell and Harpster, 2001; Seymour et al., 1990) but, until now, the precise roles of most of the polysaccharide and glycoprotein components are still not entirely understood (McCann and Rose, 2010). Biochemical models, based on enzymes activity during fruit ripening, suggested that mainly two enzyme families, such as polygalacturonases and the pectin methylesterases, might play the major role in controlling texture changes in tomato (Brummell, 2006; Giovannoni, 2001).

In addition, another important contributor to texture and fruit firmness is cellular turgor that is mainly governed by the water status within fruit and the relative symplastic and apoplastic distribution of water. In order to extend shelf life and generally enhance texture factors that influence fruit turgor during development and ripening should be studied.

2.3. Flavour

Fruit and vegetable flavour depends upon taste (balance between sweetness and sourness or acidity, and low or no astringency) and aroma (concentrations of odour active volatile compounds). Although taste and aroma are well integrated in their contribution to the overall flavour, aroma is often considered to play a dominant role in flavour (Kader, 2008).

Perceived sweetness is mainly determined by the concentrations of the predominant sugars, which are ranked relative to sucrose in the following order of sweetness: fructose (1.2) > sucrose (1.0) > glucose (0.64). Acidity or sourness is determined by the concentrations of the predominant organic acids, which are ranked relative to citric acid in the following order of sourness: citric (1.0) > malic (0.9) > tartaric (0.8) (Kader, 2008). Taste may also be influenced by the presence of some amino acids and also by minerals such as calcium, phosphorus, and potassium. Glycine and alanine have a sweet taste, valine and leucine have a bitter taste, and aspartic acid and glutamate have sour and umami tastes, respectively. Although called a bitter amino acid, valine has a slightly sweet taste as well. Combination of amino acids with their respective tastes is a key determinant for the taste of food. Relationships between amino acids and taste have been explored since the discovery of glutamate as an umami ingredient.

In fruits and beverages, tactile sensation of astringency is provoked primarily by flavanol polymers (proanthocyanidins or condensed tannins). Variations in proanthocyanidin composition, such as polymer size, extent of galloylation, and formation of derivatives, affect astringency (Lesschaeve and Noble, 2005).

The relative importance of each of the flavour quality factors and their interactions depends upon the commodity. New fruit genotypes with better flavour, which means high sugars and moderate to high acids and enough of the desirable odour-active volatiles for good aroma, are needed to satisfy today's consumers. Since flavour quality involves perception of the tastes and aromas of many compounds, it is much more challenging to manipulate it than other quality factors. This has been true for traditional plant breeding- and it will continue to be so with biotechnology approaches. This may be the reason that improvement of flavour quality of fruits has received much less attention than e.g. textural quality from biotechnologists so far (Kader, 2008).

High priority should be given to replacing poor flavour cultivars with good flavour cultivars from among those that already exist and/or by selecting new cultivars with superior flavour and good textural quality. Flavour is a complex, multigenic trait providing unique challenges to breeders. Selection for yield, fruit size, and shelf life characteristics has had unintended negative consequences on fruit flavour (Goff and Klee, 2006). Breeders need more information and analytical tools in order to select for flavour quality. Use of wild relatives may be necessary in breeding programs to recover flavour characteristics that have been lost.

After genotype, the maturity stage at harvest is the second most important factor influencing flavour quality of fruits and vegetables. Taste is generally better when products are harvested fully ripe since sugar/acid ratio and the synthesis of aroma volatiles increases with ripening. Therefore, an additional challenge for breeders is optimizing fruit flavour upon delivery to the consumer, selecting fruit varieties that maintain or enhance flavour during storage and marketing. Generally, the longer the time between harvest and fruit consumption,

the greater the losses of characteristic aroma volatiles and greater the off-flavours developmentIn (Kader et al., 1978; Pelayo-Zaldivar et al., 2005).

Plant volatile organic compounds (defined as volatiles) generated from both primary and secondary metabolites are generally low molecular weight compounds. More than 7000 flavour volatiles have been identified and catalogued from foods and beverages (Goff and Klee, 2006). Many volatiles are produced in plant tissues at specific developmental stages, for example, during flowering, ripening, or maturation. Although a single fruit or vegetable synthesizes several hundred volatiles, only a small subset generates the "flavour fingerprint" that helps animals and humans recognize appropriate and avoid dangerous food choices. Flavour perception is often described as a combination of taste and smell, nevertheless appearance, texture, temperature, mouth feel, and past experience also play a major role, indicating that multiple distinct sensory inputs are processed to generate the overall sensation.

Aroma perception depends not only on food chemical composition but also on food structure and on the oral physiology parameters (Foster et al., 2011; Poinot et al., 2009; Taylor, 2002) since flavour compounds are released from the matrix and then transported to the receptors in the mouth and nose (Buettner et al., 2008). The perception of food aroma is a complicated physiological and psychological process resulting from the concurrent chemical stimulation of orthonasal and retronasal receptors (Sheperd, 2006). Orthonasal olfaction is the perception of odours that occurs during sniffing as opposed to retronasal olfaction, commonly associated with the sense of taste, which is the perception of odours emanating from the oral cavity during eating and drinking (Sheperd, 2006). Volatiles delivered by these two pathways are not perceived by the brain in the same way. It is retro-nasal olfaction, and not orthonasal olfaction, that is essential to flavour (Klee and Tieman, 2013). The perception of the odour and flavour cannot be exhaustively explained by simple linear models since human olfactory receptors are simultaneously influenced by hundreds of compounds interacting with each other.

Tomato taste: non-volatile compounds

Sugars, organic acids, free amino acids, and salts are the main non-volatile components contributing to tomato taste (table 1). About 50% of the dry content in tomato is composed of sugars, primarily the reducing sugars: fructose and glucose, while organic acids comprise about 15% (Petro-Turza, 1987). Citric and malic acids are the major organic acids, in addition to several other carboxylic acids, sugar acids, and alicyclic acids. Malic acid has been reported to be 14% more sour than citric acid, but it has less influence on tomato taste because of its lower concentration at the red ripe stage where the malic to citric acid ratio is 0.5 or even lower. It is generally accepted that there must be an appropriate balance of sufficient sugars and acids in order to achieve good flavour (Stevens et al., 1977; Petro-Turza, 1987).

Free amino acids, glutamic acid, ϒ-amino butyric acid, glutamine and aspartic acid, form about 2-2.5% of the total dry matter of tomatoes (Petro-Turza, 1987). The two amino acids, glutamate and aspartic acid are essential to the taste of tomato. The ratio of the two amino acids is also important - a glutamate-to-aspartic acid ratio of 4:1 makes the tomato taste the best and brings out the genuine tomato taste (Baines and Seal, 2012). The higher glutamic acid concentrations in fruit picked at the breaker or earlier stages were parallel to higher scores for "off-flavour" as described by a sensory panel (Kader et al., 1978b), while "tomato fruitiness

intensity" was significantly correlated to reducing sugars / glutamic acid ratio and content. Free amino acid content of tomato fruit pericarp increases markedly during ripening transition of tomato fruit (Boggio et al., 2000), suggesting a high protein turnover. In particular, free glutamate content of ripe tomato fruit is much higher in all the cultivated varieties than in tomato wild species (Sorrequieta et al., 2010). The concentration of this amino acid is higher in tomato compared with many vegetables such as carrots, onions or pepper. In addition, glutamate provides the characteristic "umami taste".

Minerals, principally potassium and phosphate, constitute about 8% of the dry matter content of ripe tomatoes. Minerals mainly influence the taste perception effecting on pH and titratable acidity and having buffering capacity as well (Petro-Turza, 1987).

Table 1. Composition of Dry Matter Content of Tomato (adapted from Petro- Turza, 1987).

Constituent	**%**
Fructose	25
Glucose	22
Saccharose	1
Citric acid	9
Malic acid	4
Protein	8
Dicarboxylic amino acid	2
Pectic substances	7
Cellulose	6
Hemicellulose	4
Minerals	8
Lipids	2
Ascorbic acid	0.5
Pigments	0.4
Other amino acids, vitamins, and polyphenols	1
Volatiles	0.1

Tomato aroma: volatile compounds

From over 400 volatile compounds identified in tomato fruits, less than 20 compounds (table 2) are considered important for flavour based on their odour thresholds (Abegaz et al., 2004). These volatiles are derived from a diverse set of precursors that include branched-chain and aromatic amino acids, fatty acids, and carotenoids (Klee and Tieman, 2013). More is known about the chemical pathways contributing to flavour in tomato than in any other fruit or vegetable. The most abundant volatiles in tomato fruits are derived from lipids through the oxylipin pathway (Baldwin et al., 2000; Buttery and Ling, 1993; Reineccius, 1991). These volatiles, associated with flavours described as "tomato", "green" or "grassy", are derived from linoleic acid (hexanal) and linolenic acid (cis-3-hexenal, cis-3-hexenol, trans-2-hexenal) via lipoxygenase activity (Chen et al., 2004).

Other important compounds that positively contribute to the tomato aroma are derived from the essential amino acids leucine, isoleucine and phenylalanine (Buttery and Ling, 1993). These volatiles, such as phenyl acetaldehyde, 2-phenylethanol, methyl salicylate and 2- and 3-methylbutanal are also important flavour constituents of many other fruits, including strawberries and apples.

A third class of tomato volatiles, the apocarotenoids (e.g. β-ionone, geranylacetone and 6-methyl-5-hepten-2-one), is derived from oxidative cleavage of carotenoids (Goff and Klee, 2006). The specific association of these volatiles with ripe fruits and their relative absence from vegetative tissues suggests a role in signalling ripeness and attracting seed-dispersing organisms, including humans. Tomato aroma can thus be viewed as a set of cues that reflect the ripeness and nutritional quality/nutrient availability of the fruit (Goff and Klee, 2006).

Tomato volatiles can also be classified into two classes based on their biochemical dynamic of synthesis: one class comprises of compounds formed during fruit during ripening (e.g. isobutylthiazole, 3-methylnitrobutane, geranylacetone, β-ionone (Buttery and Ling, 1993)) and another class comprises of compounds formed when the fruit is macerated either by cutting or by eating (Galliard et al., 1977; Brauss et al., 1998). Among them, six carbon compounds, produced by the lipid oxidation pathway, play a major role giving tomato its fresh 'top-note' (Boukobza et al., 2001). In a recent work, Tieman et al. (2012), investigated the role of volatiles in tomato aroma perception and suggested that the impact of aldehydes is less important than it was previously considered. The metabolic suppression of the lipoxygenase activity in transgenic tomato fruit blocked the production of six carbon volatile compounds without significant altering the overall final consumer preference (Tieman et al., 2012). This indicates that there is not always a clear correlation between volatile content and consumer flavor perception (Klee and Tieman, 2013).

Table 2. Tomato volatile compounds and aroma descriptors generated in previous studies [a].

Volatile compound	Aroma/taste descriptor
3-Methylbutanal	Unpleasant, sweet, malty
1-Penten-3-one	Green, Bitter, Pungent
Hexanal	Green, grassy, ripe aroma, sweetness, tomato flavour
Cis-3-Hexenal	Fresh green, sweet, sour, bitter, overall aroma intensity, green, grassy.
Trans-2-Hexenal	Green, leafy, raw green, overall aroma intensity, fruity, green apple
Trans-2-Heptenal	Green, ripe aroma, sweetness
6-Methyl-5-Hepten-2-one	Sweetness, floral, nutty, raw green
2-Isobutylthiazole	Musty, sharp, green, tomato leaves
Geranylacetone	Sharp, overall taste intensity, ripe aroma, sweetness, tomato flavour
β-Ionone	Sour, overall aroma intensity, ripe aroma, floral, violet
Phenilethanol	Floral, fruity
Acetone	Glue, sweet
3-methylbutanol	Sweet, fresh
Acetaldehyde	Sweet, floral

[a] (Mayer et al., 2004; Maul et al., 2000; Baldwin et al., 1998; Krumbein and Auerswald, 1998; Tendon et al., 2000; Baldwin et al., 2008)

2.4. Nutritional quality and antioxidant

Providing higher nutritional content in fruits and vegetables at affordable prices is likely to increase their consumption, which would be good for producers, growers and the logistic chain as well as for consumers. At the same time more attention to flavour and taste quality has to be devoted. A new diet-health paradigm is evolving which places more emphasis on the positive aspects of diet. We are in the middle of a revolution that is changing the concept of food and our way of eating. In fact, in addition to their nutritional and sensory properties, foods have recently been recognized as acting as protective agents as well. Food have now assumed the status of functional foods, which should be capable of providing additional physiological benefit, such as preventing or delaying onset of chronic diseases, as well as meeting basic nutritional requirements (Kaur and Kapoor, 2001; Dillard and Bruce German, 2000; Galland, 2013; Hurtst and Hurst, 2013).

Numerous epidemiological studies have shown that there is a considerable association between fruit and vegetable consumption and lower risk of many diseases. Phytochemicals are associated with the prevention of certain chronic diseases, including cardiovascular diseases, cancer, diabetes, osteoporosis and vision diseases, which are especially severe in Western

countries. These diseases are mainly attributed to a high-fat and high-sugar diet, lack of exercise, smoking and other unhealthy lifestyles.

The pleiotropic mechanism of action of phytochemicals imply that the chemo preventive properties that are associated with fruits and vegetables consumption are complex and likely arise from synergistic combinations from several distinct molecules, not only within a given food but also from the overall composition of the diet (Lee et al., 2004; Liu, 2003; McCullough and Giovannucci, 2004; Boivin et al., 2009). Moreover, the presence of high amounts of bioactive compounds in fresh tissue does not assure their bioavailability once they are consumed since only a proportion of these food components can be absorbed and utilized.

Nutrients and phytochemicals accumulate in plants in an organ-specific manner. Besides this organ-specific distribution, the accumulation of nutrients and phytochemicals is governed by a variety of pre- and postharvest conditions. So it is important to consider that the antioxidant content of fresh tissues can be affected by maturity, agricultural practices, temperature and storage conditions. The types of stresses to which fruits and vegetables are exposed, such as high temperature storage, ultraviolet-C irradiation, hormone treatment, among others, also affect their antioxidant capacity (Dangl et al., 2000; Wang et al., 2007; Cho et al., 2007).

Only recently, breeding for phytochemicals in horticultural crops has been identified as an important factor in the development of new cultivated lines of quality and disease-resistant fruits and vegetables especially because the plant's genotype is considered of primal importance in the determination of its phytochemical profile, often surpassing the impact of cultural practices such as irrigation or fertilization. Horticultural crops such as strawberry, apple, tomato, potato, cabbage, broccoli, lettuce, onion, cranberry and raspberry are currently the subject of breeding programs in which the phytochemical content was considered a key component (Crosby et al., 2007; Patil et al., 2012).

Another factor that influences the phytochemicals content and the antioxidant capacity of fruit and vegetables is the postharvest storage condition; at this point the fruit senescence phase starts (Hodges and deLong, 2007). Senescence, a type of programmed cell death of plant organs, is defined as a genetically regulated and orderly loss of structure and function leading up to the death of cells, organs, or whole organisms (Noodén and Guiamét, 1989; Palma et al., 2006). The production of reactive oxygen species (ROS) has been associated with senescence of both fruits and vegetables, with lipid peroxidation often among the earliest detectable senescence symptoms (Cabello et al., 2006; Hodges and Forney, 2000). As increases in plant ROS levels are also a response to stress, stresses can induce senescence. Pigment bleaching, loss of membrane integrity, cessation of photosynthesis, changes in respiration, degradation of proteins and other macromolecules, and increases in ROS levels are all symptoms of plant senescence (Hodges et al., 2004).

The majority of published work to date indicates that antioxidant potential often declines during postharvest senescence, while remaining relatively unchanged during postharvest fruit ripening (as opposed to preharvest fruit ripening) despite ripening-related increases in levels of ROS-scavenging compounds such as carotenoids and anthocyanins (Hodges and deLong, 2007). Presumably, a decreased antioxidant capacity in conjunction with an increased potential to produce ROS influences the senescence-related quality decline in postharvest fruits and vegetables.

Tomato nutritional quality

The consumption of tomatoes and tomato products has been inversely related to the development of some types of cancer (Clinton, 1998; Giovannucci, 1999) and to plasma lipid peroxidation (Parfit et al., 1994; Balestrieri et al., 2004).

The beneficial effects of tomato consumption are generally attributed to carotenoids. Two main carotenoids are present in tomato are lycopene, which is the major carotenoid compound (80-90 %), giving the red colour to the fruit, and β-carotene, which is 7-10% of the total carotenoid content. Because of the presence of long-chain conjugated double bonds, lycopene has been reported to possess higher antioxidative activity than luteolin or β-carotene. Vitamin C and various phenolic compounds are also thought to be health-promoting factors with antioxidant properties (Frusciante et al., 2007). Tomato antioxidant content depends on the cultivar (genetic factors), maturity, and both agronomic and environmental conditions during cultivation (Martínez-Valverde et al., 2002; Dumas et al., 2003).

During ripening, tomatoes change in colour from green, typical of chlorophylls, through pink-orange to bright red, owing to the development of carotenoids. At the turning stage, lycopene content considerably increases and can reach 80–100 mg kg^{-1} fresh matter at the red stage. So far, little is known about the effect of agricultural practices and soil/climate factors on the oxidant content of tomatoes. It is clear, however, that factors such as watering, fertilisation, temperature and light have a bearing on carotenoid level in tomatoes, as have variety, degree of maturity, harvest date, fruit growth and post-harvest storage. Lycopene in fresh tomato fruits occurs essentially in the all-trans configuration. It needs to be protected from excessive heat and extreme pH conditions, exposure to light, oxygen, and lipid-degrading enzymes in order to prevent its oxidation and isomerisation, which are the main causes of tomato lycopene degradation during processing (Shi and Le Maguer, 2000). Thermal processing generally causes some loss of lycopene in tomato-based foods. The cis-isomers increase with temperature and processing time. In general, dehydrated and powdered tomatoes have poor lycopene stability unless carefully processed and promptly placed in a hermetically sealed and inert atmosphere for storage. Frozen foods and heat-sterilised foods exhibit excellent lycopene stability throughout their normal temperature storage shelf life (Shi and Le Maguer, 2000).

Vitamin C, including both ascorbic and dehydroascorbic acid, is important in the protection of the tomato itself against autoxidative damage that might increase with ripening due to enhanced respiration (Slimestad and Verheul, 2005). Ascorbic acid is relatively stable in tomatoes because of the acidic conditions found in the tissue. However, it is easily destroyed by oxidation, exposure to light, or high temperatures. Significant losses of ascorbic acid can occur during the post-harvest storage period. Reducing the temperature from room temperature (20°C) to chill (4°C) or further reduction to freezer temperatures (–18° C) decreases the rate of loss (Davey et al., 2000)

Tomato also contains high amounts of phenolic compounds, which also exhibit a strong antioxidant activity. Kahkonen et al. (1999) reported the total phenolic content of tomatoes is up to 200 mg of gallic acid equivalent per 100 g (as dried weight). Tomato polyphenols, mainly phenolic acids, are present in free soluble form and in insoluble form when they are bound to the fibre. Moreover, tomato contains flavonoids, in particular rutin and naringenin. Some papers

pointed out that tomato flavonoids, due to their high antioxidant power and to the significant biological activities, can have a substantial role in the health benefits attributed to the tomato consumption (Frusciante et al., 2007).

3. Quality in the production chain

Many pre-harvest, harvest, and post-harvest factors influence the quality of tomato fruit. These include genetic and environmental factors such as climatic condition (temperature, light, CO_2, and relative humidity) and cultural practices (type of substrate, nutrient and water supply, harvesting methods). Maturity stage at harvest and postharvest handling also directly affect tomato quality.

Genotypic variation in fruit texture at harvest and softening pattern is important factor in determining storability and acceptance of tomatoes. Cultivars that maintain firmness will permit picking the fruits at more advanced ripeness stages with consequently better flavour since ripeness stage at harvest affects fruit composition and quality. Field-ripened tomatoes have better overall quality than room-ripened ones (Bisogni et al., 1976) mainly because fruit accumulate organoleptic compounds, such as acids and sugars, during ripening on the vine (Sakiyama and Stevens, 1976).

The effect of genetic variation (e.g cultivars) and growing conditions on tomato flavour is not completely understood. The main reasons are the complexity of evaluating their effects on quality and the difficulty in developing a consistent methodology for sensory evaluations (Brauss et al., 1998). Lack of flavour in tomato fruit is a serious consumer concern (Hobson, 1988). Tomato flavour has declined as genotype selection has emphasized yield, fruit size, firmness, lack of defects, disease resistance, and processing performance and not the sensory aspects of fruit quality (Stevens and Rick, 1986; Shewfelt, 2000). For instance all tomato cultivars with non-ripening genes (e.g. the *rin* and *nor* mutants) generally produce fruits with extended shelf-life but limiting flavour quality. Support of this breeding strategy also comes from consumers that appear to be conflicted in their desires; while taste is given high importance, fruit of poor appearance will not be chosen even if the taste can be “guaranteed” (Bruhn, 2002). In addition sensory parameters that could assist the breeders in an efficient selection for flavour have not been extensively characterized yet. The definition and use of markers that correlate with tomato flavour could improve this situation and provide breeders with analytical tools for flavour enhancement of fresh and processed tomatoes (Bucheli et al., 1999).

The two best-known and studied environmental factors influencing the nutritional value and quality of tomato are light and temperature. Less conclusive studies are reported on the direct effect of high CO2 and air humidity on organoleptic compounds. For field tomato crops, changes in quality levels are often described in terms of variation in geographic location or season, which include interactions among several factors, making interpretation difficult. Phytonutrients of tomato such as vitamin C, carotenoids and phenols are strong positively affected by the intensity, duration, and quality of light (Dorais et al., 2008) that directly promotes the photosynthetic production of carbohydrates. Temperature has a direct influence on plant metabolism and, thus, affects tomato fruit development and its quality value (Heuvelink and Dorais, 2005). As carbon availability is required for the biosynthesis of certain

phytonutrient compounds, such ascorbic acid and carotenoids, an increase in sugars through reduced respiration under lower temperatures may result in a higher level of phytochemicals. Nevertheless temperature regulation of fruit and plant metabolism is cultivar specific. For instance lycopene synthesis in tomato fruit is highest when the temperature ranges between 16 and 24° while lower and higher temperatures reduce the carotenoid synthesis. The optimum temperature depends on the genotype and interactions with environmental and cultural factors. (Dorais et al., 2001; Krumbein et al., 2006)

However, producing high quality fruit is not sufficient to guarantee the consumer a final high quality product. After genotype and environmental growth conditions, storage is the third important factor that affects fruit quality. Postharvest losses in quality are related to immaturity at harvest, incidence and severity of physical damage, improper climate storage conditions (e.g temperature, and relative humidity) and delays between harvest and consumption. Mostly, the longer the time between harvest and fruit consumption, the greater the loss of quality. Senescent tomatoes usually have more off-flavor and less "tomato-like" flavour than fresh ones (Kader et al. 1978). Nevertheless avoiding incorrect storage practices along all the distribution chain may be not enough to prevent product quality reduction as consumers usually have limited knowledge on how to prolong the shelf life of fruit (Johnson et al., 2008). More effort has to be spent to increase the available information for consumer to reduce waste as well.

Assessing consumer responses to product quality will demonstrate to supply chain stakeholders how improving product quality management can improve the performance of the whole chain. This needs foremost quantification of quality attributes of fruit and vegetables in distinct segments of the production chain. It implies synergy of approaches from different branches of knowledge, such as genetic, physiology, analytical chemistry and sensorial analysis to improve and optimize quality upon delivery to the consumers. Analytical techniques to evaluate the quality of food are needed to ensure the appropriate physicochemical properties of a product (Greenfield and Southgate, 2003). The quality and desirability of a food product is determined mainly by subjective interactions with sensory organs of consumers and techniques that simulates as closed as possible human perception are needed. Improving analytical tools to assess fruit quality will be important not only for quality related studies and breeding programs. Accurate, rapid and user friendly techniques for phenotyping genetic traits related with quality are crucial to develop markers or to help classical breeding screening and some of these analytical tools will be developed in this thesis. These tools will be used with attention to the high biological variation present in tomatoes by following the quality traits of a sample non-destructively so that the same sample can be measured repeatedly.

4. Scope of the research and thesis outline

The aim of this study is to enhance the knowledge and understanding of some major pre- and post-harvest factors affecting tomato quality as perceived by the consumer. We focused on several part of the production chain that, up to our knowledge, were still poorly or far from completely studied that affect the consumer assigned quality. In figure 2 a schematic representation of the framework of this research is described.

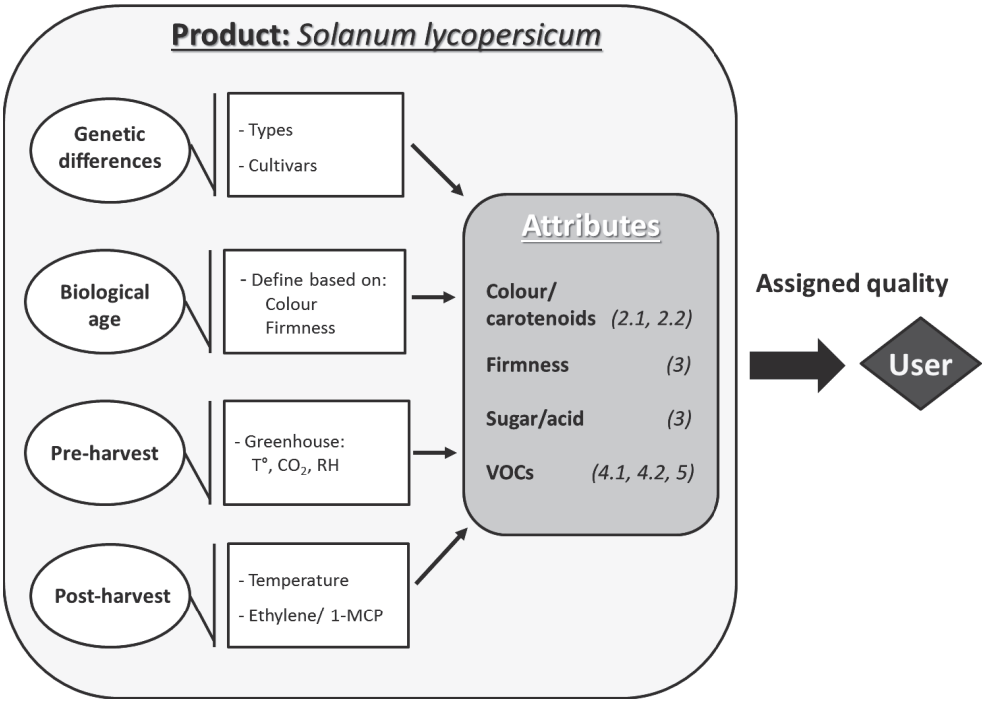

Figure 2. research framework. Factors and quality attibutes considered in this research; in brakets the relative thesis chapters.

Chapter 2 focuses on one of the most studied parameter of tomato quality: the colour. Colour was assessed by regularly used techniques, such as tristimulus colour measurements and RGB image analysis, and also with remittance VIS spectroscopy that permitted a non-destructive quantification of the main tomato colour pigments. In **chapter 2.1** we focused on the discoloration of ripe tomato fruit due to chilling temperature storage, since the conservation of subtropical fruit at home refrigerator condition is still a common habit. Discoloration of fruit, beyond diminishing the appearance quality, decreases also the nutritional quality due to the lycopene depletion. In **chapter 2.2** we described the tomato colour transformation process expressed as changes in lycopene and chlorophyll levels using a kinetic modelling approach as influenced by temperature.

Chapter 3 aims to investigate how different semi-closed greenhouse climate management may affect the postharvest quality of tomatoes during the whole production season. Quality attributes under investigation were fruit size, firmness, and taste (expressed as sugar and acid levels) of fruits harvested fully ripe (in terms of colour).

Chapter 4 describes the development of a rapid and accurate method, based on proton-transfer reaction-mass spectrometry (PTR-MS), suitable to dynamically monitor the emission of volatiles defining the tomato aromatic profile. In **chapter 4.1** we adapted the analytical method based on PTR-MS, already used in other researches related to food quality, to tomato

VOC profiling. The method was developed by evaluating several tomato varieties and cultivars at different ripening stages with both PTR-MS and GC-MS. In **chapter 4.2** we developed an analytical system to study the tomato aroma profile that mimic, as closed as possible, the release of volatiles during chewing in the human mouth and the consequent retronasal olfaction perception.

Chapter 5 demonstrates how a storage management of tomato fruit at low temperatures can affect the overall aroma of the fruit. VOCs profiling was assessed including flavour and off-flavour compounds evaluated with GC-MS and PTR-MS techniques.

Chapter 6 is the general discussion of the results described in chapter 2-5. Physiological perspective and practical implications of this study are debated.

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Chapter 2

Tomato colour and postharvest management

Chapter 2.1

Low temperature-induced lycopene degradation in red ripe tomato evaluated by remittance spectroscopy

Abstract

Nowadays tomatoes are mostly harvested ain the orange and red-ripe stage. A survey among consumers indicated that tomatoes are most often stored in the refrigerator well below 10 °C, a temperature considered harmful for chilling sensitive products such as tomato. Also during distribution, tomatoes may be exposed to chilling temperatures. The effects of storage at chilling temperatures on quality aspects of tomatoes was investigated. The colour and lycopene content of red ripe tomatoes of two cultivars (cocktail and round type) was evaluated during twenty days of storage at 4, 8, 12 and 16 °C. Colour was repeatedly measured over time by tristimulus colour measurements, RGB image analysis and colour intensity was scored by eye using a consumer panel. Lycopene content was repeatedly assessed by following the NAI index over time. This index, obtained from remittance VIS spectroscopy, was found to relate closely to the lycopene level as measured by HPLC measurements of pericarp tissue. Temperatures below 12°C resulted in lycopene loss in ripe-red tomatoes and substantial color loss well assessed by visual evaluation. Colour measurement using tristimulus colour measurements and RGB image analysis did not correlate well with lycopene content. Prior hot water treatment did not prevent lycopene loss. Results show that storage of red ripe tomatoes at chilling temperatures reduces the nutritional and presumed health promoting value and affects the fruit's visual quality.

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1. Introduction

Storage of tomatoes and other tropical or subtropical products between 0 and 12 °C induces chilling injury (King and Ludford, 1983). The major chilling injury symptoms in tomatoes appear in mature-green and breaker tomato. These symptoms include a failure to ripen normally, surface pitting and increased fungal decay (Jackman et al., 1992; Lurie et al., 1997; Saltveit, 2002). Chilling injury symptoms in non-ripe tomatoes can be alleviated by short term exposure to high temperature (Whitaker, 1994; Lurie et al., 1997; Saltveit, 2001; Saltveit, 2005;; Lu et al., 2010; Luengwilai et al., 2012). Nowadays tomatoes are mostly harvested at the orange and red stage and often stored in the consumer's refrigerator before consumption. Due to the sensitivity to chilling temperatures tomato storage in the refrigerator is not recommended (Parnell et al., 2004), but in practice it is common. In a survey among over 1800 respondents, more than 60% stated that they store tomatoes in the refrigerator (table 1 supplementary material).

Next to ascorbic acid and β-carotene, lycopene is one of the main antioxidants in ripe tomatoes (Clinton, 1998; Sies and Stahl; 1998; Ilahy et al., 2011). Lycopene is an acyclic carotenoid with eleven conjugated double bonds. The long chromophore in the polyene chain accounts for the red colour and for its antioxidant activity (Rice-Evans et al., 1997). Epidemiology studies have revealed an inverse correlation between consumption of lycopene rich diets and the incidence of several cancers and coronary heart diseases (Barnley, 2000; Hu et al., 2008; Karppi et al., 2009). Low temperature induced reactive oxygen species (ROS) production (Suzuki and Mittler, 2006) might affect the lycopene content of red ripe tomatoes when stored in the refrigerator. Lycopene loss was found in fresh cut watermelon stored at low temperatures preceding the appearance of pitting and lesion development (Perkins-Veazie and Collins, 2004; Perkins-Veazie, 2007).

High performance liquid chromatography (HPLC) is currently used for lycopene content assessment. Although HPLC offers high sensitivity, it is also an elaborate, costly and destructive technique thus prohibiting the repeated assessment of lycopene of the same tomatoes. Lycopene accounts for the majority of the tomato's red colour (Gray et al., 1994). As most lycopene in the tomato is found in the pericarp, efforts have been undertaken to link colour measurements with lycopene content. Arias et al. (2000) reports a number of well-established correlations between lycopene content and chromaticity values (a*, a*/b* and $(a^*/b^*)^2$), although only one cultivar was tested. The relation between lycopene content and a*/b* was found to be noisy and cultivar dependent (D'Souza et al., 1992) or, for fourteen cultivars, relatively weak (López et al., 2001). Based on the results of Pflanz and Zude (2008) we used remittance VIS spectroscopy as a technique to non-destructively assess lycopene content in the tomato pericarp tissue. This technique has been successfully applied to assess the chlorophyll content in apple (Zude et al., 2002; Kuckenberg et al., 2008) and banana (Zude, 2003) and for determination of carotenoids in carrot (Zude et al., 2007).

Aim of this research is to investigate the influence of low temperature storage on the lycopene content of red ripe tomato of two types (round truss and cocktail truss) and to investigate whether remittance VIS spectroscopy can provide accurate estimates of the lycopene content. The normalized anthocyanin index (NAI) derived from the remittance VIS spectroscopic method is tested as an indicator for the lycopene content as measured by HPLC

and compared with chromaticity, RGB image analysis and visual judgement.

2. Materials and methods

2.1. Plant material

Tomatoes (Solanum lycopersicum L.) of the cvs. Cappricia RZ (round truss) and Amoroso RZ (cocktail truss) from breeding company RijkZwaan BV, The Netherlands, were obtained from a greenhouse in the south east of The Netherlands in June 2010. Both cultivars were grown under similar, commercial, growing conditions.

2.2. Lycopene measurements

Lycopene content of pericarp samples was determined according the method described by and Lana et al. (2005). The lipophilic fraction of 20 mg of freeze-dried tomato pericarp was extracted by a 5 ml solution of tetrahydrofuran and methanol (1:3). The lycopene and β-carotene concentration was measured by HPLC with an Alltima C18, 3 µm, 100 mm x 4.6 mm column (Alltech, Illinois, USA) at 25 °C. The mobile phase used 75% methanol + 0.05 M ammonium acetate + 0.05% TEA and 25% ethyl acetate + 0.05% TEA. The flow rate was 1 ml min-1 and detection of lycopene was at 470 nm.

2.3. Remittance VIS spectroscopy and colour measurements

Remittance and chromaticity measurements were performed on three positions of the equatorial region of each tomato. A hand-held photodiode array spectrophotometer (Pigment Analyzer PA1101, CP, Germany) was applied for recording remittance spectra of tomato fruit between 350 to 1100 nm. Remittance was assessed at 570 (R570) and 780 (R780) nm by calculating the NAI index which is a normalized value between -1 and 1.

$$NAI = \frac{R780 - R570}{R780 + R570} \qquad \text{(Eq. 1)}$$

L*, a*, b* system chromaticity values were measured using a tristimulus chromameter (CR-400, Minolta, Japan). Tomato colour was expressed as either a* or a*/b* (Arias et al., 2000, Gómez et al., 2001). Tomato colour, expressed as RGB values from image analysis measurements, was measured on individual tomatoes using a colour video camera (JVC KY-F30 3CCD) in a controlled light environment and expressed as 1000/R (Schouten et al., 2007).

2.4. NAI-lycopene correlation

To determine the relationship between the lycopene content and the NAI index, 45 tomatoes per cultivar were collected varying in colour from dark green to dark red. Prior to the destructive lycopene assessment the remittance was measured three times on the equator of each fruit.

2.5. Storage experiment

A batch consisting of 110 red ripe (NAI > 0.4) tomatoes per storage temperature (4, 8, 12, 16 °C) and per cultivar were stored for twenty days. Remittance and colour were measured repeatedly on thirty tomatoes. On day 0, 5, 10, 15 and 20 fifteen randomly taken fruits per batch were used for lycopene assessment by HPLC.

2.6. Visual colour evaluation

An attribute rating test on the evaluation of the red colour intensity was conducted on red ripe tomatoes at harvest and after ten and twenty days of storage at 4, 8, 12, 16 °C. A thirty person consumer panel evaluated the tomato colour using a 10 cm unstructured scale, anchored at the two extremes with colour descriptors (0 cm – light red, 10 cm - dark red). Samples were randomly presented in an open area laboratory setting illuminated with cool white florescent lights. Each person evaluated four fruits per cultivar, one for each storage treatment. The significant differences in sensory attribute between the storage treatments were determined by the non- parametric statistical Kruskal-Wallis test with a significant level of 0.05 using SPSS 19.

2.7. Hot water treatment

Twenty red ripe tomatoes per cultivar were held in hot water at either 40, 45 or 50 °C for either 2, 5 or 10 minutes (Lurie et al., 1997; Saltveit, 2005) prior to twenty days of storage at 4 °C. Remittance was assessed on day 0, 5, 10, 15 and 20 during the storage and compared with non-heat-treated tomatoes.

3. Results and Discussion

3.1. Non-destructive lycopene assessment

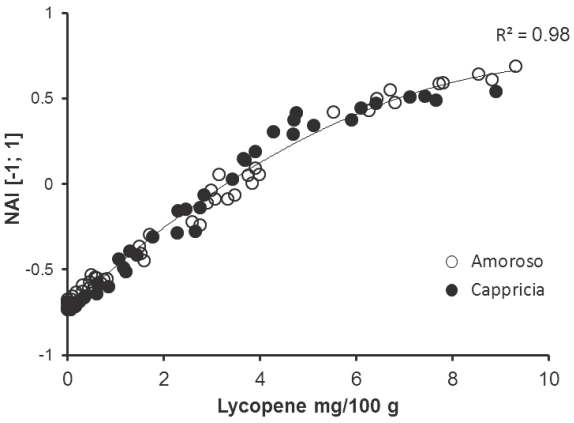

Figure 1. Correlation between NAI values and lycopene content measured by HPLC in the pericarp of the tomato cvs. Cappricia and Amoroso.

HPLC measurements of lycopene content of individual tomato fruit, showed a clear relation with the averaged NAI values per tomato, for both cultivars (Figure 1). Apparently, the NAI index, that corresponds to the absorption at 570 nm, is an excellent non-destructive indicator of the pericarp lycopene content as it was previously found by Plfanz and Zude (2008).

3.2. Effect of low temperature storage on lycopene

A decreasing lycopene content as measured by HPLC was observed over time at low storage temperatures (Figure 2). This trend was also reflected in the NAI values (Figure 3). In cv. Cappricia lycopene loss was already detected for tomatoes stored at 12 °C whereas for tomatoes of cv. Amoroso lycopene loss was not visible at this temperature. This indicates that cv. Cappricia is more chilling sensitive than Amoroso. Tomatoes of cv. Amoroso stored at 16 °C showed an increase in lycopene content over time, presumably due to continued ripening. Temperature did not clearly affect the β-carotene content during storage (Figure 3). The decrease of the lycopene content induced by low temperature storage may be caused by lycopene fragmentation. Lycopene quenches highly reactive singlet oxygen ($O2^{\cdot -}$) and traps peroxyl radicals ($ROO^{\cdot}$) that results in break down products like acetone, methyl-heptanone, leavulinic aldehyde and glyoxal (Conn et al., 1991; Palozza, 1998). This presumed degradation of lycopene during storage not only reduces the nutritional and presumed health promotional value of the product but also affect the visual quality since the colour is one of the main consumer quality attribute.

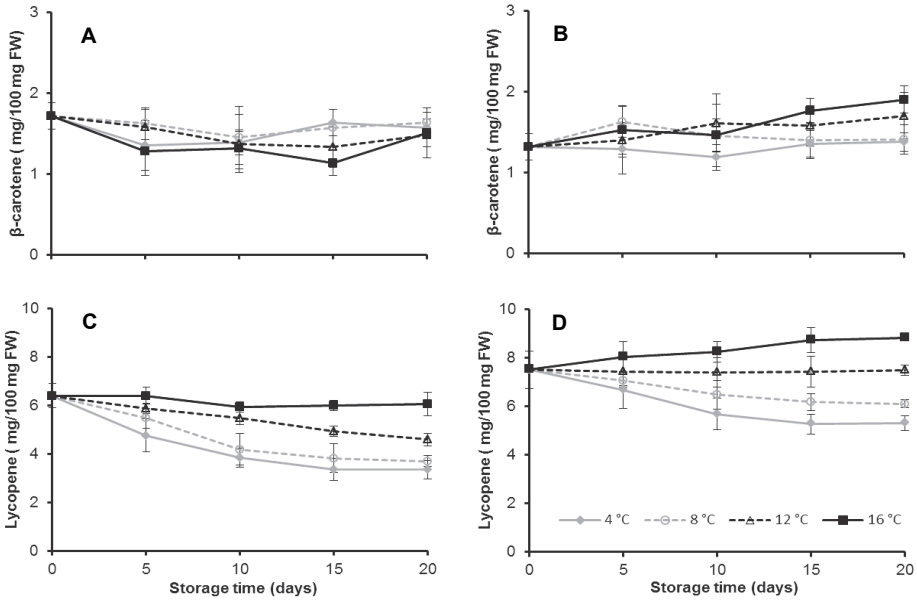

Figure 2. Effect of storage temperature on carotenoid (lycopene and β-carotene) content of red ripe tomato cvs. Cappricia (panel A and panel C) and Amoroso (panel B and panel D) measured by HPLC. Values are mean with standard deviation of 15 tomatoes.

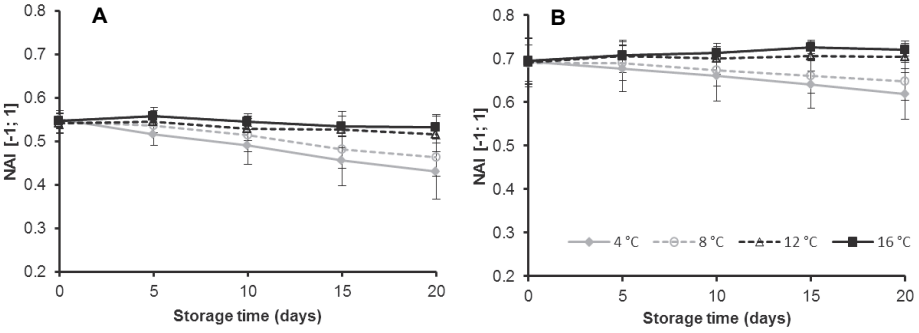

Figure 3. Effect of storage temperature on NAI values for red- ripe tomatoes of cvs. Cappricia (panel A) and Amoroso (panel B). Values are mean with standard deviation of 30 tomatoes.

Heat-shock treatments at 45 °C prior to storage at 4 °C did not stop lycopene degradation (Figure 4). The other hot water temperature treatments (at 40 and 50 °C) had very similar results (data not shown). Although symptoms of chilling injury can be alleviated in tomatoes in earlier ripening stages (Luengwilai et al., 2012), heat shock, apparently, does not prevent lycopene degradation in red ripe tomato. Heat shock treatments induce the expression of heat shock proteins that may act as antioxidants under oxidative stress conditions (Levine et al., 1996). The expression of various tomato heat shock proteins is mainly induced in tomato fruits at the breaker stage when chloroplasts are transformed in chromoplasts (Neta-Sharir et al., 2005). Heat treatments in red ripe tomatoes might therefore not be accompanied by the induction of sufficient heat shock proteins or the lycopene decay at chilling temperature is not caused by oxidative stress.

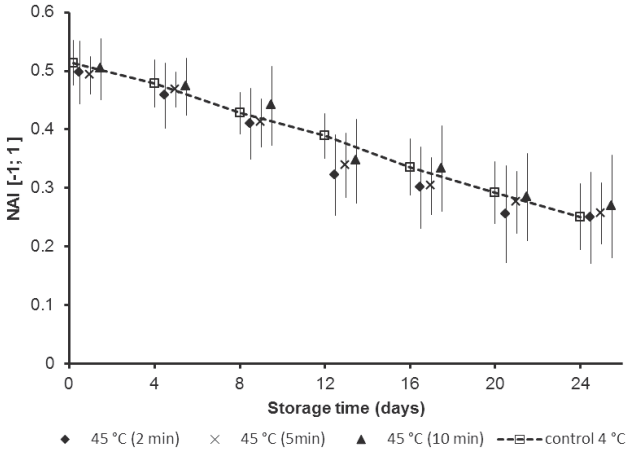

Figure 4. Effect of pre storage hot water treatment at 45 °C on NAI values over time during cold storage (4 °C) for red ripe tomatoes of cv. Cappricia. To improve the graphical representation data points have been slightly shifted over the time axis; actual measurements were carried out simultaneously every four days. Each data point is the mean with standard deviation of 20 tomatoes.

3.3. Effect of low temperature storage on colour.

The analytical measurements of lycopene during storage with HPLC and with the Pigment Analyzer were linearly correlated with a high correlation coefficient for both cv. Cappricia (R^2= 0.88) and cv. Amoroso (R^2= 0.83) (Figure 5).

Colorimetric analysis with Minolta Lab was not always useful for detecting these color differences since the chromaticity indexes (L^*, a^*, b^*, C^*, h^*) were not suitable to monitor the color change during the cold storage (data not shown). Even the combination of these indexes (Arias et al. 2000), such as a^*/b^* ratio, did not give useful results for both cultivars (Figure 5). The correlation between lycopene content and a^*/b^* for the cv. Cappricia was similar to the one obtained with the Pigment Analyzer (R^2= 0.86), while for the cv. Amoroso there was no correlation (R^2= 0.25).

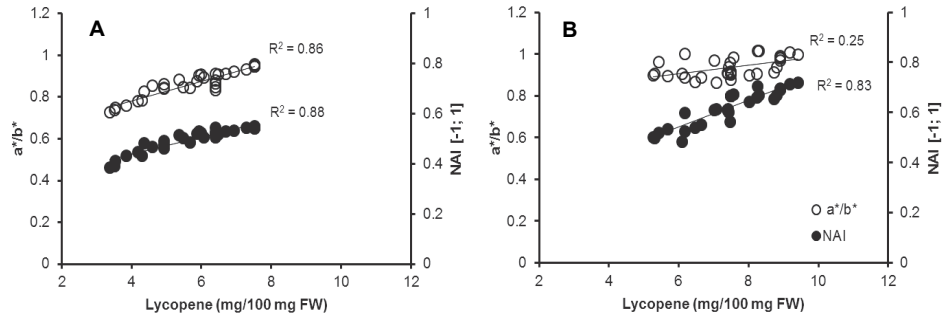

Figure 5. Correlation between lycopene content, measured by HPLC, and color index NAI and a*/b* (measured by Pigment Analyzer and Minolta chromameter respectively). Color readings and lycopene content are taken from red ripe tomatoes of cv. Cappricia (panel A) and cv. Amoroso (panel B) stored for 20 days at four temperatures (4, 8, 12, 16°C).

Figure 6 shows chromaticity, RGB and NAI values graphically expressed in ranges that can be expected for orange to red tomatoes (Arias et al., 2000; Schouten et al., 2007). Chromaticity a* values measured over time for cv. Cappricia were consistently higher than those of cv. Amoroso which is not in accordance with the lower lycopene levels in cv. Cappricia compared to cv. Amoroso. The a*/b* values over time for cv. Amoroso were unaffected by the cold treatment, again not in accordance with the decrease in lycopene content. Colour expressed as 1000/R was rather constant over time for both cultivars and not a useful indicator of lycopene content. The nature of remittance spectroscopy likely allows for probing the pericarp for a significant part in the radial direction. In the case of reflectance based measurements such as chromaticity and RGB data are much more likely to be influenced by cuticle thickness or surface structure that varies per cultivar (Allende et al., 2004; Hetzroni et al., 2011). In addition, possible differences between lycopene levels in peel and pericarp (Sharma and Le Maguer, 1996; Markovic´ et al., 2010) may cause different results when using different methods to assess colour. This might explain why the link between chromaticity values and lycopene level is sometimes strong (Arias et al., 2000) and sometimes weak (López et al., 2001).

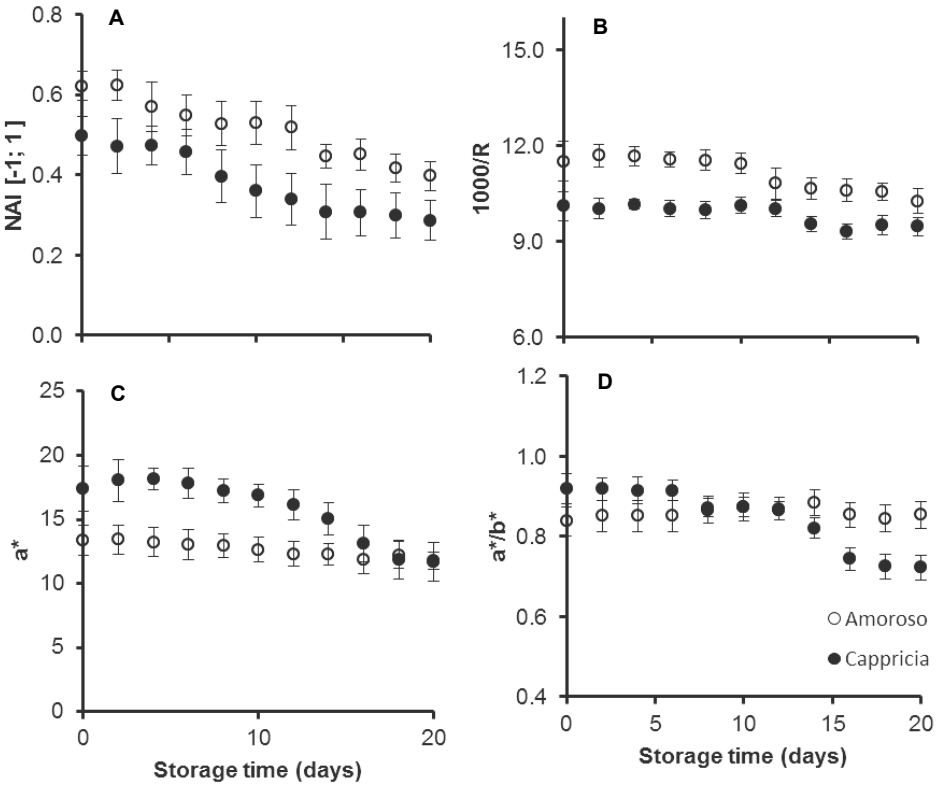

Figure 6. Evaluation of low temperature (4 °C) storage of red ripe tomatoes of cvs. Cappricia and Amoroso on NAI values (panel A), colour measurements expressed as 1000/R (panel B), a* (panel C) or a*/b* (panel D). Values are mean with standard deviation of 10 fruits.

For tomatoes of both cultivars stored at low temperatures a loss of red colour over time was visible by eye as evaluated by the consumer panel (Figure 7). A good correlation was found between the scores by visual judgement of color and the lycopene content. It seems from these experiments that the human eye is better able to assess colour/lycopene in tomato than tristumulus and RGB measurements. Only the NAI values showed a pattern very similar to the change in lycopene content.

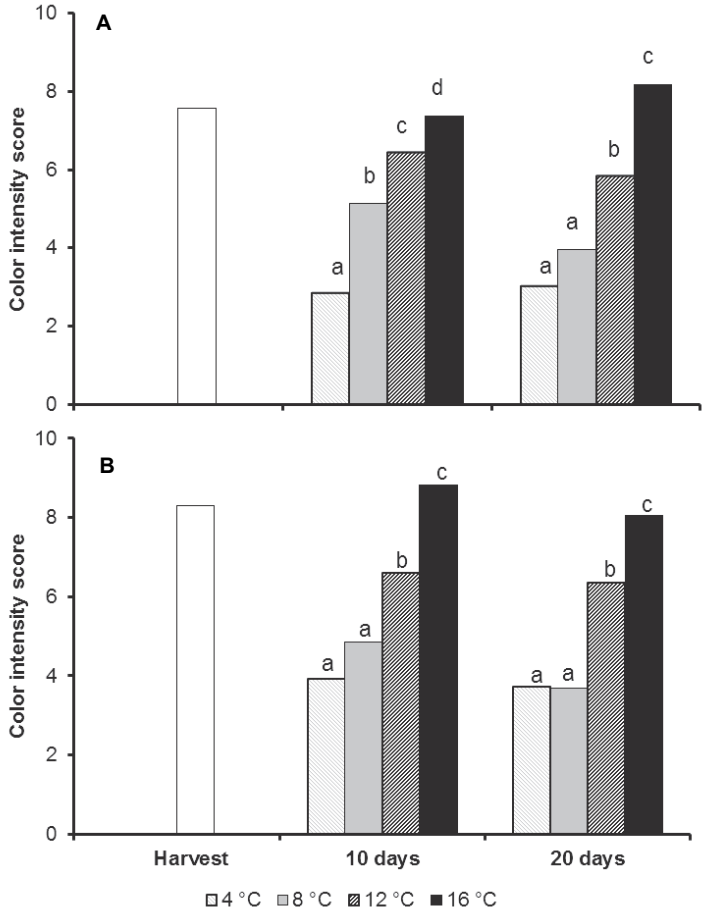

Figure 7. Consumer panel evaluation scores of the colour intensity of red ripe tomatoes of cvs. Cappricia (panel A) and Amoroso (panel B) at harvest and following 10 and 20 days storage at different temperatures. Values are the mean of 30 evaluations with indicated statistical differences (significance level 0.05).

4. Conclusions

Storage of tomatoes at temperatures below 12 °C, a common practice for consumers, induces lycopene degradation and, consequently, a reduction of the presumed health promoting value and external visual quality. Lycopene content of pericarp tissue can be assessed accurately and non-destructively by visible remittance spectroscopy. Comparison with tristimulus and RGB image analysis measurements turned out favourable for remittance spectroscopy likely due the deeper penetration depth into the pericarp tissue**.**

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6. Supplementary material

A questionnaire was setup to determine how people store fruit and vegetables at home. The one question questionnaire ('Where do you store the following fruits/vegetables?') was distributed via e-mail and social networks. Total number of respondents was 1883.

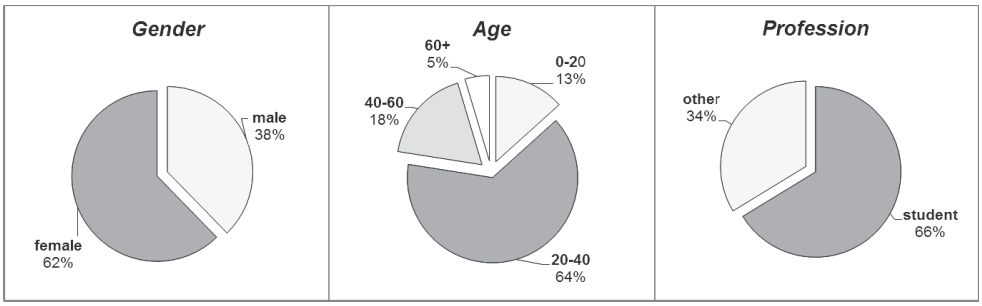

Figure S1: General characteristics of questionnaire respondents.

Table S1: How consumers store fruit and vegetables at home.

	Refrigerator (%)	Room temperature (%)	Other (%)
Tomato	62.2	34.5	3.3
Bell pepper	67.9	25.6	6.5
Cucumber	77.8	18.4	3.8
Apple	22.0	72.7	5.3
Kiwifruit	20.6	75.7	3.7

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Chapter 2.2

Quantifying lycopene synthesis and chlorophyll breakdown in tomato fruit using remittance VIS spectroscopy

Abstract

The aim of this study is to increase the understanding of chlorophyll breakdown and lycopene synthesis on a quantitative level in Solanum lycopersicum fruit. To accomplish this, a kinetic model is proposed describing the transition from chloro- to chromoplast. Remittance VIS spectroscopy was used to assess chlorophyll and lycopene levels non-destructively in cocktail and round type tomatoes. Tomatoes were stored at constant temperatures between 4 and 24 °C or at a stepwise changing temperature between 4 and 16 °C. Chlorophyll and lycopene levels were measured repeatedly over time and used to calibrate the kinetic model that describes how an autocatalytic enzyme system links chlorophyll breakdown to lycopene synthesis, including breakdown of lycopene precursor and lycopene itself. Increasing storage temperatures increases the reaction constant for lycopene synthesis more than that of chlorophyll breakdown for both tomato types. The reaction constants describing chlorophyll breakdown and lycopene synthesis are considerably larger, and the estimated enzyme levels are lower for the round type. This allows round tomatoes to quickly resume lycopene synthesis after a cold storage period when enzyme levels are low. Lycopene breakdown was established for the round type while the cocktail type showed lycopene precursor breakdown. Chlorophyll breakdown and lycopene synthesis, as affected by storage temperature and tomato type, is covered well by the model for both tomato types. We hypothesize that the postulated enzyme system, responsible for the direct link between chlorophyll breakdown and lycopene synthesis, is due to STAY-GREEN proteins. Remittance VIS spectroscopy is, in combination with a parameter estimation tool, suited to screen tomato genotypes for intended colour transformation performance or as tool in chloroplast to chromoplast transition studies.

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1. Introduction

Colour is one of the most important food quality attributes affecting consumer acceptance, taste and flavour perception (Francis 1995; Grossman and Wisenblit 1999; Bayarri et al., 2001; Crisosto et al., 2003; Leon et al., 2006; Costa et al., 2011; Nisha et al., 2011). Tomato (*Solanum lycopersicum*) fruit undergo significant changes during ripening including a typical climacteric rise in respiration and ethylene, volatile production, texture changes and most evidently a colour transformation (Campbell et al., 1990; Alexander and Grierson 2002). This colour transformation has been quantified based on colour measurements expressed in the $L^*a^*b^*$-colour space (Tijskens and Evelo, 1994; Hertog et al., 2004, Pinheiro et al., 2013) or in the RGB colour space (Lana et al., 2006, Schouten et al., 2007). Most approaches are very similar and describe tomato colour development as one process where a green pigment complex is converted into a red pigment complex resulting in a description of the colour transformation as a logistic curve. These kinetic models incorporate the effect of temperature (between 2 and 25 °C) and also describe how the often large differences in developmental age at harvest between individual fruit affect the colour formation after harvest. Recently, Farneti et al. (2012) showed that the normalized anthocyanin index (NAI) obtained from remittance VIS spectroscopy is closely related to the lycopene level in pericarp tissue as measured by HPLC. Another index, the Normalized Difference Vegetation Index (NDVI) is linked to the chlorophyll content (Zude, 2003). Calculating NAI and NDVI values from a tomato remittance VIS spectrum is expected to assess the level of lycopene and chlorophyll simultaneously. Repeated non-destructive assessment over time of lycopene and chlorophyll levels of individual tomatoes allows building a physiologically more correct kinetic model describing the transition from chloro- to chromoplast.

The regulation of the pathways that govern chlorophyll breakdown and lycopene synthesis has been an area of considerable interest. Carotenoid formation in tomato fruit during ripening has become a model system for chromoplast-containing tissues (Bathgate et al., 1985; Pyke and Howells, 2002; Forth and Pyke, 2006; Kahlau and Bock, 2008; Egea et al., 2010). The changes during the chloro- to chromoplast transition have been monitored extensively. Several studies have been dedicated to ultra-structural events underlying this transition. These events include the biochemical transformation linked to carotenogenesis (Giuliano et at. 1993; Fraser et al., 1994; Ronen et al., 1999; Bramley, 2002), the rearrangement of the internal membrane system (Spurr and Harris, 1968; Harris and Spurr 1969; Bathgate et al., 1985; Marano et al., 1993) and proteome changes (Barry et al., 2008, Barsan et al., 2010; Barry et al., 2012; Wang et al., 2013). The chloroplast to chromoplast transition has been monitored in both isolated plastids and intact live mesocarp tissues using green fluorescent protein and confocal microscopy (Marano et al., 1993; Pyke and Howells, 2002; Forth and Pyke, 2006; Pyke, 2007; Egea et al., 2011). The plastid transition starts by an accumulation of carotenoids while the chlorophyll level is still high in plastids of a single cell. At the ripening breaker stage the lycopene begins to appear and simultaneously chlorophyll disappears (Fraser et al., 1994). Ethylene is required for ripening of tomatoes by regulating biochemistry, morphology, and gene

expression (Hoeberichts et al., 2002; Alba et al., 2005; Barry and Giovannoni, 2007; Klee and Giovannoni, 2011; Martel et al., 2011). Studies performed on tomatoes treated with an ethylene receptor inhibitor, 1-methylcyclopropene, as well as on tomato pleiotropic non-ripening mutants demonstrated that climacteric ethylene signalling plays a central role in the coordination of the molecular processes involved in pericarp carotenogenesis (Fraser et al., 2001; Alba et al., 2005; Martel et al., 2011; Barry et al., 2008; Barry et al., 2012; Fujisawa et al., 2013; Pan et al., 2013; Luo et al., 2013). Concomitant with fruit ripening, ethylene causes an increase of carbon flux into the carotenoid pathway and alters the kinetics of the pathway such that lycopene accumulates preferentially over β-Carotene due to a decrease in the accumulation of transcripts that encode lycopene cyclase activities (Alba et al., 2005). Additionally, hormones such as auxin, cytokinin, abscisic acid and jasmonates mediate the onset of chloroplast to chromoplast transition (Kubo and Kakimoto, 2000; Lisso et al., 2006; Symons et al., 2006; Zhang et al., 2009; Chai et al., 2011; Jia et al., 2011; Liu et al., 2012). Increasing storage temperatures increase the rate of the chloroplast to chromoplast transition. Previous studies have found that temperatures below 12 and above 25 °C adversely affect the plastid conversion causing mainly an inhibition of lycopene synthesis related to the reduction of mostly phytoene and phytofluene (Shewfelt et al., 1988; Dumas et al., 2003; Gautier et al., 2008). Storage temperatures below 12 °C may cause chilling injury, a disorder characterised by lower radical scavenging activities (Tijskens et al., 1994) that increases membrane permeability especially in green tomato fruit (Soto-Zamora et al., 2005; Polenta et al., 2006). Storage temperatures below 12 °C decrease lycopene levels (Farneti et al., 2012).

The aim of this study is to increase the understanding of chlorophyll breakdown and lycopene synthesis on a quantitative level in tomato fruit. One of the intriguing properties of the chloroplast to chromoplast transition is how the synchronisation is arranged between chlorophyll breakdown and lycopene synthesis. A kinetic model is proposed combining relevant, parallel occurring, physiological processes that affect chlorophyll breakdown and lycopene synthesis. The model is calibrated for fruit of two tomato cultivars based on repeated non-destructive chlorophyll and lycopene measurements obtained during storage at constant temperatures between 4 and 24 °C or at a stepwise changing temperature between 4 °C and 16 °C. The nature of the synchronisation between chlorophyll breakdown and lycopene synthesis will be discussed.

2. Materials and methods

2.1. Plant material

Tomato trusses of the cultivars Amoroso RZ (cocktail type) and Cappricia RZ (round type) were obtained from a greenhouse in Steenbergen (south-east of The Netherlands) during April and September 2010 (first two experiments) and May 2011 (third experiment). All tomatoes were grown in one and the same greenhouse using commercial growing conditions.

2.2. Remittance VIS spectroscopy and chlorophyll and pigments levels

A hand-held photodiode array spectrophotometer (Pigment Analyzer PA1101, CP, Germany) was applied to record remittance spectra of tomato fruit between 350 and 1100 nm. Remittance

was assessed at 570 (R_{570}), 660 (R_{660}) and 780 (R_{780}) nm to calculate the normalized difference vegetation index (NDVI, eqn 1) and the normalized anthocyanin index (NAI, eqn 2).

$$NDVI = \frac{R_{780} - R_{660}}{R_{780} + R_{660}} \quad (1)$$

$$NAI = \frac{R_{780} - R_{570}}{R_{780} + R_{570}} \quad (2)$$

Remittance VIS spectroscopy of fruit was assessed at harvest and subsequently at a two or three day interval for tomatoes stored between 4 and 16 °C and at a one or two day interval for tomatoes stored at 20 and 24 °C. Measurements were carried out on three positions on the equator of each tomato and reported as an average NDVI and NAI value per tomato.

The relation between NDVI and NAI measurements and lycopene and total chlorophyll concentration was estimated for the cultivars mentioned above. Small batches (45 'Amoroso' and 'Cappricia' tomatoes) varying in maturity from mature green to deep red were collected. Prior to pigment analysis remittance was measured three times on the equator of each fruit and pericarp samples were collected and stored at -80 °C. Lycopene content of pericarp samples was determined by HPLC analysis according the method described in Farneti et al. (2012). Chlorophyll content was estimated according to the method described in Nagata et al. (1992). Tomato fruit pericarp samples (1 g) where homogenized with 15 ml of acetone-hexane (4:6) solvent. The optical density (A) of the solution was measured at 645 and 663 nm using a UV-Vis spectrophotometer (Varian 4000, Agilent Technologies, USA). Total chlorophyll content (in mg 100 ml^{-1}) was calculated by summing up Chl a and Chl b according to eqn (3) and eqn (4).

$$Chl\ a = 0.999\ A_{663} - 0.0989\ A_{645} \quad (3)$$

$$Chl\ b = -0.328\ A_{663} + 1.77\ A_{645} \quad (4)$$

2.3. Temperature experiments

For the first experiment one batch of 'Amoroso' tomatoes and one batch of 'Cappricia' tomatoes each consisting of 330 tomatoes were harvested. Tomatoes were labelled and divided in 24 equally sized sub-batches based on maturity stage at harvest (mature green, breaker, pink and red) and temperature treatments. Tomatoes in each of the maturity classes were dark stored at 4, 8, 12, 16, 20 or 24 °C.

The second experiment consisted of one batch of 'Amoroso' tomatoes and one batch of 'Cappricia' tomatoes each consisting of 175 tomatoes harvested at the mature green, breaker, pink or red maturity stage. Tomatoes were subdivided into five sub-batches of 35 tomatoes with each sub-batch containing an equal number of green and red tomatoes. The first sub-batch was stored at 4 °C, the second sub-batch at 16 °C. The other sub-batches were initially stored at 4 °C and later moved to 16 °C after five (sub-batch 3), ten (sub-batch 4) or 15 (sub-batch 5) days.

2.4. Ethylene experiment

For the third experiment five trusses of 'Amoroso' and 'Cappricia' tomatoes were harvested and individual tomatoes were binned in mature green, breaker, pink and red maturity classes. Five 'Amoroso' and 'Cappricia' tomatoes per maturity class were placed in 1.2 L airtight glass containers for one hour to accumulate ethylene. Ethylene levels for each tomato were assessed by injecting 1 ml headspace samples into a Focus GC (Thermo Electron S.p.A., Italy) equipped with a Restek RT-QPLOT 15 m x 0.53 mm I.D. column.

2.5. Model formulation

Translating the chloroplast to chromoplast transition into processes is complex due to the biophysical and biochemical nature of the events and therefore considerable simplifications are needed for model development. It is proposed that an autocatalytic enzyme system links both chlorophyll breakdown and lycopene synthesis leading to logistic behaviour for the chlorophyll breakdown and lycopene synthesis over time. This approach shows similarity with the tomato colour modelling approaches that have been used before (e.g. Hertog et al., 2004, Schouten et al., 2007) but differs from them by introducing the lycopene precursor and by the flexibility in how the enzyme system affect the chlorophyll breakdown and lycopene synthesis separately. This flexibility also allows the description of more complex behaviour covered by assuming breakdown of the precursor, by lycopene breakdown or by both.

The main reactions that describe the conversions of the chlorophyll (C) and lycopene (L) during postharvest storage of tomatoes by an enzyme system (E) are represented by Eqs. (5-6). The breakdown reaction of lycopene (L) and the lycopene precursor (P) is shown in Eqs. (7-8).

$$C + E \xrightarrow{k_c} 2E \tag{5}$$

$$P + E \xrightarrow{k_l} L + E \tag{6}$$

$$P \xrightarrow{k_{pd}} nil \tag{7}$$

$$L \xrightarrow{k_{dl}} nil \tag{8}$$

kc, kl, kdl and kdp are the reaction rate constants for the breakdown of chlorophyll, the formation of lycopene and the breakdown of lycopene and the lycopene precursor, respectively. A set of ordinary differential equations (Eqs. 9-12) can be deduced from these model reactions (Eqs 5-8) using the basic rules of chemical kinetics.

$$\frac{d[C]}{dt} = -k_c [C][E] \quad with\ [C](t=0) = C_0 \tag{9}$$

$$\frac{d[E]}{dt} = k_c [C][E] \quad \text{with } [E](t=0) = E_0 \tag{10}$$

$$\frac{d[E]}{dt} = k_c [C][E] \quad \text{with } [E](t=0) = E_0 \tag{11}$$

$$\frac{d[L]}{dt} = k_l [E][P] - k_{dl} [L] \quad \text{with } [L](t=0) = L_0 \tag{12}$$

All four reaction rate constants (k_c, k_l, k_{dl} *and* k_{dp}) depend on temperature (T) assumed according to Arrhenius' Law (Eq.13).

$$k_i = k_{i,ref} \cdot e^{\frac{E_i}{R} \cdot \left(\frac{1}{T_{ref}} - \frac{1}{T} \right)} \tag{13}$$

For each reaction (index i) the reaction rate constant $k_{i,ref}$ is the value of k_i at an arbitrary chosen reference temperature T_{ref} (here 287.15 K or 14 °C), and E_i the energy of activation (in kJ mol^{-1}). R is the universal gas constant (8.314 J mol^{-1} K^{-1}).

2.6. Statistical Analysis

The equations of the model formulation were developed using Maple (release 12, Waterloo Maple Software, Canada). Unfortunately, no analytical solution is available. The developed model was therefore analysed and calibrated using OPTIPA (Hertog et al., 2007), a kinetic modelling tool developed for use with Matlab (Matlab R2007b, The MathWorks, Inc., Natick, MA, USA) to solve ordinary differential equations and for parameter estimation. During optimisation the Runge–Kutta solver for non-stiff systems (ODE45) with a termination tolerance of 0.001 was used.

3. Results

3.1. Calibrating the handheld spectrophotometer for chlorophyll and lycopene

Figure 1 shows the non-linear relationships between the NAI and NDVI values and the lycopene and chlorophyll levels for both tomato cultivars expressed in nmol per gram fresh weight. Remarkably, the (third degree polynomial type) relationship between NAI values and lycopene is independent of cultivar while the (second degree polynomial type) relationship between NDVI values and chlorophyll is cultivar dependent. This might be related to the thickness of the pericarp as this differs per tomato type.

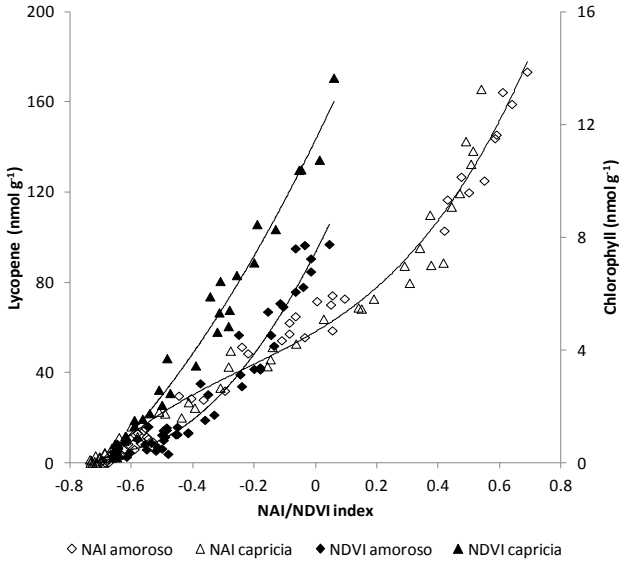

Figure 1. Relations between estimated lycopene and chlorophyll levels in tomato pericarp tissue and NAI and NDVI values obtained from remittance VIS spectroscopy.

3.2. Overview of the chlorophyll and lycopene data

An overview of the chlorophyll (left column) and lycopene levels (right column) of tomatoes stored at constant temperatures (upper four plots) or when a switch in temperatures is applied (lower four plots) is shown for 'Amoroso' (Figure 2) and 'Cappricia' (Figure 3) tomatoes. Elevated storage temperatures result in increased chlorophyll breakdown and lycopene synthesis both according to a sigmoid pattern for mature green tomatoes. Chlorophyll eventually fully disappears when lycopene levels are still increasing. The maximum lycopene level is higher for 'Amoroso' while the maximum chlorophyll level is higher for 'Cappricia' tomatoes (first row of Figure 2 and 3). This means that 'Amoroso' tomatoes, although initially containing less chlorophyll, eventually synthesize more lycopene than 'Cappricia' tomatoes. The lycopene levels of red tomatoes show differences as function of temperature and cultivar. Above 8 °C the lycopene levels increases to a maximum value while for lower temperatures the lycopene levels appear to be constant for 'Amoroso' tomatoes. Red 'Cappricia' tomatoes show a gradual loss of lycopene over time that increases with decreasing temperature. Red tomatoes do not show a discernible chlorophyll level (second row of Figure 2 and 3).

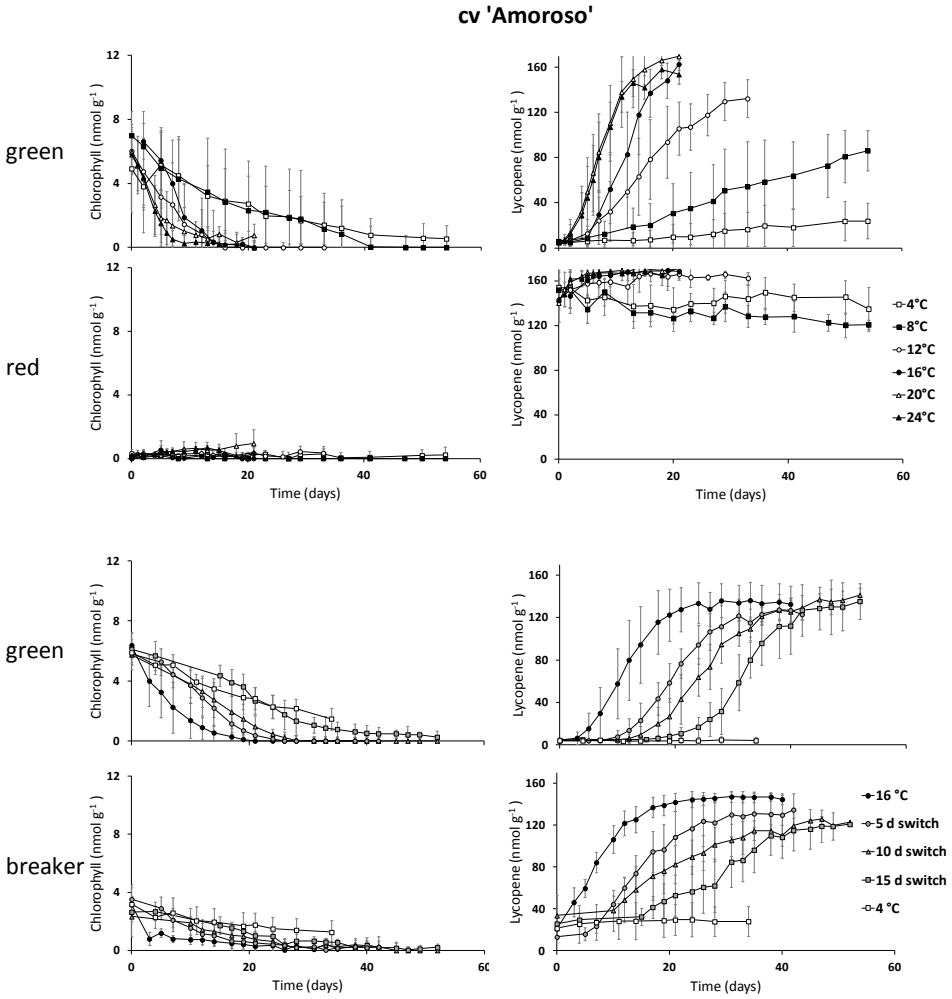

Figure 2. Development over time of chlorophyll and lycopene levels with indicated standard deviation for mature green and red 'Amoroso' tomatoes stored at constant storage temperatures (upper four plots). The lower four plots indicate chlorophyll and lycopene changes of mature green and breaker tomatoes after a switch from 4 to 16 °C after 0, 5, 10 or 15 days or no switch.

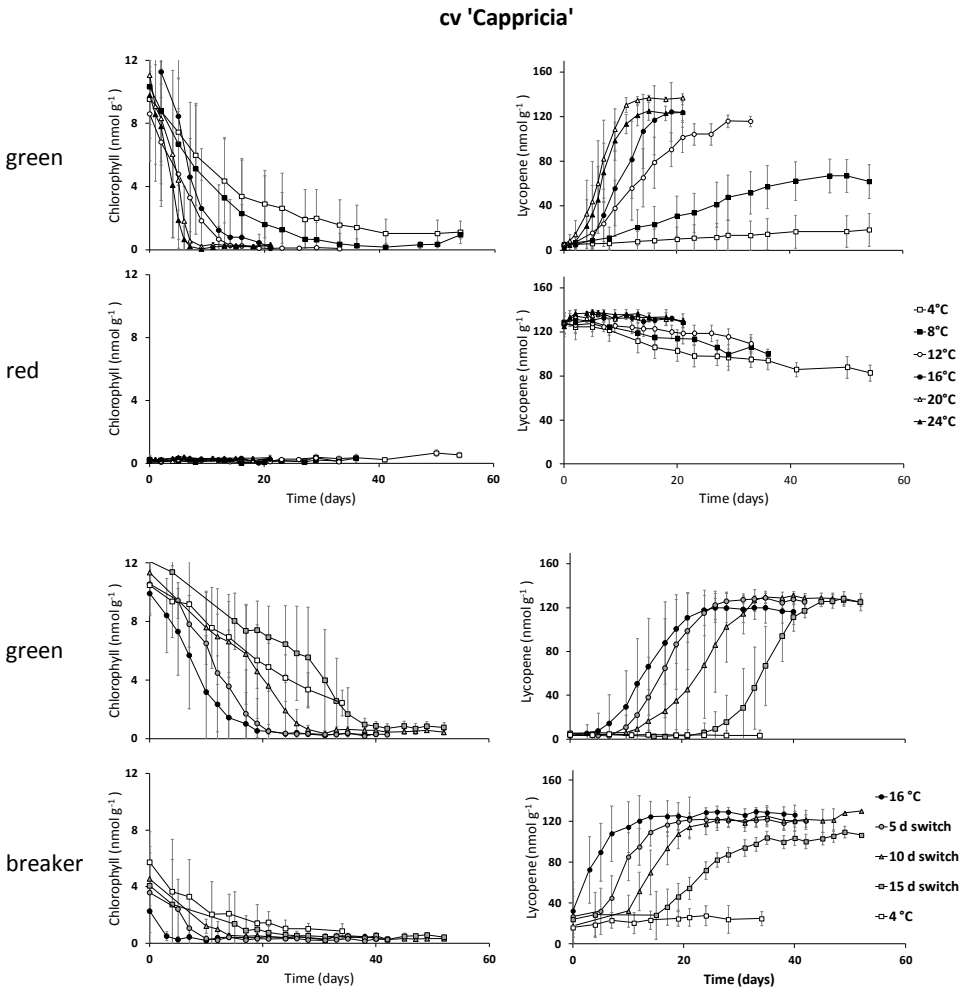

Figure 3. Development over time at constant temperatures of chlorophyll and lycopene levels with indicated standard deviation for mature green and red 'Cappricia' tomatoes stored at constant storage temperatures (upper four plots). The lower four plots indicate chlorophyll and lycopene changes of mature green and breaker tomatoes after a switch from 4 to 16 °C after 0, 5, 10 or 15 days or no switch.

Switching temperatures from 4 to 16 °C after varying times of storage time shifts the time at which lycopene synthesis starts for mature green and breaker tomatoes. Mature green 'Amoroso' tomatoes all reach the same maximum lycopene level regardless of the time of the temperature switch while a slight decrease in lycopene levels is encountered for mature green 'Cappricia' tomatoes for the constant 16 °C treatment. This indicates that prolonged storage of mature green tomatoes at 4 °C does not lead to irreversible aberration of the colouring process. Chlorophyll levels of mature green and breaker tomatoes from both cultivars decrease faster at

higher storage temperature. Differences in lycopene accumulation can be observed between the two cultivars. The longer it takes to switch the temperature from 4 to 16 °C the lower the maximum lycopene level for breaker 'Amoroso' tomatoes. A lower maximum lycopene level can only be observed for the 15 day switch for breaker 'Cappricia' tomatoes (last row of Figures 2 and 3). This indicates that the prior storage of breaker tomatoes at 4 °C irreversibly affected lycopene synthesis for 'Amoroso' tomatoes. This effect is also visible for pink 'Amoroso' tomatoes (data not shown).

3.3. Model calibration

Model parameters were estimated simultaneously using the model formulation of Eqs. 9-12 together with the temperature dependence according to the Arrhenius equation (Eq. (13)) using normalised chlorophyll and lycopene levels. Chlorophyll and lycopene values were normalised by dividing the measured values by the mean of the values of that compound to assure that equal weights are assigned to all data during multi-response regression analysis. The model was calibrated for each cultivar using temperature and time simultaneously as explaining variables for both cultivars. Kinetic parameters were estimated in common. The parameter estimation was refined in several steps using the possibility to increase the indexation of model parameters. Indexation is a critical part of OPTIPA and allows assigning variation encountered in the experimental data to specific sources. The variation in the chlorophyll and lycopene data can either be assigned to individual parameters (e.g. individual tomatoes), treatment parameters (e.g. initial maturities) or common parameters (e.g. reaction rate constants) or a combination of the above.

During the first step model parameters were estimated in common, except the initial level of chlorophyll (C_0), lycopene (L_0), lycopene precursor (P_0) and enzyme (E_0) using all available data of the first experiment. C_0, L_0, P_0 and E_0 were estimated per initial maturity stage (mature green, breaker, pink and red) using Eqs. (9-13) and setting $k_{dl,ref}$ and $k_{dp,ref}$ to zero. In this way the effect of temperature and the synchronisation between the chlorophyll breakdown and lycopene synthesis was roughly estimated. In further steps the optimisation was started with saved parameter estimates from the previous steps. In the second step all available data from the first and second experiment were pooled per cultivar and all model equations were used (Eqs. 9-13). In this step P_0 and E_0 were estimated per initial maturity stage and C_0 and L_0 were estimated per tomato. The pooling is carried out with a specific reason. Without pooling the data from both experiments, the variation in e.g. maximum lycopene levels of green tomatoes stored at constant temperatures or after a temperature switch (right hand side plots of Figures 2 and 3) will be hard to allocate to variation in P_0, to lycopene breakdown or to lycopene precursor breakdown. With pooling the variation in maximum lycopene levels can be allocated with a higher degree of certainty to the different sources of variation. During the second calibration step k_{dl} and $E_{a,dp}$ for 'Amoroso' and k_{dp} and $E_{a,dl}$ for 'Cappricia' tomatoes were estimated to be small, about the same magnitude as their standard error. During subsequent optimisation these model parameters were therefore fixed at 0. This indicates that the lycopene level of 'Amoroso' tomatoes is only affected by temperature insensitive lycopene precursor breakdown and that 'Cappricia' tomatoes only show temperature insensitive lycopene breakdown. An overview of the estimated kinetic parameters is shown in Table 1 (set 1). Estimated P_0 and E_0 levels expressed per initial maturity after completing the second step are shown in Figure 4. In the

third and final step, a representative subset was selected from the first and second experiment covering all storage temperatures and initial maturities. This subset was about 10% of the size of the dataset used in first and second step. This was necessary because of computational limitations. In this last step the kinetic parameters were estimated in common and all other parameters were estimated per tomato (C_0, L_0, P_0 and E_0). The estimated kinetic parameters estimated after completing the second step (first set of estimates) and third step for both cultivars are shown in Table 1 (set 2). Measured and simulated chlorophyll and lycopene data for tomatoes of both cultivars for constant storage temperatures (first experiment) are shown in Figure 5. The simulated data were gained by applying the estimated kinetic parameters from the second set of parameter estimates shown in Table 1 and the estimated C_0 and L_0 levels for individual tomatoes. Figure 6 shows the measured and simulated chlorophyll and lycopene data when tomatoes of both cultivars are switched from 4 to 16 °C after varying times of storage (second experiment).

Table 1. Overview of the kinetic parameter estimates and their standard error of estimates (S.E.) per cultivar for the colour model.

	'Amoroso'				'Cappricia'			
	set 1		set 2		set 1		set 2	
Kinetic parameters	Estimate	S.E.(%)	Estimate	S.E.(%)	Estimate	S.E.(%)	Estimate	S.E.(%)
k_l ($nmol^{-1}g^{-1}day^{-1}$)	0.0360	17.7	0.0384	28.1	0.118	7.2	0.134	6.0
k_e ($nmol^{-1}g^{-1}day^{-1}$)	3.50	11.4	5.95	6.4	15.2	8.8	14.4	5.2
k_{dl} (day^{-1})	0	fixed	0	fixed	0.0694	13.2	0.0659	15.5
k_{dp} (day^{-1})	0.196	16.7	0.177	25.1	0	fixed	0	Fixed
E_l ($kJ\ mol^{-1}$)	105.1	6.1	87.8	17.2	109.9	6.3	76.7	8.3
E_c ($kJ\ mol^{-1}$)	26.5	11.7	59.6	28.1	49.1	4.2	53.5	10.0
E_{dl} ($kJ\ mol^{-1}$)	0	fixed	0	fixed	0	fixed	0	fixed
E_{dp} ($kJ\ mol^{-1}$)	0	fixed	0	fixed	0	fixed	0	fixed
Administrative information								
N	9828		1128		10876		1244	
R^2_{adj} (%)	89.3		97.5		87.9		97.1	

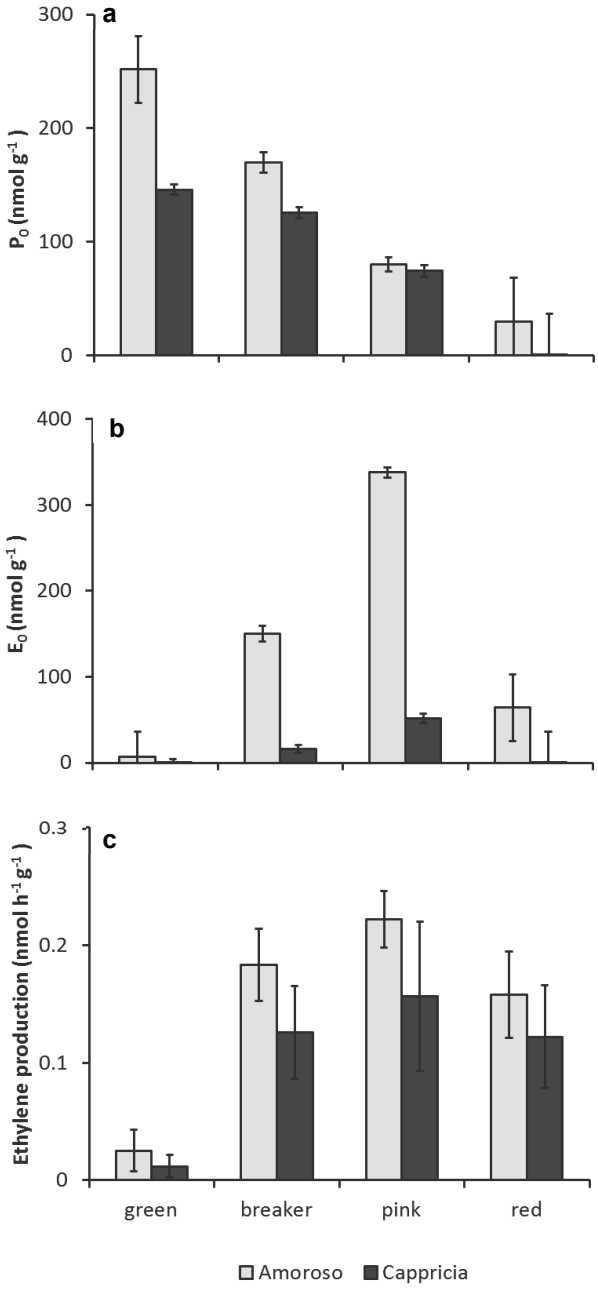

Figure 4. Estimated initial levels of lycopene precursor (a), initial levels of enzyme (b) and measured ethylene production (c) as function of the initial maturity of tomatoes. Standard error of estimation is indicated for the left hand side and middle plot. Standard deviation (n=5) is indicated for the right hand side plot.

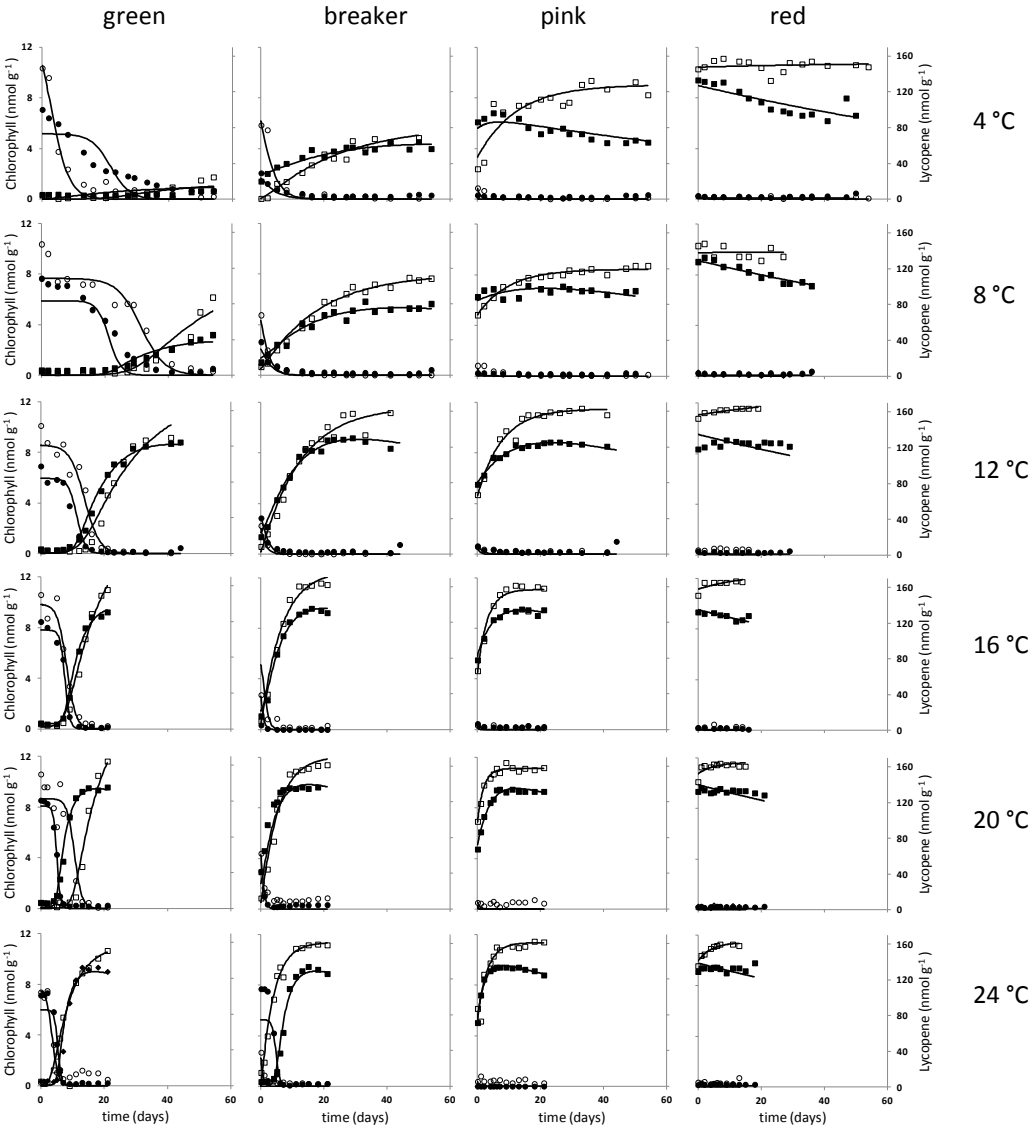

Figure 5. Development over time at constant temperatures of chlorophyll (circles) and lycopene (boxes) levels for 'Amoroso' (open symbols) and 'Cappricia' (closed symbols) tomatoes varying in initial maturity. The lines represent simulated chlorophyll or lycopene levels using the model parameters of Table 1.

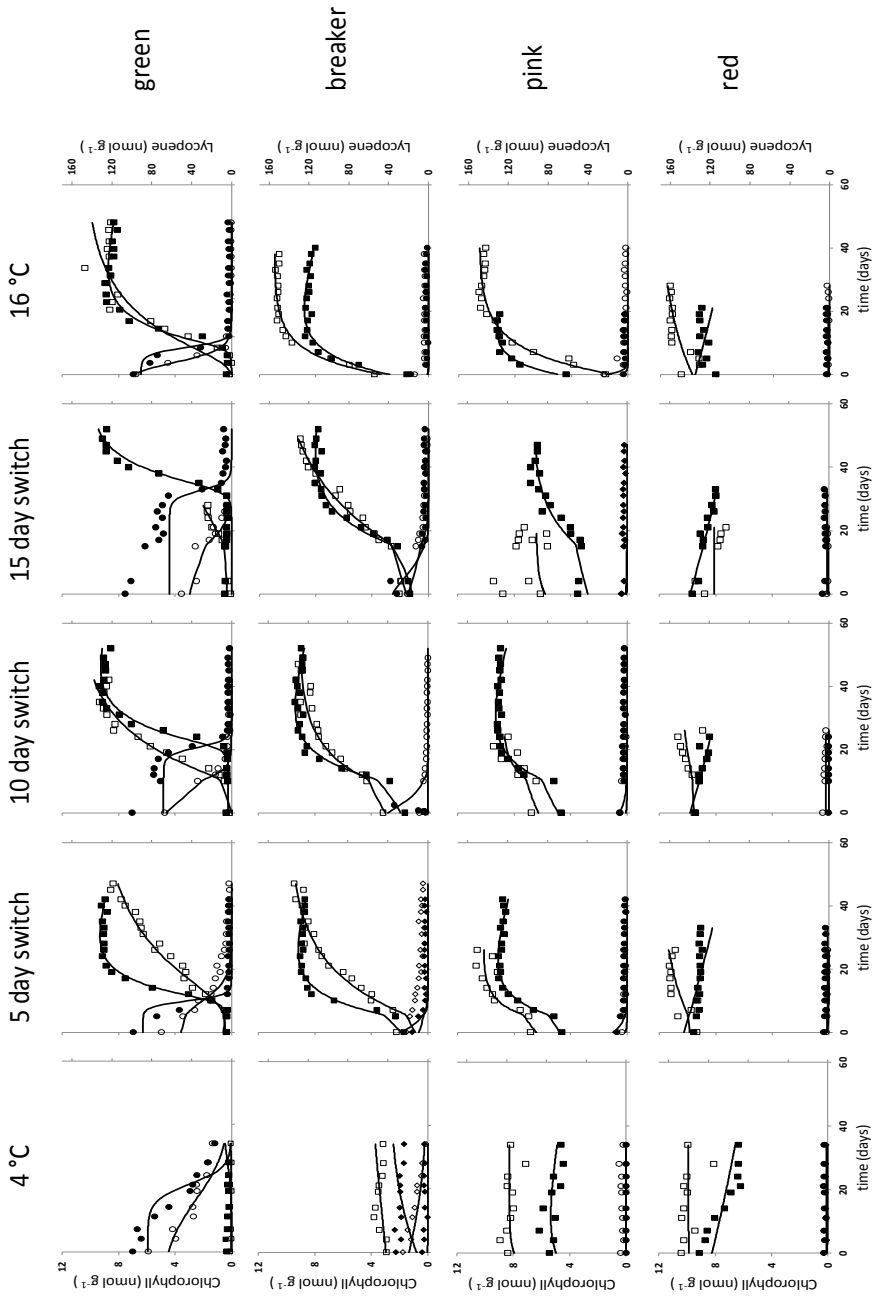

Figure 6. Development over time after a switch from 4 to 16 °C after 0, 5, 10 or 15 days of chlorophyll (circles) and lycopene (boxes) levels for 'Amoroso' (open symbols) and 'Cappricia' (closed symbols) tomatoes varying in initial maturity. The lines represent simulated chlorophyll or lycopene levels using the model parameters of Table 1

4. Discussion

4.1. Model performance

The observed changes in the levels of both the chlorophyll and lycopene as a function of initial maturity and (constant and stepwise changing) temperature for both cultivars are well covered by the model (Table 1, Figures 5 and 6). The first set of parameter estimates shown in Table 1 for 'Amoroso' and 'Cappricia' tomatoes uses five kinetic parameters estimated in common for the whole dataset, two initial levels (E_0 and P_0) estimated per maturity stage and two initial levels (C_0 and L_0) estimated per tomato with standard error of estimates often considerable smaller than 20% of the parameter estimates. The second set of parameter estimates results in a considerably higher percentage variation accounted for (R^2_{adj}) but is based on almost double the amount of model parameters since E_0 and P_0 are now also estimated for each tomato. Nevertheless, the first and second set of parameter estimates show considerable similarity which indicates that over parameterisation in the case of the second set of parameter estimates is not likely.

4.2. Synchronization between chlorophyll breakdown and lycopene synthesis

The value for k_l, the reaction rate for lycopene synthesis, increases faster with increasing temperature than k_c, the reaction rate for chlorophyll breakdown, due to a higher activation energy for both cultivars ($E_{a,l} > E_{a,c}$ Table 1). In other words, higher storage temperature shifts the synchronisation towards more lycopene synthesis. The picture is, however, more complex when the cultivars are compared. The values for $k_{l,ref}$ and $k_{c,ref}$, the reaction rate constants for lycopene synthesis and chlorophyll breakdown at the reference temperature, are about a factor three to four larger for cv. Cappricia than for cv. Amoroso (Table 1). Higher reaction rate constants for the chlorophyll breakdown and the lycopene synthesis suggest a much faster colour change for 'Cappricia' tomatoes. However, the differences in chlorophyll breakdown and lycopene synthesis between the two cultivars are much smaller when considering the measured data (Figure 5). The ratio of $k_{c,ref}$ to $k_{l,ref}$ is similar for the two cultivars. This indicates that the larger values for $k_{c,ref}$ and $k_{l,ref}$ for cv. Cappricia compared to cv. Amoroso are counterweighted by a common factor affecting both reaction rates. This common factor is the initial enzyme level (E_0) that is about a factor eight higher for 'Amoroso' than for 'Cappricia' tomatoes (Figure 4). In other words, chlorophyll and lycopene changes will occur at lower enzyme levels in 'Cappricia' tomatoes. This can be observed when tomatoes are switched from 4 to 16°C (Figure 6). After storage at 4°C the enzyme level produced will be limited due the slow chlorophyll breakdown (Eq. (5)) causing the lycopene synthesis to be slower for 'Amoroso' than for 'Cappricia'.

4.3. The nature of the enzyme system

The description of complex pathways needs acceptable simplifications to enable quantification with today's enzyme-kinetic modelling technology (Schallau and Junker, 2010). From a modelling point of view, only green and red coloured compounds and their precursors are of interest. Acceptable simplifications might be those that focus on chlorophyll breakdown and assuming one rate limiting step for the lycopene synthesis with two extensions dealing with

either lycopene precursor or lycopene breakdown. The question whether the description of the enzyme system is an acceptable simplification can be answered better when the nature of the enzyme system is known. Since ethylene can be regarded as inducer of carotenogenesis enzymes, the first candidate for the enzyme system is ethylene. Estimations of E_0 as a function of maturity for both cultivars exhibit a similar pattern as observed for ethylene production, including the larger E_0 values and higher ethylene production for 'Amoroso' tomatoes as a function of initial maturity (middle and right hand side plots of Figure 4). The link between ethylene and chlorophyll breakdown in tomato is, however, weak. Ethylene induces chlorophyllase (Trebitsh et al., 1993; Jacob-Wilk et al., 1999), considered a rate-limiting enzyme during chlorophyll catabolism (Harpaz-Saad et al., 2007), but this is in citrus type fruit, not in tomato.

We hypothesize that the enzyme system postulated in the model is not actually an enzyme, but represents a family of proteins: STAY-GREEN (SGR) proteins. Silencing of the SGR gene, *SlSGR1*, in tomato resulted in reduced chlorophyll degradation (Hu et al., 2011). SGR proteins play a role during tomato ripening in the regulation of chlorophyll degradation (Barry et al., 2008; Hortensteiner, 2009). SGR proteins might regulate ROS generation and initiate chlorophyll degradation by inhibiting the recovery of β-carotenes in the light-harvesting complexes of photosystem II. Plastid numbers in fruit pericarp cells of SGR silenced plants were significantly higher than in WT fruits and the chloroplasts converted earlier into chromoplasts, resulting in higher pigmentation of *SlSGR1*-repressed fruit. SGR proteins play a role in lycopene accumulation through direct interaction with the rate limiting enzyme of the carotenoid pathway, phytoene synthase (Luo et al., 2013). So it appears that the synchronisation functions by first inducing chlorophyll breakdown by a high SlSGR1 level during early tomato ripening but at the same time blocking lycopene synthesis. At the moment the SlSGR1 level lowers, lycopene synthesis inhibition is released. This behaviour is simulated in the kinetic model by first having to create sufficient enzyme during chlorophyll breakdown before being able to start lycopene synthesis. Observing the large differences in enzyme levels between the two cultivars (Figure 4), it seems that substantial plasticity is available in the expression of *SlSGR1* assuming that SGR proteins indeed represent the postulated enzyme system. Manipulation of the transcription level of *SlSGR1* by transgenic approaches might not only enrich the lycopene content, resulting in added nutritional value for the consumer, but might also be needed to test the enzyme system hypothesis..

4.4. Lycopene and lycopene precursor breakdown

Lycopene breakdown is observed for 'Cappricia' tomatoes but no temperature dependence for $k_{dl,ref}$ was established ($E_{a,dl}$=0). This lack of temperature dependence for this cultivar is unexpected as it seems from viewing the measured data that the lycopene breakdown is somewhat faster at lower temperatures in red 'Cappricia' tomatoes (Figure 3). There might be two reasons for this. Red harvested 'Cappricia' tomatoes still contain lycopene precursor (Figure 4) which results in lycopene synthesis at higher storage temperatures. At lower temperatures lycopene synthesis is very limited and lycopene breakdown occurs. Likely, lycopene is broken down as the result of chilling-induced membrane damage (Nishida and Murata, 1996) and it might, at the same time, be involved as an antioxidant. 'Cappricia' tomatoes might suffer from some chilling-induced lycopene loss not taken into account by the

model. High (30-35 °C) storage temperatures have been reported to cause yellow discolouration in tomatoes (Tijskens and Everlo, 1994). Likely, small amounts of lycopene breakdown observed at low (4-8 °C) temperatures and small amounts of lycopene breakdown at 24 °C are covered in the model by establishing a small lycopene breakdown over the whole temperature range. A separate description of the lycopene breakdown at low (4-8 °C) and high (24-35 °C) temperatures would require more temperature treatments than the ones included in this study.

The absence of lycopene breakdown estimated for 'Amoroso' tomatoes (Table 1, Figs 6-7) in this experiment is different from our previous observations. Lycopene breakdown at 4 and 8 °C, expressed both as NAI values and HPLC lycopene measurements, was observed in both 'Amoroso' and 'Cappricia' tomatoes by Farneti et al. (2012). Low temperature lycopene breakdown in tomato has a genetic component since there are differences between genotypes grown at the same growing conditions. On the other hand, also greenhouse conditions play a role as growing conditions seem to influence the post-harvest chilling tolerance of several horticultural products such as peach (Lurie and Crisosto, 2005; Campos-Vargas et al., 2006; Martınez-Garcìa et al., 2012), apple (Bramlage and Weis, 1997), avocado (Woolf et al., 1999), and cucumber (Kang et al., 2002). Recently, it was shown that monodehydroascorbate reductase (MDHAR), an enzyme involved in restoring the gluthatione and ascorbate pools, is connected to chilling tolerance in tomato by studying transgenic lines with reduced MDHAR activity (Airaj et al., 2013).

5. Conclusion

Remittance VIS spectroscopy is an accurate and fast method to non-destructively assess both the chlorophyll and lycopene levels in pericarp tomato tissue, although there is a cultivar specific interaction between NDVI values and chlorophyll levels. The conversion of chlorophyll to lycopene and the breakdown of either the lycopene precursor or lycopene itself are well characterised for two types of tomatoes. The kinetic model, in combination with the VIS spectroscopy, could be of interest in studies where the chloro- to chromoplast transition is studied in terms of phytohormones, mutants or transcription patterns. A small series of typical tomato photographs over time is often included in these publications without actual quantification of lycopene synthesis and chlorophyll breakdown. Tomato breeders can screen existing and new genotypes for the colour performance per genotype x growing condition using the kinetic model in combination with remittance VIS spectroscopy. Quantifying the colour performance would allow classification of genotypes that e.g. can be harvested early due to already having high amounts of lycopene precursor at harvest. Screening for genotypes with e.g. low values for $E_{a,l}$ and $E_{a,c}$ would allow for genotypes that ripen irrespective of the temperature in the logistic chain. Genotypes that are sensitive for lycopene breakdown at low temperature storage can be identified. Molecular markers that quantify the model parameters such as shown in Table 1 could be the key to develop genotypes with an intended colour transformation performance..

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Chapter 3

Greenhouse climate control affects postharvest tomato quality

Abstract

In this study important quality properties such as firmness, sugar and acid levels were measured and analysed of tomatoes harvested from three greenhouses during a five month period and stored at 16 °C for over twenty days. Tomatoes were harvested from three identical, neighbouring, greenhouses which were either conventionally ventilated (open greenhouse) or mechanically cooled (semi-closed greenhouses). Sugar and acids levels were hardly affected by greenhouse type. Compared to the open greenhouse, semi-closed greenhouses produced heavier and less mature (firmer) fruit at the commercial harvesting stage based on colour. Fruit maturity differences could be linked to the vertical temperature gradient and to CO_2 levels in the different greenhouses. This indicates that CO_2 levels and temperature affect the synchronisation between colour and firmness maturity at harvest. The acceptance period, i.e. the time period both tomato colour and firmness are considered acceptable by consumers, will likely be positively affected when growers switch from conventionally ventilated to semi-closed production systems. Additional to greenhouse effects also effects of the harvest month were observed. The sugar to acid ratio was highest and glucose to fructose ratio was lowest in July, the month with the highest irradiance, irrespective of greenhouse type. The estimated value for the maximum firmness (F_{max}) varied from 17.9 N in August to 31.2 N in June. This monthly variation in F_{max} explains an important part of the variation found in the postharvest behaviour of tomatoes. Interestingly, the monthly variation in F_{max} showed the same trend as found for the monthly initial sugar levels. It might be hypothesized that the monthly variation in glucose and fructose levels causes variation in that part of firmness that is generated by cell turgor. The monthly variation in F_{max}, sugar and acid levels could not be linked to climate conditions and remains to be elucidated.

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1. Introduction

Greenhouse climate control strategies have mainly been used to optimise crop characteristics and yield but little attention has been paid to effects on fruit quality. How greenhouse climate control affects tomato quality during postharvest storage and handling is unclear. Only a weak relation was found between the cumulative photosynthetically active radiation and firmness, soluble solids content and total phenolic compounds whereas a strong relation was found between these quality properties and the cumulative temperature during the last 45 days before harvest (Riga et al., 2008). Firmness and titratable acidity at harvest were considered season dependent with tomatoes being significantly firmer in autumn than in spring at the same greenhouse climate control regime (Anza et al., 2006). This indicates that seasonal effects may mask the effects of greenhouse climate control. To elucidate the role of greenhouse climate control on quality issues, seasonal effects should be eliminated by comparing fruit from identical greenhouses in close vicinity of each other that vary only in their greenhouse climate control.

The concept of closed or semi closed greenhouses has recently been developed to reduce energy consumption. In this system, the excess of solar energy in summer is collected and stored in aquifers to be reused in winter to heat the greenhouse. Heat exchangers and air treatment units can transport cold air directly into the top of the greenhouse or do so via air distribution ducts below the gutters (De Zwart, 2009). The reduced rates of ventilation result in higher CO_2 concentrations, a lower vapour pressure deficit and a vertical temperature gradient when cooling is applied in the lower part of the greenhouse (Opdam et al., 2005; Heuvelink and Bakker, 2008; De Gelder et al., 2012). Here, the effects of greenhouse climate control on the most important tomato quality issues are investigated during a five month period at three identical greenhouses positioned next to each other. The first, open, greenhouse is window-ventilated according to conventional practice. The other two greenhouses are managed according to the semi-closed concept and differ only in terms of top or bottom cooling. Here the question is investigated how the three greenhouse types affect the postharvest quality of tomatoes. Quality attributes under investigation are those considered most important for tomato quality, firmness and taste (expressed as sugar and acid levels) of fruits harvested fully ripe (in terms of colour).

2. Materials and methods

2.1. Plant material and greenhouses conditions

Truss tomato plants (*Lycopersicon esculentum* (Mill.)) cultivar Cappricia RZ grafted on the rootstock 'Emperador' were planted in rockwool on December 23, 2008 in three identical experimental Venlo type greenhouses located in Bleiswijk, The Netherlands. Stem density was initially 2.5 stems m^{-2} (1 stem per plant). By maintaining side stems, the stem density was increased to 3.3 stems m^{-2} eight weeks after planting. Truss initiation rate was 0.9 truss $week^{-1}$. The first truss flowered five weeks after planting. Fruit number was restricted to six fruits per truss by manual pruning. Fruits were harvested weekly starting fifteen weeks after planting.

At the start of the cultivation, crops in all greenhouses were grown under identical climate conditions. The temperature control strategies started thirteen weeks after planting when cooling or ventilation was necessary to maintain the temperatures at the required levels. Treatments consisted of a conventional open greenhouse (O) and two semi-closed with heath exchange units (cooling capacity 350 W m^{-2}). Each greenhouse has an area of 144 m^2 (15 x 9.6 m) with a gutter height of 5.5 m. In the semi closed greenhouses, air from the top of the greenhouse is cooled and dehumidified in the air treatment units and returned to the greenhouse. The two semi closed greenhouses (SC) differ in how the cooled air is returned to the greenhouse, either at the bottom, or at the top. In the 'cooling from below' greenhouse (SC_below) treated air is returned through five plastic cooling ducts placed horizontally beneath the growing gutters. Each duct has six holes (diameter 16 mm) per meter. In the 'cooling from above' greenhouse (SC_above) cooled air is returned at the top of the greenhouse using five blowers near the roof. In both treatments, ventilation windows were opened to support cooling if the cooling capacity was insufficient. Pure CO_2 was supplied with a maximum capacity of 230 kg ha^{-1} h^{-1} during day time with a set point of 1000 ppm in all three greenhouses. CO_2 concentration, air temperature and humidity inside the greenhouse were recorded at 5 min intervals. In addition, in each greenhouse four temperature and humidity sensors (Hoogendoorn, Vlaardingen, The Netherlands) were placed at different heights in the canopy (1.0, 1.9, 3.2 and 4.2 m). The sensor at the highest point was situated above the top of the canopy. The sensor at the lowest point was situated between the lowest truss on the plant and the rockwool slab.

2.2. Fruit selection and storage conditions

On May 14, July 16 and September 27, 180 tomatoes per greenhouse were selected and randomly divided into six equal sized sub batches. Five sub batches were used for HPLC analysis (sugar and acid measurements) every five days and one sub batch for (non-destructive) firmness measurements every five days during twenty or twenty-five days. Additionally, on June 18 and August 27, 60 tomatoes per greenhouse were harvested and randomly divided into two sub batches. One sub batch was used for sugar and acid levels measurements directly after harvest and one sub batch for (non-destructive) firmness measurements every five days during twenty days. All tomatoes were stored at 16 °C and a relative humidity of about 80%.

Selection of fruits was based on colour at harvest. Only uniformly colored fruits of the middle part of each truss were selected using a hand-held photodiode array spectrophotometer device (Pigment Analyzer PA1101, CP, Germany) (Kuckenberg et al., 2008, Zude-Sasse et al., 2007). Remittance was assessed at 570 (R570), 660 (R660) and 780 (R780) nm as an average of two readings on the equatorial region per tomato to calculate the normalized difference vegetation index (NDVI, Eq. 1) and the normalized anthocyanin index (NAI, Eq. 2).

$$NDVI = \frac{R780 - R660}{R780 + R660} \quad \text{(Eq. 1)}$$

$$NAI = \frac{R780 - R570}{R780 + R570} \quad \text{(Eq. 2)}$$

Only red ripe tomatoes were selected with a NAI value higher than 0.4 and a NDVI lower than -0.65 (Farneti et al., 2012).

2.3. Sugar and acid measurements

Quarter pieces of individual tomatoes were flash-frozen using liquid nitrogen and stored at -80˚C until analysis. After grinding, 0.2 g was extracted for 20 minutes with 5 ml 75% ethanol in a 10 mM HEPES buffer, pH 7.1 at 80˚C. The supernatants were vacuum dried using a SpeedVac AES 2000 (Savant Instruments, Farmingdale, NY, USA) and resolved in Milli-Q water. Samples were analysed on a Dionex HPAEC system (Dionex Corporation, Sunnyvale, CA, USA) equipped with a GS50 pump, a PED detector and a CarboPac PA1 (250 x 4 mm) column eluted with 100 mM NaOH to determine carbohydrates. For anions, samples were analysed on a Dionex HPAEC system equipped with a GS50 pump, an ED50 detector and an IonPac AS11-HC (250 x 2 mm) column eluted with a gradient starting with 16 mM NaOH.

2.4. Measurement of weight and firmness

Weight at harvest and during storage was determined using a standard Sartorius laboratory balance (Goettingen, Germany). Firmness of individual fruits was measured at two orthogonal selected spots using a Zwick Z2.5/TS1S materials testing machine (Ulm, Germany) with a cylindrical probe (Ø 15 mm). Tomatoes were placed on plastic ring to keep the tomatoes upright during measurement. Firmness was determined as the maximum force needed to compress the tomato 1 mm at 40 mm/min, after lowering the probe until the tomato skin was touched (Schouten et al., 2007a).

2.5. Model development

The rate of softening of tomatoes as measured by non-destructive, limited compression firmness measurements has been linked with processes involved in cell wall degradation (Van Dijk and Tijskens, 2000). Softening has been described as first order mechanism (exponential function with a fixed invariable part) as often only this part of softening is observed (Schouten et al., 2007a, Schouten et al., 2010). Another approach is to apply a sigmoid model as softening of most fruit seems to follow this behaviour, including tomato (Hertog et al., 2003, 2004a). Indeed, tomatoes from this cultivar (Cappricia RZ) stored at various temperatures and harvested at green or breaker stages showed sigmoid behaviour (data not shown). So, the observed exponential behaviour can be seen as the final part of the sigmoid curve. Such as sigmoid curve can be described as an autocatalytic process where firmness is broken down by a general enzyme that is produced during the softening. The ordinary differential equations can be solved to describe the firmness behaviour over time (Eq. 3).

$$F = F_{max} - \frac{F_{max} - F_{min}}{1 + \frac{F_0 - F_{min}}{F_{max} - F_0} e^{-k \cdot t \cdot (F_{max} - F_{min})}} \qquad \text{(Eq. 3)}$$

with the asymptotic firmness values F_{max} (N) and F_{min} (N) the maximum and the minimum asymptotes, k the reaction rate constant (day^{-1}) and the initial firmness value at harvest, F_0 (N). It might be assumed that preharvest firmness loss follows the same mechanism. In that case the

firmness at the end of the preharvest period, F_0, can be regarded as the starting firmness for the postharvest period (Eq. 4).

$$F_0 = F_{max} - \frac{F_{max} - F_{min}}{1 + \frac{F_{ref} - F_{min}}{F_{max} - F_{ref}} e^{-k \cdot \Delta t \cdot (F_{max} - F_{min})}} \quad \text{(Eq. 4)}$$

with F_{ref} (N) an arbitrary reference firmness value. Δt (day) is the biological age, or maturity, expressed as the time needed to change the firmness from F_{ref} to F_0. Substituting Eq. 4 into Eq. 3 results in an expression for the postharvest firmness behaviour as function of (storage) time and the maturity at harvest (Eq. 5).

$$F = F_{max} - \frac{F_{max} - F_{min}}{1 + \frac{F_{ref} - F_{min}}{F_{max} - F_{ref}} e^{-k \cdot (t - \Delta t) \cdot (F_{max} - F_{min})}} \quad \text{(Eq. 5)}$$

Eq. 5 allows the variation encountered in initial firmness values to be described as variation in maturity (Schouten et al., 2004, Hertog et al., 2004b). This approach is increasingly applied in postharvest quality modelling and was reviewed by Hertog et al. (2007).

2.6. Statistical analysis

Two-way analysis of variance (ANOVA) was carried out to determine the effects of harvest month and greenhouse type on initial weight, initial firmness, and initial sugar and acid levels (version 20, SPSS, USA). The model equations (Eq. 3-5) were developed using Maple V (release 12, Waterloo Maple Software, Canada). Firmness data over time were analysed using an indexed version of the nls (non-linear regression) procedure of R (R Development Core Team, 2012). This adapted version estimates model parameters as function of indexed levels and is available on request. This allows parameters to be estimated in common, per treatment, preharvest month or for every individual tomato. Starting values for the parameters were obtained by applying the standard nls procedure using averaged firmness data.

3. Results and Discussion

3.1. Growth conditions

Temperature and CO_2 data are displayed as averages for the six weeks preceding each of the five harvests (left hand side of Fig. 1) with additional climate data available as supplementary Fig 1. During the whole growing period the average temperature was 0.5 to 1 °C higher for the open greenhouse compared to the two semi-closed greenhouses. The cooling strategies applied to the two semi-closed greenhouses caused a vertical temperature distribution for SC_below (right hand side of Fig. 1) with up to 5 °C difference between top and bottom of the greenhouse during the middle of a sunny day. CO_2 levels in the semi-closed greenhouses were higher

compared to the open greenhouse and the difference increased over the growing season to more than 100 ppm.

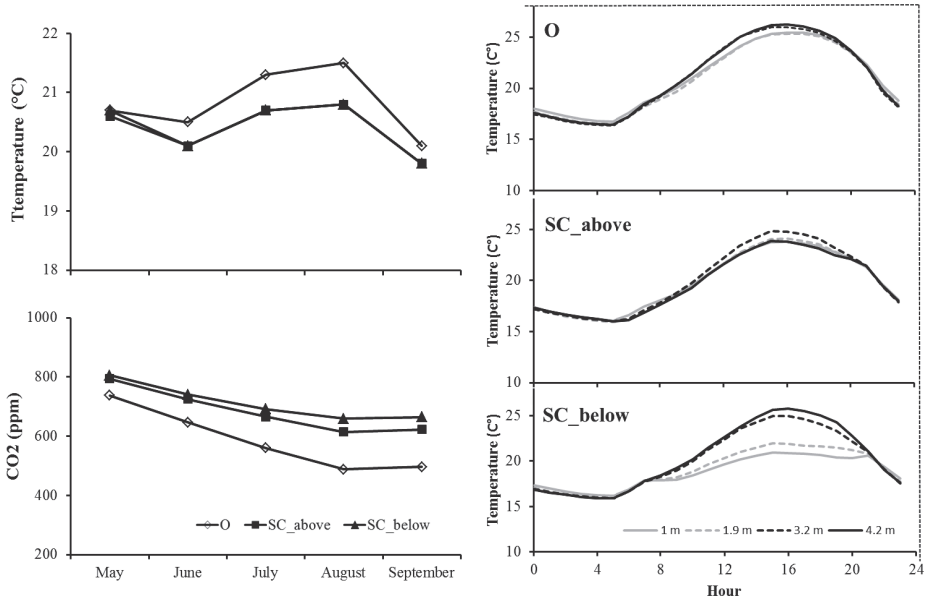

Figure 1. Average growing conditions during six weeks preceding each harvest for the three greenhouses are shown on the left hand side. The right hand side plot shows the hourly vertical temperature gradient between May and July measured at different heights in each greenhouse type.

3.2. Weight at harvest

Fruit from the semi closed greenhouses showed, on average, a higher weight at harvest (SC_above ±11% and SC_below ±15%) compared to the open greenhouse (upper panel of Fig. 2). This difference between the semi-closed and open greenhouses was largely explained by the difference in CO_2 levels (Qian et al., 2011). Islam et al. (1996) found that raising the CO_2 level from 350 to 850 ppm increased the tomato weight up to 30% for two out of three cultivars. The open greenhouse showed the highest temperature during the whole growing period (Fig. 1). The differences in growing conditions between the semi-closed greenhouses are small, especially in May, June and July. The higher fruit weight for fruits from SC_below (upper panel of Figure 2) is therefore likely related to the vertical temperature gradient (Fig. 1) since the temperature in the lower part of the canopy in the later stages of fruit development was lower. Weight loss was monitored during storage. No differences in percentage weight loss over time were observed as function of month or greenhouse type (data not shown).

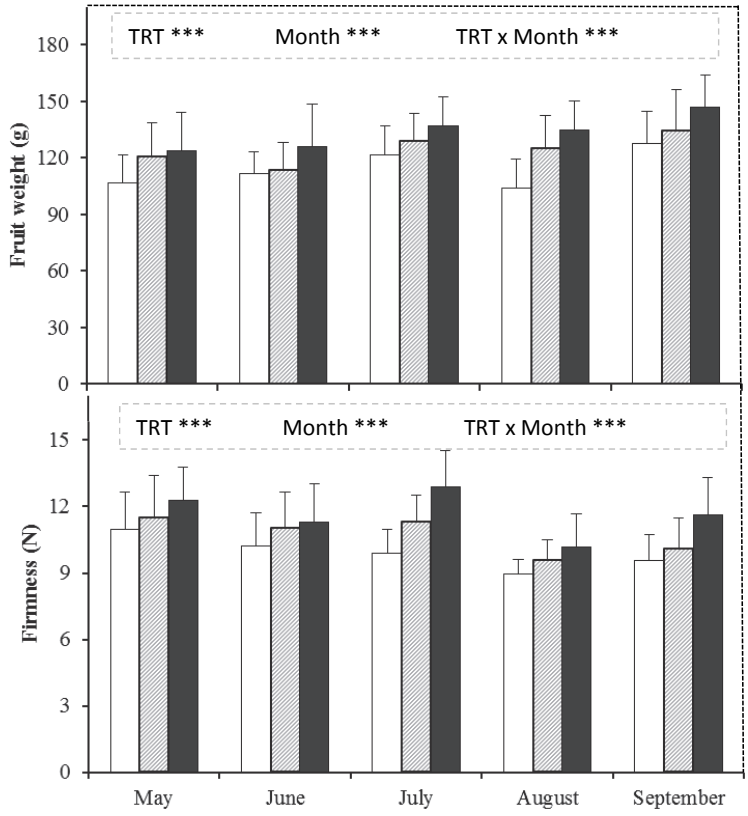

Figure 2. Average fruit weight and firmness at harvest with indicated standard deviation for tomatoes (N= 45) grown in different types of greenhouses (O: □, SC_above: ▨ and SC_below: ■). Level of significance ($p < 0.001$) is represented by ***.

3.3. Analysis of firmness behaviour

Apart from being bigger, fruit from the semi-closed greenhouses were, on average, significantly firmer than fruit from the open greenhouse with fruits from SC_below being the most firm (lower panel of Fig. 2). No relation was found between weight and firmness at harvest for tomatoes from different greenhouses (data not shown).

Softening exhibits an exponential nature, for instance in June and September, whereas it is more linear in May and August (Fig. 3). All tomatoes seem to have the same firmness end value (F_{min}). The firmness model was calibrated using all available firmness data in two steps. The average initial firmness value for all tomatoes was used for F_{ref}. At first, F_{min} and F_{max} were fixed at plausible values and k and Δt were estimated in common (k) or per individual tomato (Δt). The second step used the estimates from the first step as starting values and estimated, next to k (in common) and Δt (per individual tomato), also F_{min} (in common) and F_{max} (per month). Parameter estimates are presented in Table 1. The standard errors of the estimated

parameters are low, mostly around 10-15% and the plots showing the measured and simulated data look well (Fig. 3). Finally, it was tested whether F_{max} varied both per month and greenhouse type, but no indication was found that greenhouse type affects F_{max}. This indicates that the differences in firmness behaviour between greenhouse types in one month can be allocated to maturity differences (see below).

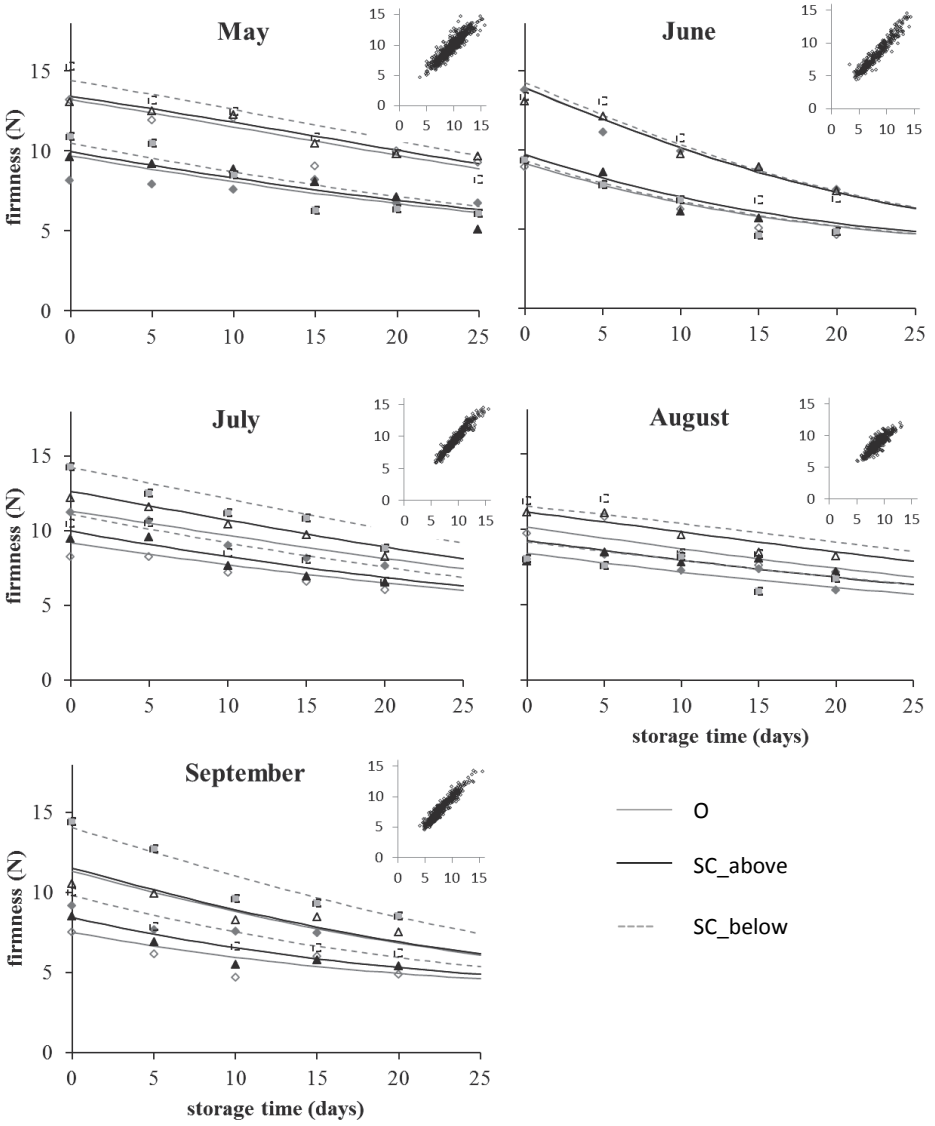

Figure 3. Simulated (lines) and measured (points) firmness change for the 10% softest and the 10% firmest tomato for each of the five harvest months and for each greenhouse type. Inlays in each plot show the measured and simulated firmness values (in N) per month.

Table 1. Overview of parameter estimates and their standard error of estimates (S.E.) or standard deviation (stdev) for the firmness model.

Parameter	in common		May		June		July		August		September	
	Estimate	S.E.(%)	Estimate	S.E.(%)	Estimate	S.E.(%)	Estimate	S.E.(%)	Estimate	S.E.(%)	Estimate	S.E.(%)
Kinetic parameters												
k (1/day)	0.00250	27.0										
F_{max} (N)			21.3	11.4	31.2	14.6	23.5	12.5	17.9	10.6	27.5	13.8
F_{min} (N)	3.538	10.5										
Batch parameters			Estimate	stdev	Estimate	stdev	Estimate	stdev	Estimate	stdev	Estimate	stdev
Δt (day) O			-3.1	8.1	0.2	4.6	1.2	4.4	10.3	4.6	3.9	4.8
Δt (day) SC_above			-6.9	8.1	-2.2	5.1	-4.5	4.3	4.6	6.0	2.1	4.7
Δt (day) SC_below			-8.7	6.3	-3.5	4.5	-8.9	4.4	1.5	8.2	-2.5	5.4
Miscellaneous param												
F_{ref} (N)	10.77											
N	2188											
R^2 (%)	86.5											

3.4. Effects of growing conditions on maturity

Although the firmness behaviour over time for different greenhouse types and harvest months could be described using one common value for k and F_{min}, other parameters, such as F_{max} and Δt, varied substantially (Table 1). Greenhouse type affects Δt with the most mature (softest) tomatoes encountered in O and the least mature (firmest) tomatoes in SC_below as indicated by positive and negative values for Δt, respectively. This difference in firmness maturity of about seven days at the same colour stage, regardless of harvest month (Fig. 4), is commercially important. Schouten et al. (2007a) showed that the firmness and colour maturity are synchronised per greenhouse, and that differences in growing conditions mainly affect firmness.

Within one greenhouse type the most mature fruit at harvest are found in August and the least mature in May (Fig. 5). This coincides with relatively low levels of CO_2 and relatively high greenhouse temperatures in August and vice versa in May (Fig 1). When the temperature set points in greenhouses are exceeded, as often happened in August, windows automatically opened resulting in lower CO_2 levels. Hurd and Graves (1984, 1985) found that the time taken for tomato fruit to mature decreased in response to higher mean air temperatures. CO_2 enrichment did not affect firmness of tomatoes, but did result in increased colouring (Islam et al., 1996). This implies that tomatoes would be firmer when harvested at the same colour stage. In other words, CO_2 might affect the synchronisation between colour and firmness maturity. This means that both lower temperature and higher CO_2 levels might give rise to firmer tomatoes (and vice versa) at the same colour stage. The acceptance period, the time period both colour and firmness are considered acceptable by consumers (Schouten et al., 2007b), will therefore likely be positively affected when growers switch from conventionally ventilated to semi-closed production systems.

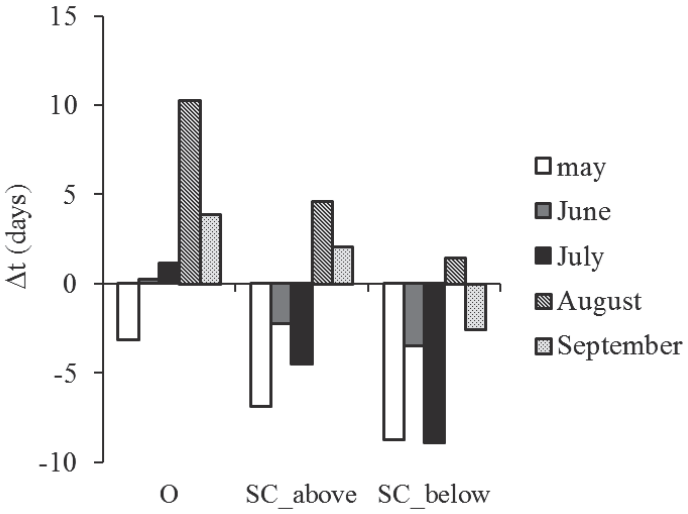

Figure 4. Estimated values for the maturity (Δt) expressed per harvest month and per greenhouse type.

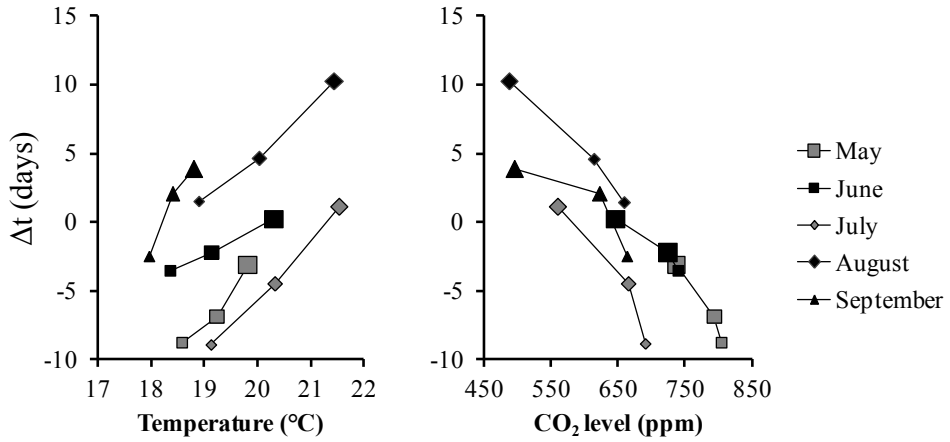

Figure 5. Effect of temperature and CO_2 levels during the last two weeks preceding harvest on maturity (Δt) expressed per month and per greenhouse type (biggest font size: O, intermediate font size: SC_above and smallest point font size: SC_below).

3.5. Effects of growing conditions on sugar and acid levels

Generally, sugar and acid levels at harvest were slightly higher in the open greenhouse (Fig. 6). Gautier et al. (2008) found that increasing the greenhouse temperature from 21 to 26 °C did not affect hexose levels but reduced the titratable acidity by 25%. Islam et al. (1996) reported higher glucose and fructose levels due to an increase in CO_2 level from 350 to 850 ppm. The higher temperatures and lower CO_2 levels in the open greenhouse (Fig. 1) should therefore result in lower acid and sugar levels compared to the other greenhouse types, although the opposite was found here. Total soluble solids of tomatoes at harvest followed the same trend observed for total sugars and were within 1% between greenhouse types (data not shown). 1% is about the difference that consumer panels can sense (Harker et al., 2002). So, the observed differences in sugars and acid levels between the different greenhouses are likely not noticeable by consumers.

The trends observed in sugars levels at harvest as a function of harvest month were similar to those found in acids levels (Fig. 6). Sugar levels in glasshouse-grown tomatoes increased with increasing irradiance during the growing season. In July the sugar to acid ratio rose to 9.8 (± 0.3) compared to, on average, 8.6 (± 0.6) for all other months. This might be connected to an increased sucrose synthase activity during periods of high temperature and radiation that raised the hexose levels in cherry tomatoes (Rosales et al., 2007).

Relatively small changes in sugar and acid levels were observed during storage (left hand side column of Fig. 7). This picture can be fine-tuned when sugars and acids are expressed as the ratio of the main sugars (glucose/fructose) and the main acids (citrate/malate) (right hand side column of Fig. 7). In May, the glucose to fructose ratio starts relatively high with a slow decrease over time, while this is the opposite in July. Although the starting point is about the same in May and July for the citrate to malate ratio, this ratio increases faster in May. Fructose is considerable sweeter than glucose (Mathlouthy, 1984; Mahawanich and Schmidt, 2004) and malate is considered by consumer panels to be more acid than citrate (Sowalsky and Noble,

1998; Noble et al., 1986). This means that the relative quick loss of glucose in July has only minor effects on sweetness. Also minor effects of the increase in citrate in July are expected. So, although differences in the sugar and acid metabolism are observed, both greenhouse type and harvest month are likely not affecting the gustative quality of stored tomatoes. Gautier et al. (2008) also found that reducing sugars and titratable acidity of stored tomatoes were hardly affected by temperature and light treatments.

Variation in total sugars and acids at harvest was mainly observed over harvest month (Fig. 6). Variation in F_{max}, the maximum asymptote estimated on the basis of firmness measurements over time, was also mainly observed over harvest month (Table 1).

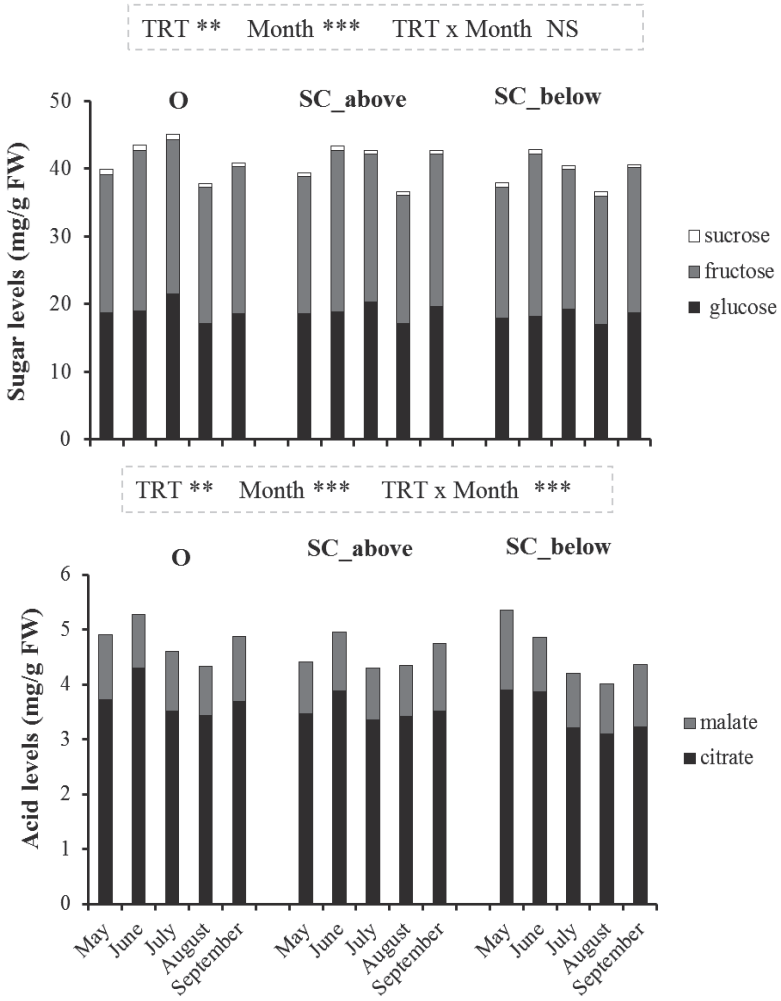

Figure 6. Sugar and acid levels at harvest as function of greenhouse type and harvest month. Levels of significance ($p < 0.01$ and $p < 0.001$) are represented by ** and ***, respectively.

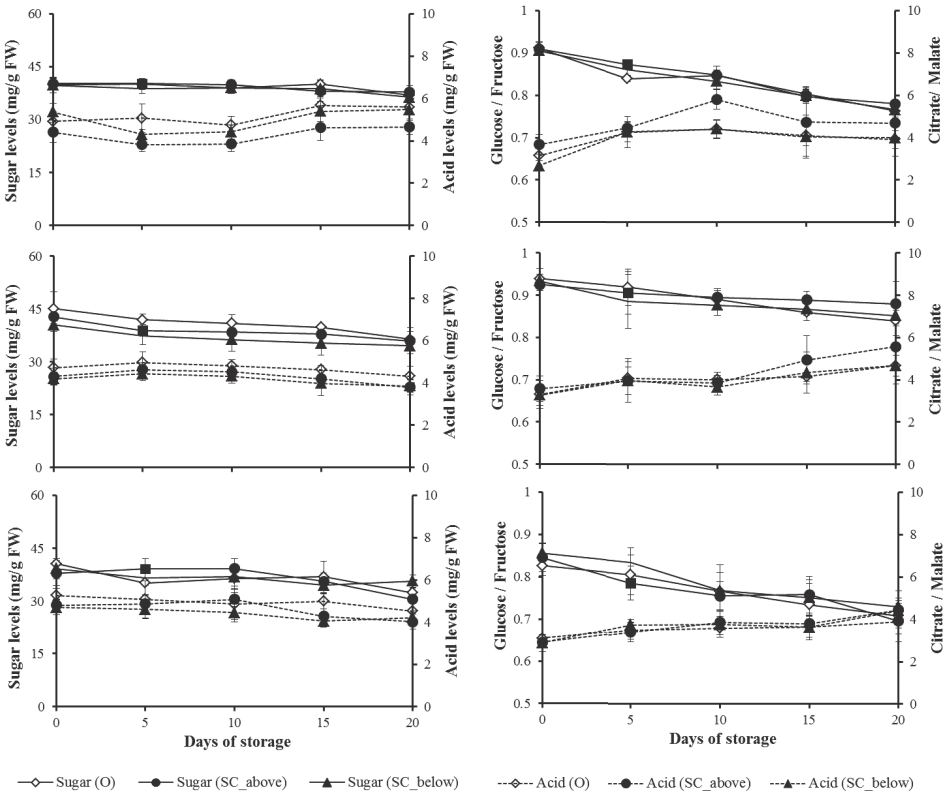

Figure 7. Left hand side plots show the total sugar and acid levels of tomatoes from different greenhouses, including standard deviations, over time in May (upper plot), July (middle plot) and September (lower plot). Right hand side plots show corresponding glucose/fructose and citrate/malate ratios.

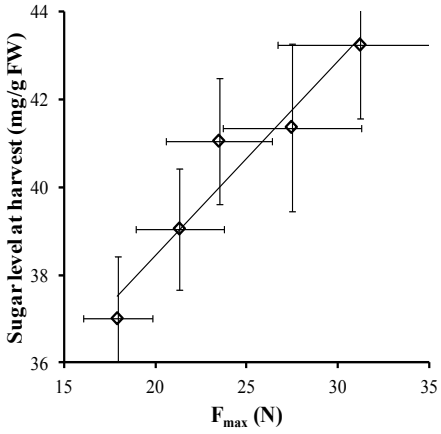

Figure 8. Estimated values for F_{max} per month with indicated standard errors of estimation (Table 1) as function of the measured initial total sugar levels with indicated standard deviation.

Intriguingly, there is a linear relation between the estimated values for F_{max} and the measured total sugar level at harvest (Fig. 8). Tomato firmness can be considered to be generated by different sources, amongst which structural components are probably the most important (Van Dijk and Tijskens, 2000). Additional to effects of structural components, also turgor (or the related tissue tension) is a source of firmness as can be observed through the reversible effects of temperature on apple texture (Johnston et al., 2001). Hertog et al. (2004a) showed that the rate of firmness loss of tomatoes using an invasive technique depended only on temperature while using a non-invasive technique this varied both with temperature and the water vapour pressure deficit. It might be hypothesized that the maximum firmness for full sized tomatoes is reached when the maximum amount of soluble sugars is available and no net cell wall breakdown occurs due to hydrolases as at that moment the maximum turgor generated firmness is achieved. In other words, the monthly variation in glucose and fructose levels might cause variation in turgor generated firmness. No clear relations between monthly variation in sugar levels and growing conditions were found in this study. This means that the large variation in F_{max} observed over harvest month remains elusive for now.

4. Acknowledgements

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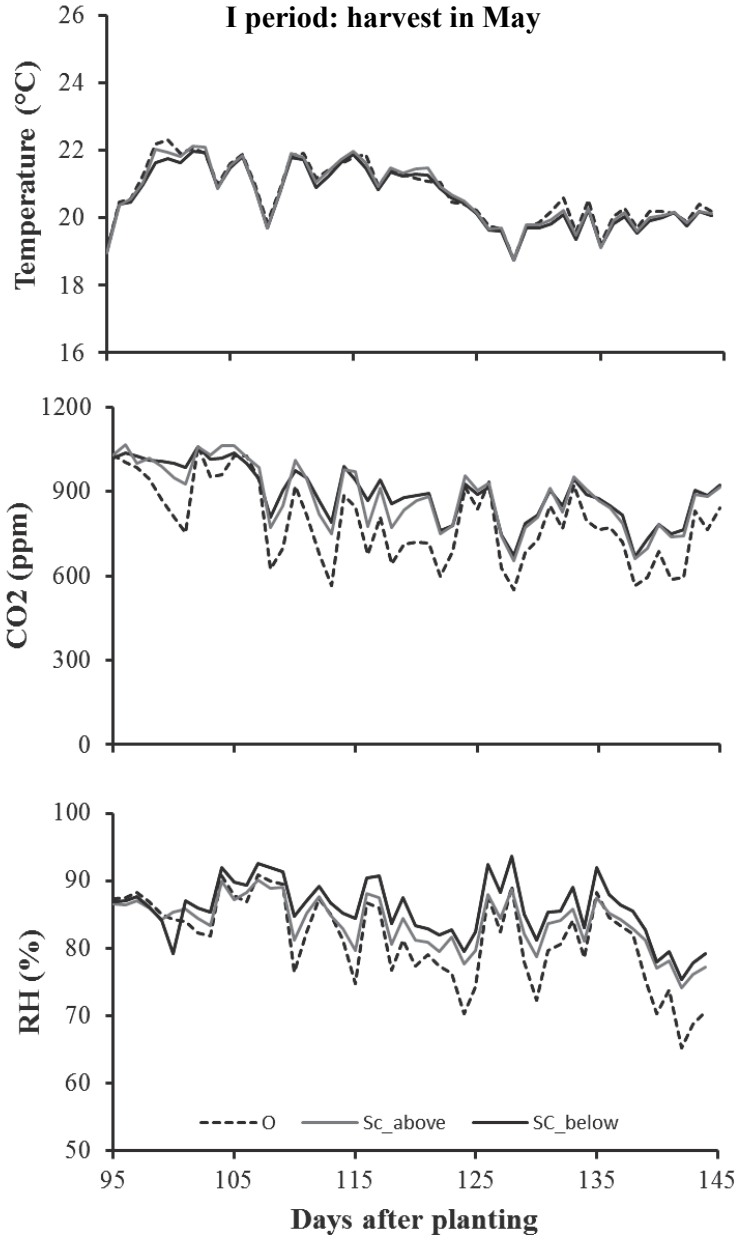
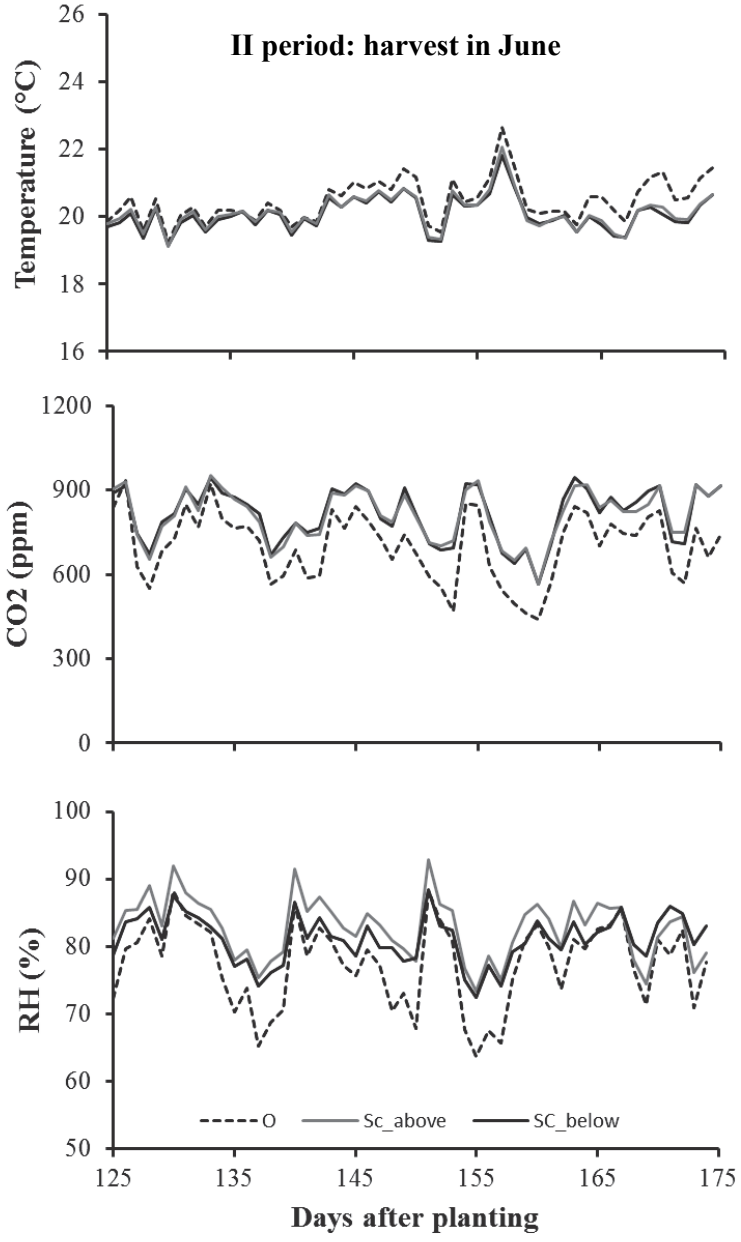
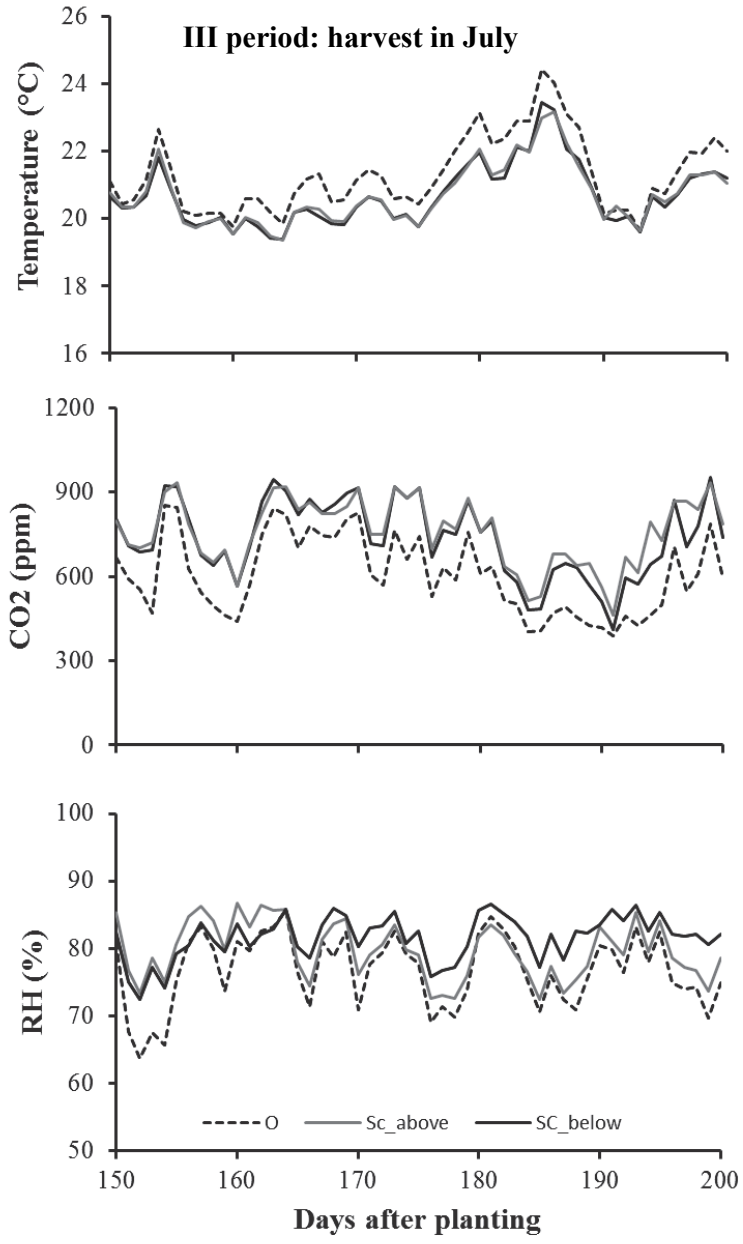
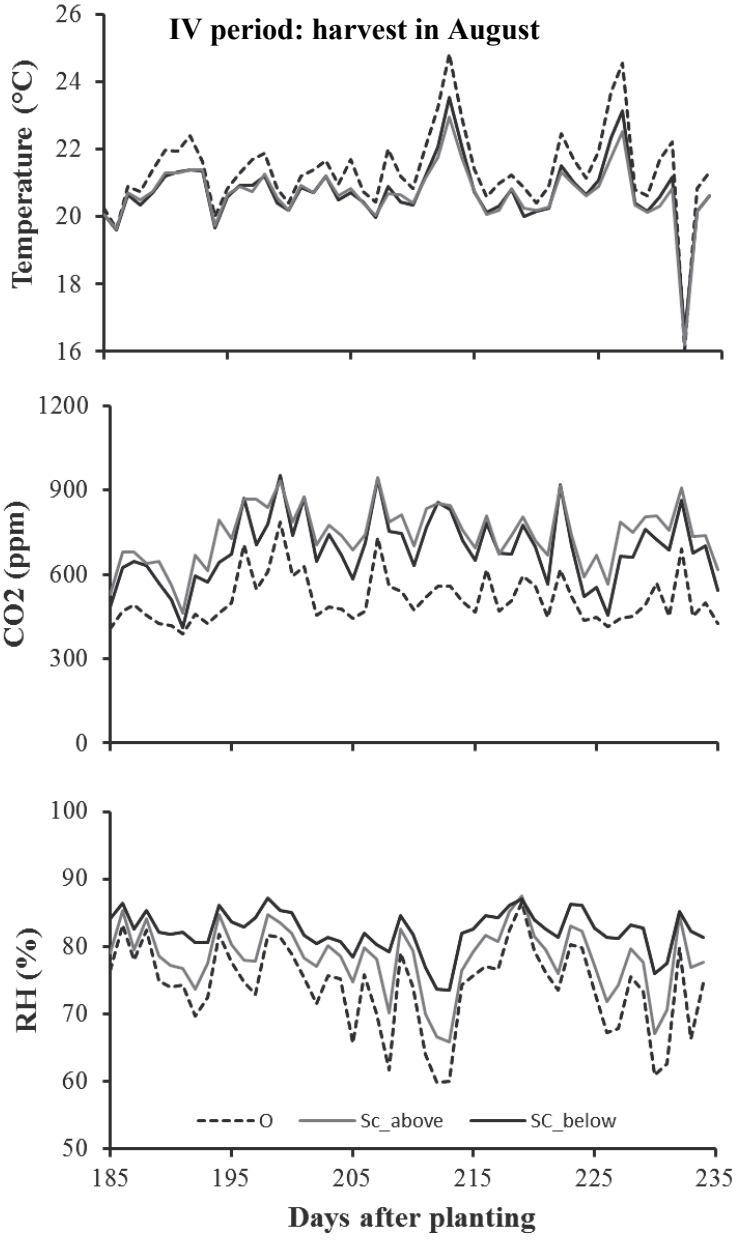
5. Supplementary material

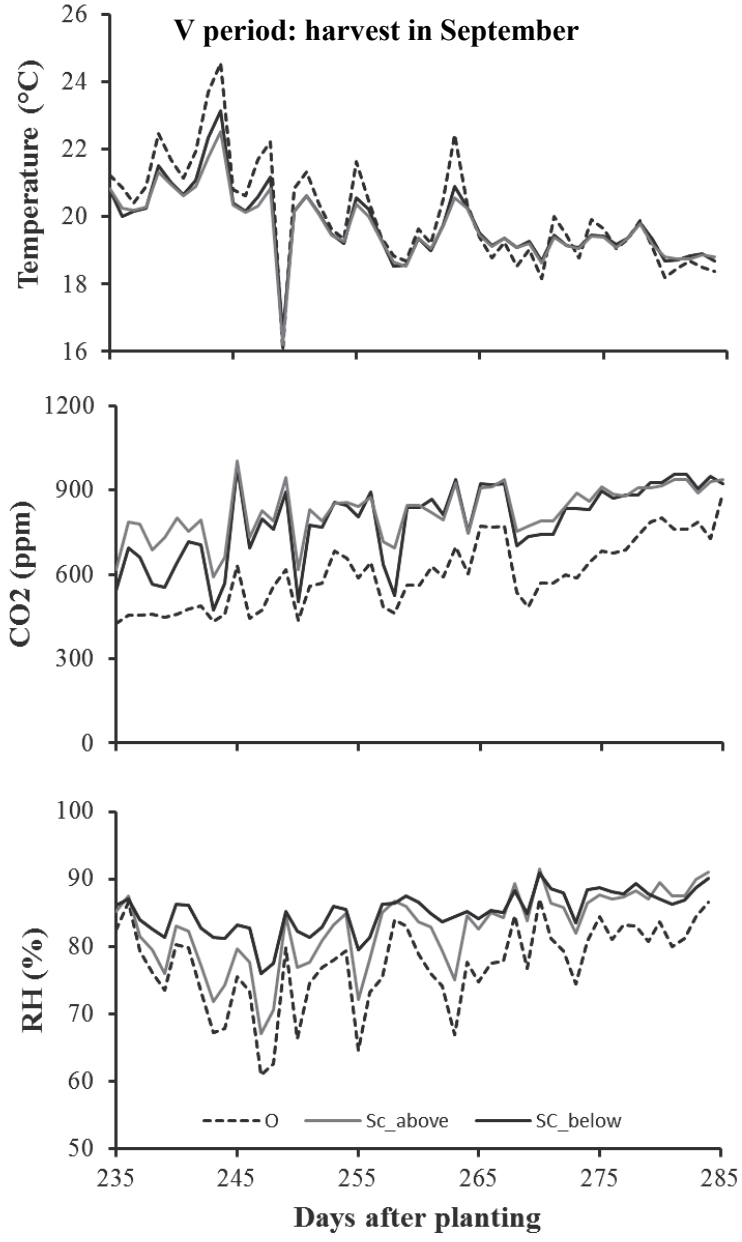
Supplemental Figure 1: Daily growing conditions during six weeks preceding each harvest for the three greenhouses for tomatoes harvested monthly between May and September 2008.




II period: harvest in June
Temperature (°C)
26
24
22
20
18
16
CO2 (ppm)
1200
900
600
300
0
RH (%)
100
90
80
70
60
50
O
Sc_above
SC_below
125
135
145
155
165
175
Days after planting


III period: harvest in July
Temperature (°C)
26
24
22
20
18
16
CO2 (ppm)
1200
900
600
300
0
RH (%)
100
90
80
70
60
50
O
Sc_above
SC_below
150
160
170
180
190
200
Days after planting


IV period: harvest in August
Temperature (°C)
26
24
22
20
18
16
CO2 (ppm)
1200
900
600
300
0
RH (%)
100
90
80
70
60
50
O
Sc_above
SC_below
185
195
205
215
225
235
Days after planting


V period: harvest in September
Temperature (°C)
26
24
22
20
18
16
CO2 (ppm)
1200
900
600
300
0
RH (%)
100
90
80
70
60
50
O
Sc_above
SC_below
235
245
255
265
275
285
Days after planting

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Chapter 4

Assessing aroma volatiles in tomato fruit by proton transfer reaction mass spectrometry (PTR-MS)

Chapter 4.1

Rapid tomato volatile profiling by using proton transfer reaction mass spectrometry (PTR-MS)

Abstract

The availability of rapid and accurate methods to assess fruit flavour is of utmost importance to support quality control especially in the breeding phase. Breeders need more information and analytical tools to facilitate selection for complex multigenic traits such as flavour quality. In this study, it is shown that proton-transfer reaction-mass spectrometry (PTR-MS) is a suitable method to monitor at high sensitivity the emission of volatiles determining the tomato aromatic profile such as hexanal, hexenals, methanol, ethanol and acetaldehyde.

The volatiles emitted by fourteen tomato varieties (at red stage) were analysed by two solvent-free headspace methods: solid-phase micro extraction/gas chromatography-mass spectrometry (SPME/GC-MS) and PTR-MS. Multivariate statistics (PCA and Cluster analysis) of the PTR-MS results allow an unambiguous separation between varieties, especially with a clear fingerprinting separation between the different tomato types: round truss, cocktail and cherry tomatoes. PTR-MS was also successfully used to monitor the changes in volatile profiles during postharvest ripening and storage.

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1. Introduction

Nowadays consumers have become more aware of food quality and, therefore, they demand a certain quality level of the products they buy. Quality of a fresh product includes sensory properties such as colour, firmness, flavour, nutritive values, chemical constituents and possible damage or defects (Abbott, 1999). Although all these intrinsic characteristics are encompassed in the definition of quality, for many years most breeding efforts have been mainly directed to improve and maintain external qualities. Selection for yield, fruit size, colour and shelf life characteristics might have had unintended negative consequences on fruit flavour (Goff and Klee, 2006).

The maturity stage at harvest is the second most important factor, after genotype, influencing flavour quality of fruits and vegetables. Taste is generally better when products are harvested fully ripe since sugar/acid ratio and the synthesis of aroma volatiles increases with maturation. Therefore, an additional challenge for breeders is optimizing fruit flavour upon delivery to the consumer, selecting fruit varieties that maintain or enhance flavour during storage and marketing. Generally, the longer the time between harvest and fruit consumption, the greater the losses of characteristic aroma volatiles. In addition, off-flavour s may develop during long-term storage (Kader et al., 1978; Pelayo-Zaldivar et al., 2005).

Tomato, apart from being one of the most consumed fruit, scientifically is a model for fruit development and ripening studies. More is known about the chemical pathways contributing to flavour in tomato than in any other fruit or vegetable. The most abundant volatiles in tomato fruits are derived from lipids through the oxylipin pathway (Baldwin et al., 2000; Buttery and Ling 1993; Reineccius 1991). These volatiles, associated with flavour s described as “tomato”, “green” or “grassy”, are derived from linoleic acid (hexanal) and linolenic acid (cis-3-hexenal, cis-3-hexenol, trans-2-hexenal) via lipoxygenase activity (Chen et al., 2004). The six-carbon aldehydes and alcohols derived from free fatty acids are also important constituents of the flavour s of a diverse group of plant products including apple, sweet cherry, olive, bay leaf, and tea.

Other important compounds that positively contribute to the tomato aroma are derived from the essential amino acids leucine, isoleucine and phenylalanine (Buttery and Ling, 1993). These volatiles, such as phenyl acetaldehyde, 2-phenylethanol, methyl salicylate and 2- and 3-methylbutanal are also important flavour constituents of many other fruits, including strawberries and apples.

A third class of tomato volatiles, the apocarotenoids (eg. β-ionone, geranylacetone and 6-methyl-5-hepten-2-one), is derived from oxidative cleavage of carotenoids (Goff and Klee, 2006). The specific association of these volatiles with ripe fruits and their relative absence from vegetative tissues suggests a role in signalling ripeness and attracting seed-dispersing organisms, including humans. Tomato aroma can thus be viewed as a set of cues that together reflect the ripeness and nutritional quality/nutrient availability of the fruit (Goff and Klee, 2006).

Therefore, the availability of rapid and accurate methods to assess these important characteristics in various phases of the production process is of utmost importance to support breeding programs, product development or quality control measures.

Generally, gas chromatography (GC) based methods are too time consuming, complex, labor-intensive, and not suited for routine field or on-line measurements. Alternative techniques to the gas chromatography - mass spectrometry (GC-MS), such as the headspace fingerprint mass spectrometry (HFMS) or the electronic nose system (E-nose), have been successfully applied for pre and postharvest profiling of tomatoes and apples (Berna et al., 2004; Berna et al., 2005; Saevels et al., 2004). E-noses, containing a number of electro chemical sensors, are useful only for discriminating one sample from another based on the volatile profile but not for compound identification/quantification (Maul et al., 1997; Maul et al., 1998); moreover the conventional electron impact ionization with consecutive mass spectrometric ion detection, such as HFMS, suffers from the strong fragmentation of organic compounds that makes results more complex to be analysed.

As an alternative, the less compound fragmentation caused by chemical ionization techniques, such as proton-transfer reaction mass spectrometry (PTR-MS), may facilitate the on-line monitoring of specific compounds in a gas mixture (Lindinger et al., 1998). Some characteristics of PTR-MS, such as the direct inlet of head space mixtures without any chemical extraction, the speed of data collection and the detection of trace compounds, allow for a rapid screening of the product headspace both for a fast aroma fingerprinting and for chemical identification (Aprea et al., 2009; Boamfa et al., 2004; Boscaini et al., 2004; Boschetti et al., 1999; Granitto et al., 2007; van Ruth et al., 2004; van Ruth et al., 2005).

The objective of the present work was to develop a fast, accurate and reliable method, based on the PTR-MS technique, for tomato volatile analysis to be applied in different phases of the production chain from breeding to postharvest quality management.

2. Materials and methods

2.1. Plant materials

Tomatoes (Solanum lycopersicum) were obtained in summer from a demo-greenhouse in the south east of the Netherlands. All cultivars were grown in the same greenhouse under identical conditions. We screened fourteen cultivars of different fruit type: eight “round truss” (Arvento RZ, Caracas RZ, Cappricia RZ, Roterno RZ, Tomala RZ, Varianto RZ, Tom 1 and Tom 2), three “cocktail” (Amoroso RZ, Brioso RZ and Delioso RZ) and three “cherry” tomatoes (Cheramy RZ, Sassari RZ and Tastery RZ). We selected homogenous batches of tomatoes for each cultivar comparing fruits with a colour card from “The Greenery”, a cooperation of Dutch fruit and vegetable auctions. This card shows predefined colour stages for tomatoes, ranging from 1 (dark green) to 12 (dark red). We ranked tomatoes according to the colour as green (colour card value 1-3), breaker (4-5), orange (6-7) or red (8-12) (Schouten et al., 2007).

Following harvest, batches of red tomatoes were stored for two days at 16°C and then analysed by GC and PTR-MS on two consecutive days to take into account the possible instrument variability. The remaining fruits (breaker and orange) of the cvs Amoroso RZ and Cappricia RZ were also analysed to test the capability of PTR-MS to distinguish the different ripening stages of fruit of the same cultivar.

To monitor the changing volatile profiles during storage by using PTR-MS, red tomato fruits of the cvs Amoroso RZ and Cappricia RZ were stored for 10 days at 4°C and 16°C, respectively, prior to measurement with PTR-MS.

2.2. Head space analysis by SPME/GC-MS

Before analysis, samples of fresh tomatoes (five fruits of each cultivar) were quickly cut into quarters and immediately frozen into liquid nitrogen. Prior to analysis, the samples were stored at -80°C and ground in liquid nitrogen in a metal electric grinder.

The profiling of volatiles was performed using a modification of the headspace solid-phase microextraction gas chromatography (SPME/GC-MS) method of Tikunov et al., (2005). Frozen fruit powder (1 g fresh weight) was weighed into a 20 mL crimp cap vial, closed, and incubated at 30°C for 10 min. The closed vials were then sonificated for 5 min. Once the samples were incubated at 60°C with agitation for 10 min, the headspace volatiles were extracted from the vial headspace and injected into the GC-MS apparatus (Trace GC Ultra, Thermo, IL, USA) equipped with a TriPlus SPME autosampler (Thermo, IL, USA). The extraction was done by exposing a Carboxen/PDMS SPME fiber (Supelco, Zwijndrecht, Netherlands) to the vial headspace for 20 min under continuous agitation and heating at 60°C. The fiber was desorbed for 15 min at 250°C in the injection port of the GC in splitless mode. Chromatography was performed on RTX-WAX (Restek, Bellefonte, PA) capillary column (60 m length x 0.32 mm i.d., 0.25 μm film thickness) with helium as a carrier gas. The GC interface and MS (DSQ II, Thermo, IL, USA) source temperature were both 250°C. The GC temperature program started at 40°C (5 min), was then raised to 240°C at a rate of 5°C per min, and finally was held at 240°C for 10 min. The total run time including oven cooling was 60 min. In preliminary runs the mass spectra in the 35 to 400 m/z range (scanning speed of 2.8 scan s-1 and ionization energy of 70 eV) was recorded in order to verify the method, check the standards (mass spectra and retention time) and define the volatiles to be analysed.

In further measurements, the headspace volatiles of the samples were analysed recording a limited number of selected masses (dwell time of 10 ms) (table 1) producing better resolution and less noisy chromatograms. Compound identification was based on mass spectra matching in the standard NIST library and retention time of authentic reference standards.

Since SPME is a semi quantitative procedure to analyse volatiles, each sample was spiked with a fixed amount of internal standard (acetonitrile) and each studied compound calibrated using the same chromatographic conditions.

Reference standards of volatile compounds studied (hexanal, E-2-hexenal, Z-3-hexenal, Z-2-heptenal, 2-octenal, pentanal, nonanal, geranial, 1-penten-3-one, 6-methyl-5-hepten-2-one, geranylacetone, β-ionone, 1-nitropentane, 2-isobutylthiazole, geraniol) were supplied by Supelco (Sigma-Aldrich and Fluka; Zwijndrecht, Netherlands) as pure compounds (92-99.5%).

Table 1. Mass Spectrometer configuration for limited mass selection gas chromatographic analysis.

Time (min)	Masses selected
0-10	27, 39, 41, 42, 43, 44, 45, 46, 55, 56, 57, 58, 61, 70, 81, 84, 93
10-15	27, 39, 40, 41, 43, 44, 55, 56, 57, 69, 70, 71, 83, 84, 91, 92, 93
15-20	39, 41, 43, 55, 67, 68, 69, 70, 72, 83, 91, 93, 99, 100, 119, 121, 134, 136
20-25	41, 43, 54, 55, 57, 61, 67, 69, 70, 71, 81, 82, 83, 93, 96, 99, 108, 110, 117, 126, 132
25-30	41, 43, 55, 56, 57, 58, 59, 68, 69, 70, 71, 81, 91, 92, 93, 95, 100, 119, 120, 121, 122, 133, 152
30-40	30, 43, 57, 60, 68, 69, 73, 91, 92, 99, 100, 104, 105, 107, 119, 120, 121, 122, 127, 128, 132, 152, 177
40-end of run	Full scan (25-300 mass range)

2.3. Proton Transfer Reaction mass spectrometer (PTR-MS)

The head space volatiles of intact fruits and of fruits cut in half on the equator (halved) were measured using a proton-transfer-reaction mass spectrometer (PTR-MS) similar to the one described by Boamfa et al., (2004). This PTR-MS consists of: (i) an ion source in which H3O+ ions are produced, (ii) a drift tube where the trace gases are ionized by proton-transfer reaction with H3O+ ions, (iii) a collision dissociation chamber, (iv) a quadruple mass filter and (v) a secondary electron multiplier. (For details, see Boamfa et al., 2004). The drift tube had a pressure of 2.08 mbar and was heated at about 55°C. The mass number of the detected ion is given by the molecular mass of the substance plus the mass of the single proton mH (mRH+ in atomic mass units, amu). Mass spectrometric data were collected over a mass range of m/z 21-140.

Based on preliminary experiments with tomato fruits, we removed the calyx and cleaned the fruits with tap water prior to measurement to avoid possible volatile contamination. Considering the differences in dimension between the three tomato types (average weight: round truss tomato, 110 g; cocktail tomato, 35 g; cherry tomato, 18 g) it was chosen to use one, three and five fruits, respectively, for the analysis.

Tomatoes (intact or halved) were put in a sealed glass vessel (500 mL) and incubated for 15 min at room temperature. To avoid possible cross contamination from one measurement to the next, the apparatus was flushed with clean air between consecutive measurements, and a different glass vessel for each sample was used.

All cultivars were measured in four independent replicates (for both intact and halved tomato), each one measured three times.

In order to increase the sensitivity and the speed of analysis, after several preliminary tests and the screening of ripe tomatoes, 21 masses considered distinctive for tomato aroma were selected.

2.4. Data analysis

The multivariate statistical software Canoco 4.5 (Biometris-Plant Research International, Wageningen) was used for principal component analysis (PCA): both GC and PTR-MS data were LOG transformed prior to the analysis.

Cluster analysis of LOG transformed data was computed by the software MVSP 3.2 (Kovach Computing Service) using the Ward's minimum-variance method.

3. Results and Discussion

3.1. Tomato volatile fingerprinting by SPME/GC-MS

The GC-MS analysis allowed for the identification and quantification of 15 volatile compounds released by fruits of all tomato varieties (table 2). The restrictive choice of only these compounds was based on literature information that consider these volatiles fundamental for the overall tomato aroma (Buttery et al., 1987; Buttery et al., 1989; Krumbein and Auerswald, 1998; Krumbein, Peters et al., 2004; Tandon et al., 2000) since they are usually present in concentrations above their odor thresholds.

In this experiment it was confirmed that aldehydes are the most abundant volatiles in tomato especially the ones derived from lipoxygenase activity: hexanal and hexenals (cis-3-hexenal, trans-2-hexenal) amount is about 85% of the total volatile profile. Our data confirm earlier reports (Gray et al., 1999) that the hexanal: hexenals ratio varies in different tomato types: 8.3, 0.9, and 0.5, for round truss, cocktail and cherry tomato, respectively.

Because SPME analysis is semi quantitative and selective with respect to compound polarity, we cannot have an absolute value of the total headspace volatiles. Volatiles measured using a SPME fiber do not necessarily reflect the actual head space composition of tomato, due to discrimination of volatiles on basis of partition coefficients and adsorption kinetics (Berna et al., 2004). For instance, some relevant compounds such as methanol, ethanol and acetaldehyde that are highly present in tomato fruits were not detected. Principal component analysis (PCA) and cluster analysis (Figure 1) were carried out to describe relations between tomato type and volatile compounds as well to reduce the data set to important components. Proximately 90% of the total variance was accounted for by the first two components. Differences between round truss tomato and cocktail and cherry ones are mainly explainable by the first principle component (79.8%). Round truss tomato, except for the cv. Caracas RZ, were clearly cluster separated from the other tomato types.

According to the PCA analysis, also 6-methyl-5-hepten-2-one and two nitrogen-containing compounds (1-nitropentane and 2-isobutylthiazole) importantly contribute to the difference in volatile profile of these tomato types. These nitro compounds are unusual in any other food than tomatoes (Buttery et al., 1987).

Table 2. Abundance of aroma volatile compounds in headspace of frozen and ground red tomato samples using SPME/GC-MS analysis technique.

Peak	Volatile compounds	Round truss tomato[a]		Cocktail tomato[b]		Cherry tomato[c]	
		mean (µg/g)	range (µg/g)	mean (µg/g)	range (µg/g)	mean (µg/g)	range (µg/g)
	Aldehydes						
1	Pentanal	2.8	*(2.5-2.8)*	2.8	*(2.7-2.8)*	2.8	*(2.7-2.9)*
3	Hexanal	208.3	*(78.8-359.2)*	122.6	*(92.5-177.8)*	96.4	*(90.6-105.3)*
4	(Z)-3-Hexenal	2.1	*(1.4-2.9)*	1.6	*(1.4-1.7)*	1.4	*(1.3-1.5)*
5	(E)-2-Hexenal	23.8	*(9.9-78.4)*	137.8	*(121.6-150.6)*	220.4	*(110.2-384.9)*
6	(Z)-2-Heptenal	3.3	*(2.9-3.9)*	3.3	*(3.2-3.3)*	3.2	*(3.0-3.4)*
9	2-Octenal	3.3	*(2.9-4.2)*	3.6	*(3.4-3.8)*	3.6	*(2.8-4.1)*
10	Nonanal	2.8	*(2.6-2.9)*	2.8	*(2.7-2.9)*	2.7	*(2.6-2.8)*
12	Geranial	1	*(0.8-1.1)*	1.1	*(0.9-1.1)*	1.2	*(1.1-1.2)*
	Ketones						
2	1-Penten-3-one	15	*(13.9-16.7)*	14.9	*(14.6-15)*	15.2	*(14.8-15.6)*
8	6-Methyl-5-hepten-2-one	1.4	*(0.8-2.4)*	1	*(0.8-1.1)*	0.5	*(0.3-0.6)*
14	Geranylacetone	2.7	*(1.7-4.2)*	2.8	*(2.1-3.3)*	3	*(1.1-5.5)*
15	β-Ionone	1	*(0.5-1.3)*	1.2	*(0.9-1.6)*	1	*(0.7-1.1)*
	Others						
7	1-Nitropentane	3.3	*(1.9-5.4)*	2.6	*(2.2-2.8)*	1.9	*(1.7-2.1)*
11	2-Isobutylthiazole	0.4	*(0.2- 0.5)*	0.2	*(0.1-0.2)*	0.1	*(0 -0.1)*
13	Geraniol	4.3	*(2.9-8.4)*	3.3	*(3.1-3.4)*	3.4	*(3.2-3.6)*

[a] Round truss tomato cv: Arvento RZ, Caracas RZ, Cappricia RZ, Roterno RZ, Tomala RZ, Varianto RZ, Tom 1, Tom 2.
[b] Cocktail tomato cv: Amoroso RZ, Brioso RZ and Delioso RZ.
[c] Cherry tomato cv: Cheramy RZ, Sassari RZ and TasteryRZ.

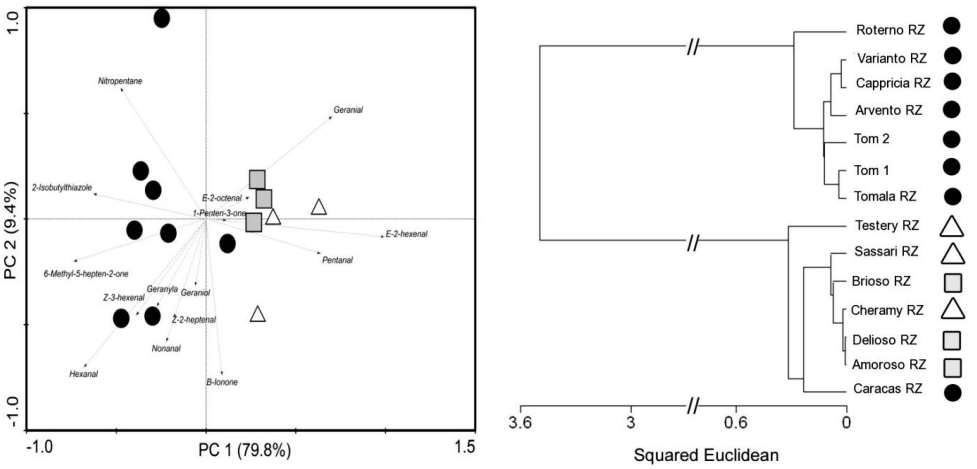

Figure 1. PCA biplot and cluster analysis of fruit powder red tomato volatiles measured by GC-MS. (●) truss tomato, (■) cocktail tomato, (△) cherry tomato. Each point is an average of four samples.

3.2. Tomato volatile fingerprinting by PTR-MS

Similar batches of tomato fruits as used for SPME/GC-MS analysis were evaluated by PTR-MS that allows for a fast headspace volatile determination by direct injection.

The headspace analysis of intact tomato volatiles did not provide a clear separation of the fruit types as justified from both PCA and cluster analysis (Figure 2). Especially from the cluster analysis, we can see many small clusters with low squared Euclidian distances. Since the tomato skin is a strong barrier for the volatile release (Hobson 1988), a majority of the volatiles has to diffuse through the scar left following excision of the calyx. Thus, the aroma volatiles released from intact tomato may not well correlate with the internal volatile composition, as it is strongly influenced by the physical characteristics of the wounded and sealed scar.

Halved tomatoes, on the contrary, were well clustering separated (Figure 3). Confirming the SPME/GC-MS analysis, volatile profiles of cocktail and cherry tomatoes appear very similar (low squared Euclidian distance) and clearly different from the round truss ones. This clustering of the tomatoes based on volatiles is graphically summarized by the biplot of the first two principal components of the PCA analysis. Based on the PCA and the compared analysis of all masses abundance for each cultivar we selected 21 main masses to describe the tomato volatile profile.

The VOCs with higher volatility (lower mass) dominate the PTR-MS spectrum in terms of signal intensity, although lower masses may also result from fragmentation of larger compounds (Figure 4). The dominant signals are listed in table 3, along with tentative compound assignments. Some apparent discrepancies between PTR-MS and GC-MS analysis are due to the presence of compounds that cannot be detected with the Carboxen/PDMS SPME fiber such as methanol (m/z 33), ethanol (m/z 47) and acetaldehyde (m/z 45). These masses in combination with mass 59 (acetone) represent almost 90% of the total headspace composition

for both intact and cut tomatoes. These compounds, even if they are not considered essential as aroma volatiles due to their high odour thresholds (Maul et al., 1998), may interact with other compounds for the final overall aroma. In particular, ethanol has been associated with enhanced sweetness perception on tomato (Tandon et al., 2000).

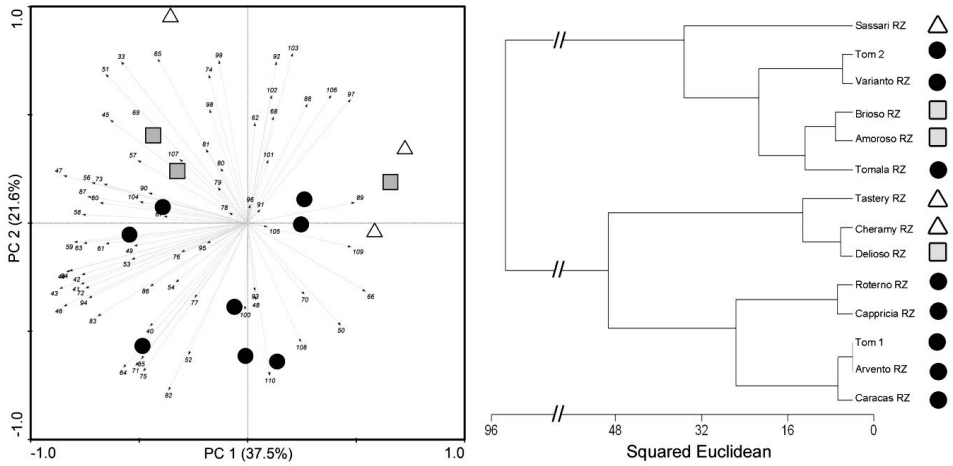

Figure 2. PCA biplot and cluster analysis of volatiles emitted by intact red tomato measured by PTR-MS. (●) truss tomato, (■) cocktail tomato, (△) cherry tomato. Each point is an average of four independent samples measured three times.

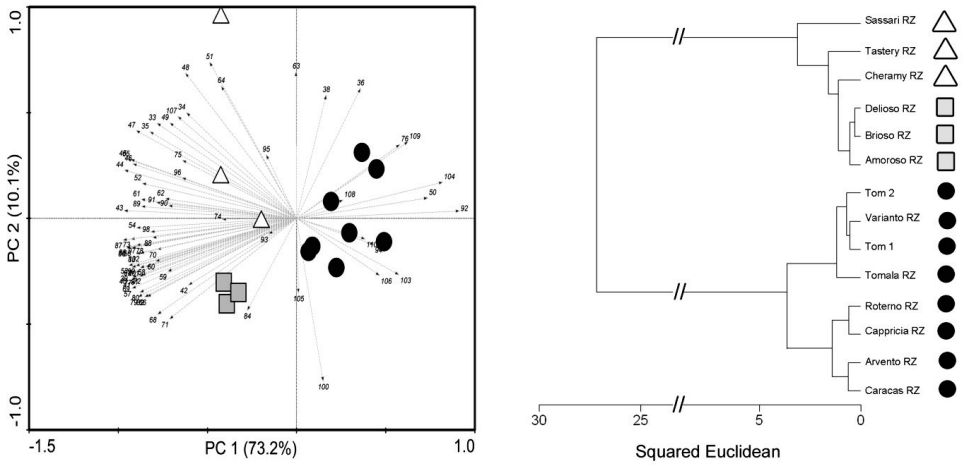

Figure 3. PCA biplot and cluster analysis of volatiles emitted by halved red tomato measured by PTR-MS. (●) truss tomato, (■) cocktail tomato, (△) cherry tomato. Each point is an average of four independent samples measured three times.

Many of the remaining masses (i.e. m/z 41, 43, 57, 81, 83, 85, 99, 101) are instead correlated with C6-tomato volatiles, such as hexanal, hexenal, hexenol and hexenyl acetate that

are also always the most abundant tomato compounds detected with GC-MS. Quantitative comparison of analysis made by PTR-MS with the SPME-GC-MS is not possible since the extraction methods are totally different. Dissimilar destruction of the food matrix and time scale of the analysis may generate an altered VOCs pattern due to the possible chemical interaction of the volatile compounds.

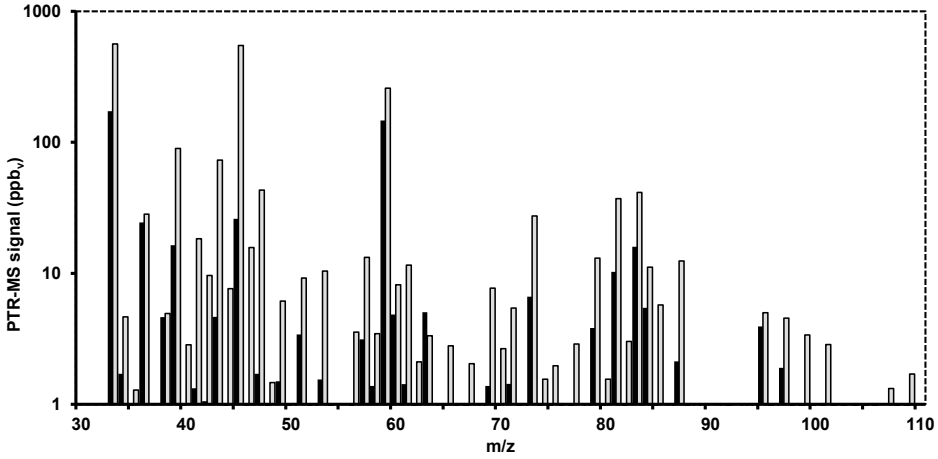

Figure 4. Comparison of PTR-MS spectra (from 30 to 110 m/z) for halved (■) truss (cv. Cappricia RZ) and (□) cocktail tomato (cv. Amoroso RZ) at red stage.

3.3. Dynamics of volatile emission during postharvest storage and ripening

Since volatiles are a plant evolution tool to attract pollinators and herbivores it is understandable why fruits increase their production during ripening. For this reason, the fruit has to permit the volatiles to be released and perceived when it is still intact and attached to the plant.

The PTR-MS headspace analysis of intact tomato fruits at different ripening stages confirms this theory. The riper the fruit is, the more masses related to aroma compounds are released. Both PCA and cluster analyses (Figure 5) reveal a clear separation between fruit of different ripening stages. According to the squared Euclidean distance values, orange tomatoes appear with respect to their aroma volatile profile more similar to breaker than the ripe fruit.

Similar results are obtained from the analysis of these fruit after being cut in half (figure 6). In this case, the separation between fruit of different ripening stages is less marked (smaller Euclidean distance); moreover, the higher biological variability of intermediate ripe fruit (orange stage) causes a less defined clusterization for both PCA biplot and cluster diagram. Tomatoes generally start to synthetize increased amounts of volatiles in the breaker and orange stages, so even one ripening day of difference may deeply influence the overall aroma profile. This difference is probably not so evident in intact fruit because of the still low permeability of membrane and skin to volatile emission.

Table 3. Abundance of aroma volatile compounds in red tomato (half-cut) using PTR-MS headspace analysis technique.

		Round truss tomato[a]		Cocktail tomato[b]		Cherry tomato[c]	
Major PTR-MS ion (m/z)	**Compounds[d]**	**mean (ppb$_v$)**	**range (ppb$_v$)**	**mean (ppb$_v$)**	**range (ppb$_v$)**	**mean (ppb$_v$)**	**range (ppb$_v$)**
33	Methanol	258.6	*(146-342)*	461.4	*(4 (41-505)*	612.5	*(332-939)*
43	Propanol	23.0	*(10.7-33.4)*	54.7	*(50.4-61.6)*	56.7	*(47.4-61.5)*
45	Acetaldehyde	38.1	*(19.9-51.7)*	229.1	*(184-300)*	528.3	*(179-988)*
47	Ethanol	3.0	*(1.2-4.4)*	12.2	*(9.1-15.8)*	39.6	*(9.7-88.4)*
57	Pentanol 1-Butanol	4.3	*(2.8-5.9)*	9.9	*(9.3-10.5)*	8.1	*(5.8-10.4)*
59	Acetone Propanal,	194.9	*(130-312)*	282.1	*(229-311)*	243.8	*(222-258)*
61	Acetic acid Ethyl acetate	4.9	*(1.8-7.7)*	9.3	*(6.5-12.1)*	10.3	*(9.3-10.9)*
69	Pentanal Nonanal	1.9	*(1.2-2.4)*	8.8	*(7.1-11.6)*	5.0	*(4.0-6.9)*
73	Butanal 2-Butanone	8.7	*(6.2-12.1)*	16.5	*(15.5-18.5)*	18.1	*(14.2-24.8)*
81	Hexenal	12.0	*(4.8-21.6)*	33.8	*(28.8-37.0)*	28.4	*(20.8-35.7)*
83	Hexanal	16.6	*(10.4-30.3)*	33.7	*(31.6-34.8)*	30.5	*(26.9-35.1)*
85	n-Hexanol 1-Penten-3-one	1.4	*(0.7-2.0)*	3.5	*(3.2-3.6)*	3.0	*(2.3-3.8)*
87	Pentanal Methybutanal 2,3-Butanedione 2-Pentanone	2.4	*(1.1-3.5)*	7.7	*(7.4-7.9)*	7.7	*(6.1-9.9)*
95	Dimethyldisulfide	4.8	*(2.2-8.6)*	5.1	*(4.0-5.6)*	4.8	*(3.7-6.6)*
99	Hexenal	1.1	*(0.6-2.0)*	2.9	*(2.3-3.4)*	2.3	*(2.1-2.6)*
101	Hexanal	1.2	*(0.6-2.3)*	2.4	*(2.1-2.5)*	2.1	*(1.7-2.8)*

[a] Round truss tomato cv: Arvento RZ, Caracas RZ, Cappricia RZ, Roterno RZ, Tomala RZ, Varianto RZ, Tom 1, Tom 2.
[b] Cocktail tomato cv: Cocktail tomato cv: Amoroso RZ, Brioso RZ and Delioso RZ.
[c] Cherry tomato cv: Cheramy RZ, Sassari RZ and TasteryRZ.
[d] Tentative identification based on results from **Table 2,** standard compounds analysis, isotope ratio and the literature.

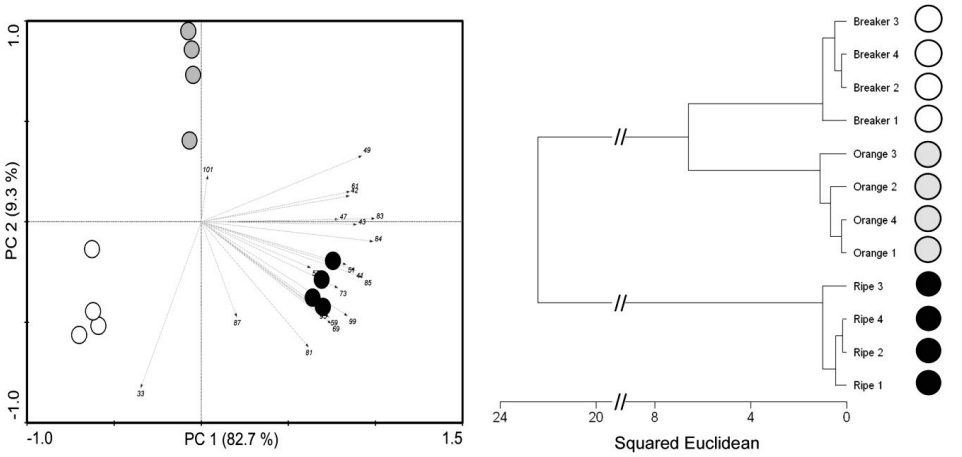

Figure 5. PCA biplot and cluster analysis of volatiles emitted by intact tomato (cv.Cappricia RZ) measured by PTR-MS at different ripening stage. (○)breaker, (◍) orange, (●) red ripe. Each point is an independent sample measured three times.

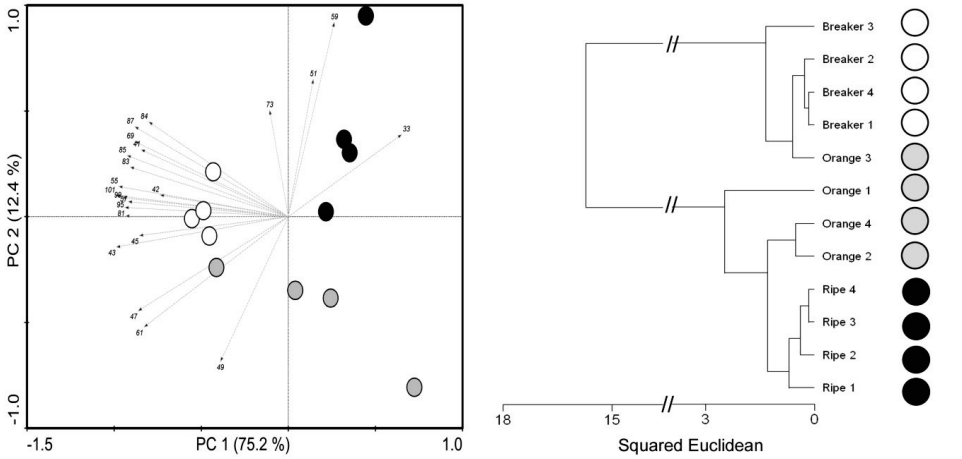

Figure 6. PCA biplot and cluster analysis of volatiles emitted by halved tomato (cv. Cappricia RZ) measured by PTR-MS at different ripening stage. (○), breaker (◍) orange, (●) red ripe. Each point is an independent sample measured three times.

Tomato aroma volatiles are formed in the intact fruits during ripening as well as upon tissue disruption (Buttery and Ling 1993; Riley and Thompson 1998). Lipoxygenase (LOX), hydroperoxide lyase (HPL), and alcohol dehydrogenase (ADH) are important enzymes responsible for synthesis of volatile compounds contributing to “green” or “grassy” and “fresh” notes in ripe fruits (Baldwin et al., 2000; Gardner 1995). This process, as well as colour change (chlorophyll degradation and lycopene synthesis), may be synchronized by the increase in ethylene production. An increase in LOX and HPL at the breaker to pink stages suggests that

there should be an increase in the volatile compounds with green, grassy odors (hexanal, cis-3-hexenal, trans-2-hexenal, trans-2-heptenal, pentanal, and cis- and trans- pentenal) at the pink to light red stages. Later development of ADH suggests that ethanol, methanol, cis-3-hexenol, hexenol, 3-methylbutanol and 1-penten-3-ol should not increase until the end of ripening (Chung et al., 1984; Maul et al., 2000).

Several reports show that ethanol and methanol are good signals for attracting frugifers and herbivores even if their odor thresholds are too high for human perception. Thus, we should consider these compounds as important physiological tools to monitor the healthy state of fruits and vegetables during ripening and storage.

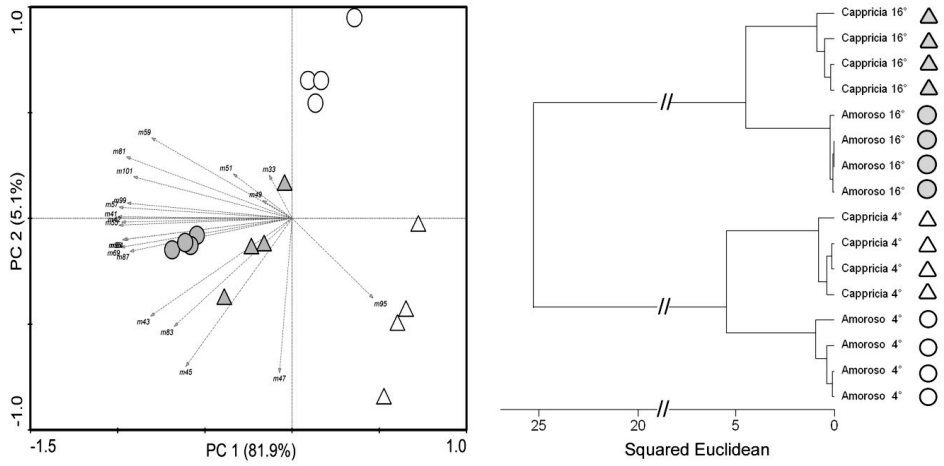

Figure 7. PCA biplot and cluster analysis of halved tomato volatiles measured by PTR-MS after 10 days of storage at 4°C (open symbols) and 16°C (closed symbols). (△, ▲) cv. Cappricia RZ, (○, ●) cv. Amoroso RZ. Each point is an independent sample measured three times.

Table 4. Abundance of aroma volatile compounds in half cut tomato, cv Cappricia RZ and Amoroso RZ, measured at the day of harvest and after 10 days of storage at 16°C and 4°C. Each data represent the average and the standard deviation of four measurements.

	Total[a] VOCs (ppb)	
	Cappricia RZ	**Amoroso RZ**
Harvest	1234 ± 47	1762 ± 62
16°C (10 days storage)	1206 ± 58	1716 ± 49
4°C (10 days storage)	659 ± 62	754 ± 43

[a] Total concentration (ppb) of the following characteristic masses representative of the tomato aroma profile: 33, 41, 42, 43, 45, 47, 49, 51, 57, 59, 69, 81, 83, 84, 85, 87, 95, 99, 101

It has been reported that one of the main consequences of poor storage practices during postharvest is the reduced production of aroma compounds. This phenomenon is enforced by inappropriate storage condition, especially low temperature (Bai et al., 2011; Boukobza and Taylor 2002; Krumbein et al., 2004; Maul et al., 2000; Zhang, et al., 2008). Our data confirm that storage and low temperature has an adverse effect on volatiles production and show that PTR-MS can be used to separate between different prior storage conditions. Both cocktail (cv. Amoroso RZ) and round truss tomatoes (cv. Cappricia RZ) stored at 4°C for 10 days, produced significantly less volatile than the ones stored at 16°C (table 4). Both PCA biplot and cluster analysis (figure 7) of the headspace volatiles highlight two main clusters corresponding to the two storage conditions. Furthermore, as a confirmation of the earlier discussed data, also the two cultivars are well cluster-separated in both storage conditions.

4. Conclusions

These results show that PTR-MS is suited to monitor at high sensitivity the emission of a large number of volatiles that describe the tomato aroma profile. The technology can easily monitor and quantify compounds related to ripening and/or senescence such as methanol, ethanol or acetaldehyde that are more difficult, expensive and time consuming to measure with other techniques such as GC-MS. Moreover, since PTR-MS measuring of volatiles is without any chemical extraction and pre-selection, differently from SPME/GC-MS, we can assume the results are more comparable with consumer quality perception. Using the mass spectrum of headspace volatiles, different tomato types and ripening stages could be distinguished. In addition, the effect of storage at chilling temperatures was clearly reflected in the mass spectrum. This study confirms research by other authors showing that aldehydes, especially hexanal and hexenals, are important volatiles in tomato. These compounds have high log odour unit and thus high impact on tomato flavour. The possibility to measure these compounds will facilitate the breeding for tomato varieties based on aroma quality.

5. Acknowledgments

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Chapter 4.2

Aroma volatile release kinetics of tomato genotypes measured by PTR-MS following artificial chewing

Abstract

The aim of this study was to develop an analytical system to study the tomato aroma profile. An artificial chewing device coupled to a PTR-MS was developed to mimic, as close as possible, the release of volatiles during chewing in the human mouth and the retronasal olfaction perception.

VOC profiles of 9 tomato lines, selected based on flavor characteristics by a sensory panel, were acquired by both a PTR-MS system following artificial chewing and by SPME-GC-MS and compared to the quantitative descriptive analysis (QDA) measured by the trained sensory panel.

Based on multivariate statistical analysis, data obtained by the PTR-MS system showed a better correlation to the outcome of the QDA than SPME-GC-MS, especially for the descriptive parameters "tomato fragrance" and "tomato flavor".

The great advantage of such an analytical system was the possibility to study the release kinetics of volatiles during eating and the possibility to consider volatile concentration similar to in vivo condition resulting on an improved characterization of the aroma profile.

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1. Introduction

The characteristic tomato fruit flavor is determined by a complex mixture of sugars, acids, minerals and volatile compounds (Baldwin et al., 2008, 1991a, 1991b). From over 400 volatile compounds identified in tomato fruits, less than 20 compounds are considered important for flavor based on their odor thresholds (Abega et al., 2004). These volatiles are derived from a diverse set of precursors that include branched-chain and aromatic amino acids, fatty acids, and carotenoids (Klee and Tieman, 2013).Tomato volatiles can be mainly classified into two classes: one class comprises of compounds formed in the fruit during ripening (e.g. isobutylthiazole, 3-methylnitrobutane, geranylacetone, β-ionone (Buttery and Ling, 1993)) and another class comprises of compounds formed when the fruit is macerated either by cutting or by eating (Galliard et al., 1977; Brauss et al., 1998). Among them, six carbon (C6) compounds, produced by the lipid oxidation pathway, play a major role giving tomato its fresh 'top-note' (Boukobza et al., 2001).

It is expected that, following chewing, volatiles will be released at different rates determined by the number of enzymatic steps required and by the activity of specific enzymes (Brauss et al., 1998). The release behavior will also be affected by the volatile compounds rate of partitioning between air and liquid according to Henry's Law (Xu & Barringer, 2010). Boukobza et al. (2001) differentiated two clear types of release behavior: some compounds (such as isobutylthiazole, 6-methyl-5-hepten-2-one, methylbutanal, methylbutanol and acetaldehyde) showed rapid release immediately after maceration, reaching maximum concentration within the first 30 s whereas the concentration of other compounds (the C6 compounds such as hexenal, hexanal and hexenol) increased at a steady rate, reaching a maximum concentration after 2 min. These different release behaviors of VOCs, may influence the human aroma perception during food consumption. Aroma perception depends not only on food chemical composition but also on food structure and on the oral physiology parameters (Foster et al., 2011; Poinot et al., 2009; Taylor 2002) since flavor compounds are released from the matrix and then transported to the receptors in the mouth and nose (Buettner et al., 2008).

The perception of food flavor and odor is a complicated physiological and psychological process resulting from the concurrent chemical stimulation of orthonasal and retronasal receptors (Sheperd, 2006). Orthonasal olfaction is the perception of odors that occurs during sniffing as opposed to retronasal olfaction, commonly associated with the sense of taste, which is the perception of odors emanating from the oral cavity during eating and drinking (Sheperd, 2006). Volatiles delivered by these two pathways are not perceived by the brain in the same way. It is retro-nasal olfaction, and not orthonasal olfaction, that is essential to flavor (Klee and Tieman 2013). The perception of the odor and flavor cannot be exhaustively explained by simple linear models since human olfactory receptors are simultaneously influenced by hundreds of compounds interacting with each other.

Right now, flavor research has mainly prioritized aroma volatiles present at levels exceeding the orthonasally measured odor threshold, ignoring the variation in the rate at which odor intensities increase above threshold (Tieman et al. 2012). During eating, a solid food product is crushed and mixed with saliva; its structure is modified and the diffusion of its volatiles from the resulting bolus to the headspace is affected. With mastication, the food

surface area exposed to the air increases, and the food matrix is separated from the water it contained initially (Arvisenet et al. 2008,; de Roos, 2003). The chewing process, which is directly related to the textural and physicochemical properties of the food matrix, has been reported as a substantial parameter affecting the in vivo flavour release (Foster et al., 2011; Mestres et al., 2006; Taylor 2002).

Considering such conditions, it seems not reasonable to compare human sensory perception with volatile compounds quantified with the traditional methodologies such as SPME-GC-MS using frozen tissue samples, long incubation times at high temperature and often the addition of salts. These methodologies, apart from measuring only the maximum amount of volatiles emitted under artificial conditions, do not consider the different release kinetics of individual volatiles from a food matrix. In vivo measurements, such as sampling out of the human mouth or nose during eating, are desirable since they more closely reflect the volatile profile interacting with the olfactory receptors and therefore such measurements may relate better to sensory perception (Boukobza et al., 2001). The high variability generally observed in consumer characteristics do not allow sensory measurement in vivo to be accepted as standard and repeatable method unless made by expensive trained panelist. Consequently, there is interest in the development of methods for rapid, repeatable and sensitive monitoring of volatile compounds emitted from food samples in a way that mimics the release in the human mouth during eating. (Benjamin et al. 2012; Poinot et al. 2009; Arvisenet et al., 2008; Salles et al., 2007; Rabe, Krings, & Berger, 2004; van Ruth & Buhr, 2004; van Ruth & Roozen, 2000; Roberts & Acree, 1995;van Ruth, Roozen, & Cozijnsen, 1995).

From a technical point of view, gas chromatography is the reference method for the analysis of food volatiles but it is still a time-consuming procedure and it generally does not allow kinetic measurements. Among the various possibilities proposed and investigated for rapid quantification and identification of VOCs, proton transfer reaction mass spectrometry (PTR-MS) is one of the most used since it allows to measure on-line, with high sensitivity, a mixture of volatile compounds in a straightforward and fast way. (Biasioli et al., 2011).Precise identification of peaks is, however, not possible with PTR-MS. Without attempting to assign chemical names to the mass peaks, PTR-MS is considered as the equivalent of an array of sensors giving a finger print of the total volatile mixture. (Biasioli et al., 2011; Granitto et al., 2007).

The aim of this work was to develop a fast and reliable system to study the volatile aroma profile of tomato fruits that mimics, as close as possible, the release of volatiles during chewing in the human mouth. The system may be used for initial screening of genotypes in breeding programs or to quantify the effects of e.g. cultivation or postharvest conditions on volatile emission. We combined PTR-MS with a “chewing device”. This allowed us to quantify the VOCs production occurring during the chewing of tomato and to study the kinetics of the release of the most significant tomato VOCs to better define their organoleptic importance. In addition, the VOCs profiles obtained with the PTR-MS system and SPME-GC-MS were compared to the quantitative descriptive analysis (QDA) of sensory attributes of the eating quality of tomatoes measured by a trained panel.

2. Materials and methods

2.1. Plant material

Tomatoes (*Solanum lycopersicum*) were obtained in summer 2010 from an experimental-greenhouse in Wageningen (The Netherlands). All cherry tomato selections were grown under identical conditions and were part of a F6 population derived from a breeding program focused on the improvement of tomato flavour. We screened nine of these genotypes that we coded Line 1 to 9. Homogenous batches of tomatoes from each genotype were selected on the basis of fruit size, colour and firmness, measured non-destructively.

2.2. Tomato quality characteristics

Total soluble solids (TSS) were measured using a digital refractometer (Atago).

Tomato firmness was measured at two orthogonal selected spots using a Zwick Z2.5/TS1S materials testing machine (Ulm, Germany) with a cylindrical probe (Ø 15 mm). Tomatoes were placed on a plastic ring to keep the tomatoes upright during measurement. Firmness was determined as the maximum force needed to compress the tomato 1 mm at 40 mm/min, after lowering the probe until the tomato skin was touched. (Schouten et al., 2007)

L*, a*, b* system chromaticity values were measured using a tristimulus chromameter (CR-400, Minolta, Japan) in two orthogonal spots of the fruit. Tomato colour was expressed as either a* or a*/b*.

2.3. Sensory analysis

A panel of nine selected panellist carried out the profiling of the different tomato lines. Selection of panellists was firstly based on performance in the recognition of basic taste and odour components and on their verbal creativity. The panellists received a sensory training program based on the recognition and quantification of the most important taste and flavour attributes of tomato fruit.

The panel, in the same session, rated the intensity of 28 sensory attributes on a 10 cm unstructured scale, anchored at each end. A balanced-block serving order across products and panellists was used, and the products (three fruits) were presented at room temperature in transparent plastic-covered cups coded with a three digit random numbers.

In this paper we consider only the attributes, scored by the panellists, related to odour and flavour such as odour strength, tomato fragrance, spicy fragrance, sweet odour, sharp odour, flavour intensity, tomato flavour, earthy flavour, green/unripe flavour and spicy flavour.

2.4. SPME/GC-MS analysis

Samples of fresh tomatoes (five fruits of each tomato line) were quickly cut into quarters and immediately frozen into liquid nitrogen. The samples were stored at -80°C and ground in liquid nitrogen in a metal electric grinder prior to analysis.

The profiling of volatiles was performed in four replicates using a modification of the headspace solid-phase micro extraction gas chromatography (SPME/GC-MS) method of Tikunov et al. (2005) described by Farneti et al. (2012). Frozen fruit powder (1 g fresh weight) was weighed into a 20 mL crimp cap vial, the vial was closed and incubated at 30°C for 10 min.

The closed vials were then sonificated for 5 min. Thereafter the samples were incubated at 60°C with agitation for 30 min and the headspace volatiles were extracted from the vial headspace and injected into the GC-MS apparatus (Trace GC Ultra, Thermo, IL, USA) equipped with a TriPlus SPME autosampler (Thermo, IL, USA). The extraction of volatiles was done by inserting a 75 µm Carboxen/PDMS SPME fiber (Supelco, Zwijndrecht, Netherlands) to the vial headspace during the last 20 min of the incubation under continuous agitation and heating at 60°C. The fiber was desorbed for 15 min at 250°C in the injection port of the GC in splitless mode. Chromatography was performed on RTX-WAX (Restek, Bellefonte, PA) capillary column (60 m length x 0.32 mm i.d., 0.25 µm film thickness) with helium as a carrier gas. The GC interface and MS (DSQ II, Thermo, IL, USA) source temperature were both 250°C. The GC temperature program started at 40°C (5 min), was then raised to 240°C at a rate of 5°C per min, and finally was held at 240°C for 10 min. The total run time including oven cooling was 60 min. Compound identification was based on mass spectra matching in the standard NIST library and retention time of authentic reference standards. Reference standards of volatile compounds studied (hexanal, (E)-2-hexenal, (Z)-3-hexenal, (Z)-2-heptenal, 3-methyl-butanal, pentanal, 5-ethyl-2(5H)-furanone, hexanoic acid, 1-penten-3-one, 6-methyl-5-hepten-2-one, geranylacetone, β-ionone, 1-nitropentane, 2-isobutylthiazole, 2-cyclohexene-1,4-dione) were supplied by Supelco (Sigma-Aldrich and Fluka; Zwijndrecht, Netherlands) as pure compounds (92-99.5%).

2.5. PTR-MS analysis

The chewing device (Fig. 1) was composed of a cylindrical glass cuvette (800 mL) sealed with a cap and a notched plunger controlled manually. The notched plunger contained 34 plastic teeth, 10 mm in height. All the parts of the device that come into contact with the fruit to be chewed are made of polytetrafluoroethylene. The head space was drawn from the chewing device at 0.5 L/h by a vacuum pump for on line analysis into the proton transfer reaction mass spectrometer (PTR-MS) described by Farneti et al. (2012). This PTR-MS apparatus consists of: (i) an ion source in which H3O+ ions are produced, (ii) a drift tube where the trace gases are ionized by proton-transfer reaction with H3O+ ions, (iii) a collision dissociation chamber, (iv) a quadruple mass filter and (v) a secondary electron multiplier. (For details, see Holger et al., 2012). The drift tube operated at a pressure of 2.08 mbar and was heated at about 55°C. The mass number of the detected ion is given by the molecular mass of the substance plus the mass of the single proton mH (mRH+ in atomic mass units, amu).

Based on preliminary experiments with tomato fruits and based on earlier reported data (Farneti et al., 2012) we selected and monitored 20 predominant masses from the overall tomato volatile spectrum (m/z 33, 41, 42, 43, 45, 47, 49, 51, 57, 59, 69, 81, 83, 84, 85, 87, 95, 99, 101,110). Mass spectrometric data were collected using a dwell time of 0.2 s per mass.

For volatile analysis after artificial chewing, one intact tomato was placed into the glass cuvette. Before crushing the fruit, the headspace VOCs concentration of the intact fruit was measured for 140 seconds to ensure reaching the equilibrium. The chewing was done through manually pressing the notched plunger 5 times within 10 seconds. VOCs analysis continued for about 3 minutes following mastication. Four replicates for each tomato genotype were analysed.

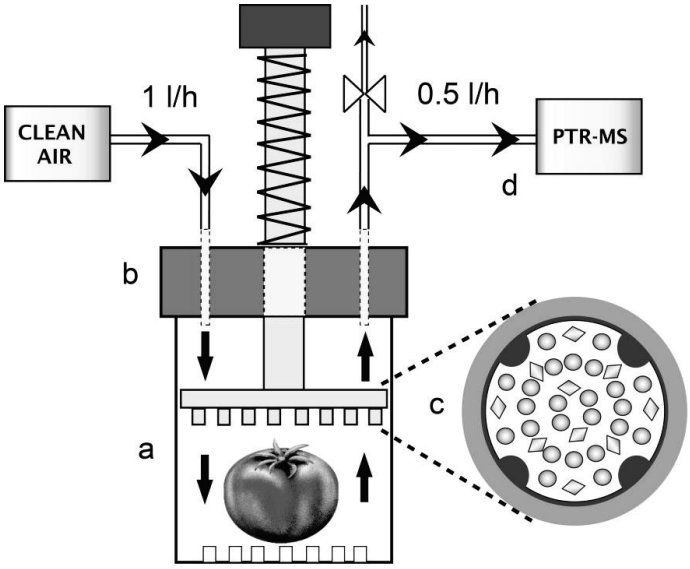

Figure 1. Schematic representation of the chewing device: cylindrical glass cuvette of 800 mL (a) sealed with a cap (b) and a notched plunger controlled manually (c). The notched plunger contained 34 plastic teeth, 10 mm in height. The head space was drawn from the chewing device at 0.5 L/h by a vacuum pump for on line analysis into the proton transfer reaction mass spectrometer (PTR-MS) (d).

2.6. Breath collection.

A preliminary test to check the validity of the "chewing device" was made by in vivo measurement of VOCs released during chewing in the human mouth. Fruit of two different cocktail tomato cultivars (designated as Tom A and Tom B) and one cherry tomato, were chewed by two persons and their breath was collected into 1 litre Tedlar air sample bag (SKC Inc., Eighty Four, Pa, USA) after they had chewed the tomato fruit for about 10 seconds. To collect the breath we used a collection device described by Cristescu et al. (2013) that consists of a mouth piece connected with teflon tubing to a sample bag. To ensure a constant exhalation flow the collection device was equipped with a pressure meter. These samples were later analysed and compared to the data obtained by PTR-MS analysis at 30, 60 and 120 and 180 seconds after artificial chewing.

2.7. Data analysis

For interpreting the sensory and instrumental results we applied principle component analysis (PCA) and partial least square regression (PLSR) on aroma descriptors (QDA) and volatile compounds (PTR-MS and SPME-GC-MS).

The dataset (QDA, GC-MS and PTR-MS results) has been arranged in four matrices. Two matrices for the PTR-MS analysis for data 30 and 120 seconds after the chewing, respectively, of 20 columns corresponding to the PTR-MS m/z (converted in ppm); one matrix

for the SPME-GC-MS results arranged in 15 columns for each volatile compound, and one matrix for the QDA results arranged in 10 columns for each flavour/taste attribute.

The multivariate statistical software The Unscrambler X 10.1 (CAMO PROCESS AS, Oslo, Norway) was used for principal component analysis (PCA) and partial least square regression (PLSR). Cluster analysis of LOG transformed data was computed by the software MVSP 3.2 (Kovach Computing Service, Wales, U.K.) using the Ward's minimum-variance method.

3. Results

3.1. Comparison between "in vivo chewing" and "artificial chewing"

Two varieties of cocktail tomato and one of cherry tomato were used to test the capability of the "chewing device" to mimic the in vivo mastication with respect to the release of volatile compounds (figure 2).

Data were log-transformed to accommodate the wide concentration range caused by the heterogeneity of volatiles released during fruit destruction. Only the most characteristic pre-selected masses were considered.

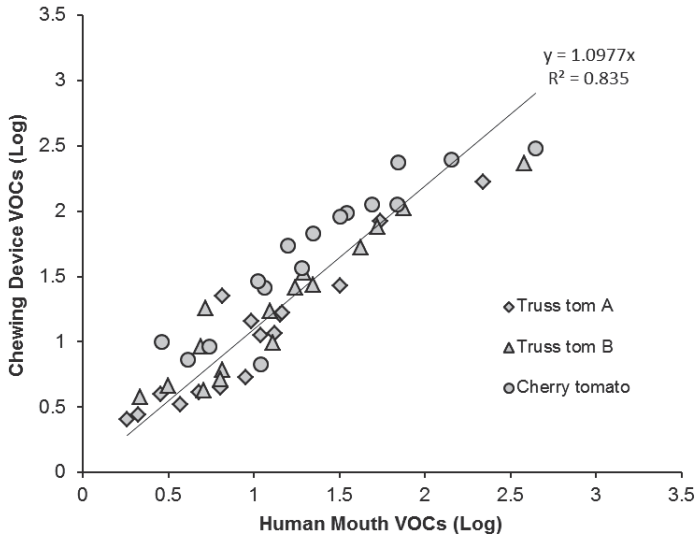

Figure 2. Correlation graph between tomato VOCs concentration measured by PTR-MS after in vivo natural chewing (x-axis) and in vitro chewing using the chewing device (y-axis) of two truss tomato batches and one cherry tomato batch. Analysis of in vivo generated volatiles were done after 10 sec of chewing; analyses with PTR-MS were done 30 seconds after start of the chewing, that in itself took about 4 to 5 seconds. Each point is an average of 3 measurements and corresponds to one of the 21 masses measured by PTR-MS.

A linear relation was found between the volatile concentrations emitted in vivo after 10 seconds of chewing and the concentrations measured 30, 60, 120 and 180 seconds after "artificial chewing", respectively. The correlation coefficients were close to 0.8 in all of the cases.

The longer the time of analysis, the greater the slope of the correlation between in vivo and artificial chewing, indicating that the short measurement times are more appropriate to simulate the human volatile perception during eating (data not shown). Volatile concentrations analysed 30 seconds after the "artificial chewing" showed a nearly 1 to 1 ratio with the volatiles detected in the human breath sample after 10 seconds chewing (figure 2).

3.2. Volatile release pattern after artificial chewing

In figure 3 the volatile release patterns of selected masses analysed by PTR-MS are shown for two tomato genotypes. Since the tomato genotypes "Tom 1" and "Tom 9" were considered at both ends of the tomato aroma spectrum as determined by sensory analysis, only data obtained from these two tomato lines are graphically presented.

In each graph the three phases of the chewing simulation analysis are labelled: i) 90 seconds measurement of volatile concentration of the empty device; ii) 120 seconds measurement of volatiles produced by the intact tomato fruit; iii) 200 seconds measurement of volatiles produced during and after the chewing, that itself lasts 4 to 5 seconds.

Volatile emission after fruit chewing was not always proportional to the intact fruit volatile production. For instance, in the case of mass 81 (most probably representing hexenal), the emission of the compound from the intact tomato of line 1 was clearly lower than emission from line 9; following the chewing, the volatile production of line 1 was significantly higher than that of line 9.

The volatile release patterns after fruit chewing were influenced both by the fruit genotype and by the volatile biochemical nature. Compounds already preformed inside the fruit, were immediately emitted after mastication with a high emission ratio in the first 30 seconds (e.g. masses 45, 59 and 84). Other compounds, most probably enzymatically produced as a consequence of fruit mashing, were released with a constant emission rate. (e.g. 81, 83 and 84).

The slope of the different phases of the emission curves were calculated for each mass. The ratios between the slope calculated from the first 30 seconds volatile release after mastication (first part of the curve) and the slope calculated from the volatile release between 30 and 120 seconds (second part of the curve) are shown in figure 4 for all genotypes together. Compounds characterized by a ratio of about 1 are linearly emitted after the mastication, whereas a higher release ratio was typical for the masses that show two different release phases, a fast one and a slower one. The large standard deviations, especially encountered for compounds characterized by high release ratio values, are caused by the variability of tomato lines and not by lack of precision on PTR-MS measurements.

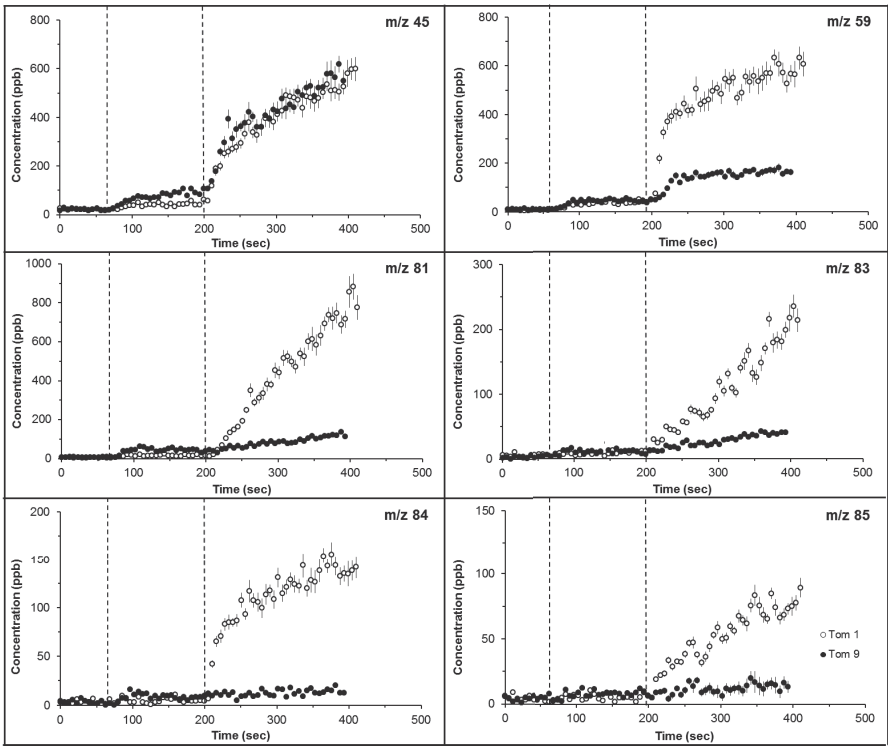

Figure 3. Comparison of the time course of the release of selected volatiles of two tomato genotypes (○ "Tom 1" and ● "Tom 9") after chewing, measured by PTR-MS. Each graph is divided by dotted lines in three phases: i)head space analysis of empty "chewing device", ii) head space analysis of intact tomato, iii) head space analysis of chewed tomato. Data are averages and standard deviation of 4 measurements.

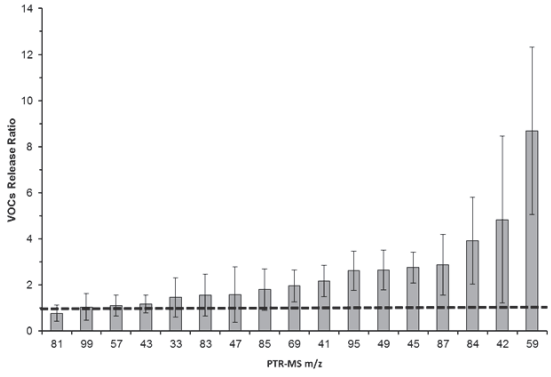

Figure 4. Ratios between the slope of the first part of the volatile release curve (first 30 seconds volatile release after mastication) and the second part of the release curve (between 30 and 120 seconds). Data are averages and standard deviation of 36 measurements (four replicates for all nine tomato genotypes).

3.3. Tomato lines description

Quality characteristics of the tomato genotypes evaluated in this research are reported in table 1. Considering the colour heterogeneity of ripe fruits, caused by the genetic differences, fruit selection solely based on red colour was not possible. Therefore, homogeneous tomato batches were selected on the base of fruit size, colour and firmness, measured non-destructively. Tomato genotypes 4, 6, 8 and 9 showed a yellow-light orange colour at the full ripe stage, reflected in the low chromaticity value of a* and a*/b*. The colour of the other tomato genotypes ranged between light red (Tom 5) and dark red (Tom 7) at the full ripe stage.

The nine tomato genotypes slightly differed also in soluble solids content; these differences are in the range between 6.4 and 7.9 °Brix.

Table 1. Quality characteristics (total soluble solids, firmness and chromaticity indexes) of the nine tomato genotypes analysed. Each figure is an average of 10 measurements.

Genotypes	Visual color	T.S.S. (°brix)	Firmness (N)	a*	b*	a*/b*
Tom 1	Red	7.6	3.7	16.1	18.5	0.88
Tom 2	Red	7.3	3.9	15.4	19.0	0.81
Tom 3	Red	6.9	3.7	14.1	16.6	0.85
Tom 4	Light orange	6.7	3.4	12.1	31.0	0.39
Tom 5	Red	7.9	3.7	11.0	18.3	0.60
Tom 6	Yellow	6.6	2.8	6.8	28.7	0.24
Tom 7	Red	6.8	3.7	20.5	18.8	1.09
Tom 8	Yellow	7.3	3.6	9.5	31.7	0.30
Tom 9	Yellow	6.4	4.1	2.2	29.8	0.07

3.4. Quantitative descriptive analysis.

A quantitative descriptive analysis (QDA) by trained panellists was carried out to qualitatively characterize the nine tomato genotypes. As the aim of this research is to link volatile production to aroma properties, only the ten characteristics related to olfactory and aroma perception were taken into account: odour strength, tomato fragrance, spicy fragrance, sweet odour, sharp odour, flavour intensity, tomato flavour, earthy flavour, green/unripe flavour and spicy flavour.

Principle component analysis (PCA) and cluster analysis were performed on the obtained dataset to describe relations between tomato genotypes and qualitative parameter as well to reduce the dataset to important components (figure 5a). The first two components account for 90% of the total variance. Qualitative differences between the tomato lines are mainly explainable by the first principle component (77%) that mostly describes the intensity scores of the parameters taken into account.

Cluster analysis (supplementary material figure S1) confirmed PCA results that seven out of the nine lines evaluated in this research have quite similar flavour characteristics. Among the two outliers Tom 8 and Tom 9, the latter shows the lowest scores for the significant tomato quality attributes, such as "odour strength", "tomato fragrance", "flavour intensity", "tomato flavour", and the highest scores for the "earthy" and "green/unripe flavour" attributes.

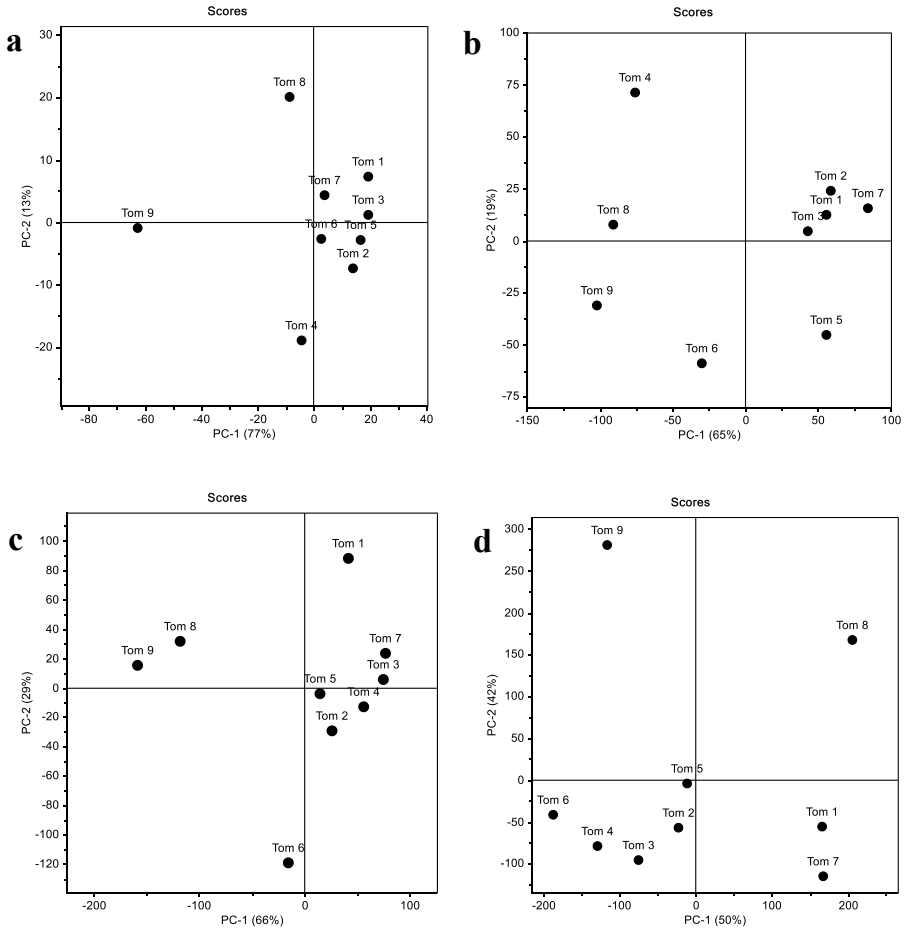

Figure 5. PCA scores of sensory analysis attributes and measured volatiles of tomato genotypes. a) Tomato quantitative descriptive analysis (QDA) attributes (each point is an average of 10 independent samples); b) SPME-GC-MS analysis of volatiles (each point is an average of four independent samples); c) and d) PTR-MS mass fingerprinting 30 and 120 seconds after chewing (each point is an average of four independent samples).

3.5. PTR-MS measurements 30 and 120 seconds after chewing

Similar batches of tomato fruit as used for quantitative descriptive analysis were evaluated by PTR-MS in combination with the chewing device.

Principle component analysis (PCA) and cluster analysis were performed on headspace volatile concentrations measured after 30 and 120 seconds from the start of chewing (figure 5 c and d). Both multivariate statistical methods, applied on mass fingerprinting after 30 seconds (Figure 5c and supplementary material figure S2 and S3), highlight the division of the examined tomato lines into two well separated clusters: one cluster, formed by Tom 1, Tom 2, Tom 3, Tom 4, Tom 5, Tom 6 and Tom 7, was characterized by high concentrations of the majority of masses analysed; the other cluster with the remaining two genotypes, Tom 8 and Tom 9. Apart for acetaldehyde (mass 45) and ethanol (mass 47), these two genotypes (particularly Tom9) produced, less volatiles, after mastication than the other genotypes (supplementary material table S1).

Similar results were obtained from the analysis at 120 seconds after chewing (figure 5d and supplementary material table S2). Different from the analysis after 30 seconds, variation between tomato genotypes were more marked. Again, Tom 9, appeared to be the outlier tomato genotype defined by less intense volatile profile.

3.6. SPME-GC-MS analysis

The SPME-GC-MS analysis allowed for the identification and quantification of 15 volatile compounds considered fundamental for the overall tomato aroma: 3-methyl-butanal, pentanal, 1-penten-3-one, hexanal, (Z)-3-hexenal, (E)-2-hexenal, (Z)-2-heptenal, 1-nitro-pentane, 6-methyl-5-hepten-2-one, 2-isobutylthiazole, 5-ethyl-2(5H)-furanone, hexanoic acid, geranyl acetone, β-ionone, 2-cyclohexene-1,4-dione.

The results confirm that aldehydes are the most abundant volatile compounds present in ripe tomato fruit: hexanal and hexenals ((Z)-3-hexenal, (E)-2-hexenal) cover about 70 to 90% of the total volatile profile of the investigated tomato genotypes examined in this research (supplementary material table S3).

The volatile profile of the genotypes characterized by yellow-orange pigmentation of ripe fruit (Tom 4, Tom 6, Tom 8 and Tom 9) was characterized by lower total amount of aldehydes and a consistently higher concentration of volatiles originated from the carotenoid biosynthesis pathway such as 6-methyl-5-hepten-2-one, geranyl acetone and β-ionone.

Principal component analysis (PCA) (Figure 5b) and cluster analysis (supplementary material figure S4) were carried out to describe relations between tomato genotypes and volatile compounds. Approximately 85% of the total variance was accounted for by the first two principle components. Differences between tomato genotypes with yellow-orange and red pigmentation are mainly explainable by the first principle component (65.5%).

3.7. PLSR modelling of scored sensory parameters and measured volatile compounds

The relationship between scored sensory parameters and measured volatile compounds evaluated by PTR-MS and SPME-GC-MS was established by PLSR (Partial Least Square Regression).

PLSR modelling between the matrices of aroma descriptors and of volatile compounds, as determined by PTR-MS analysis at 30 and 120 seconds after chewing, provided a two-factor model explaining respectively 95% and 92% of the variance in X (volatile compounds) and 58% and 70% of that in Y (sensory descriptors) (figure 6). Similarly, for the SPME-GC-MS data the two-factor model totally explained 81% of variance of X and 53% of Y (data not shown).

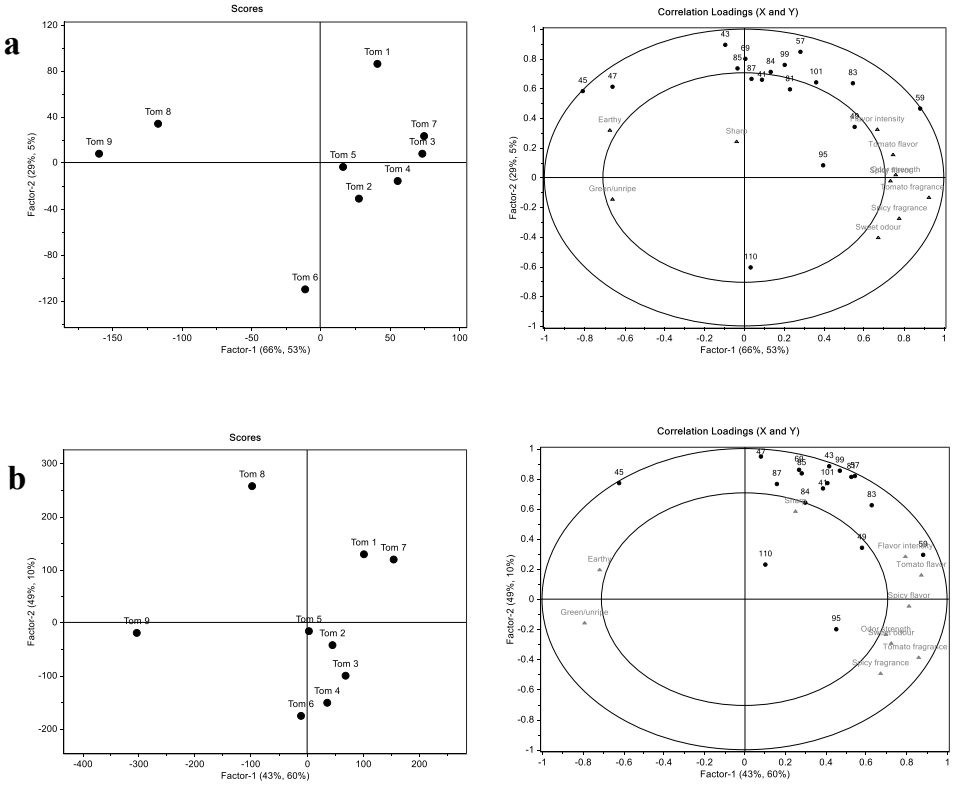

Figure 6. PLSR score and correlation loading plots of tomato QDA attributes and tomato volatiles measured by PTR-MS after 30 seconds (a) and 120 seconds (b) from the start of chewing. The inner and outer ellipses represent 50% and 100% of explained variance, respectively.

The cross-validated estimation (leave-one-out) based on PLSR analysis indicates that the minimum RMSECV (Root Mean Square Error of Cross Validation) is obtained using two PLSR scores. Adding a third score did not improve meaningfully the prediction of the sensory scores. As a final result we compared the odour-aroma scores obtained by the trained panellists and the results obtained by the cross-validated estimation based on PTR-MS and SPME-GC-MS data (figure 7 and 8).

In figure 7, estimated scores of two of the main odour-aroma tomato attributes, tomato fragrance and tomato flavour, are plotted against the scores obtained by the panellists.

Predictability of the model based on PTR-MS data gave higher fitting than the one based on SPME-GC-MS that revealed a Pearson correlation coefficients proximal to 0.

With respect to PTR-MS analysis, measured and predicted sensory scores were better correlated using data derived at 30 seconds after chewing rather than using data from 120 seconds after chewing. Pearson correlation coefficients for tomato fragrance and tomato flavour were respectively 0.86 and 0.68 for analysis using 30 seconds PTR-MS data; for 120 seconds data Pearson correlation coefficients were 0.77 and 0.46 (figure 7).

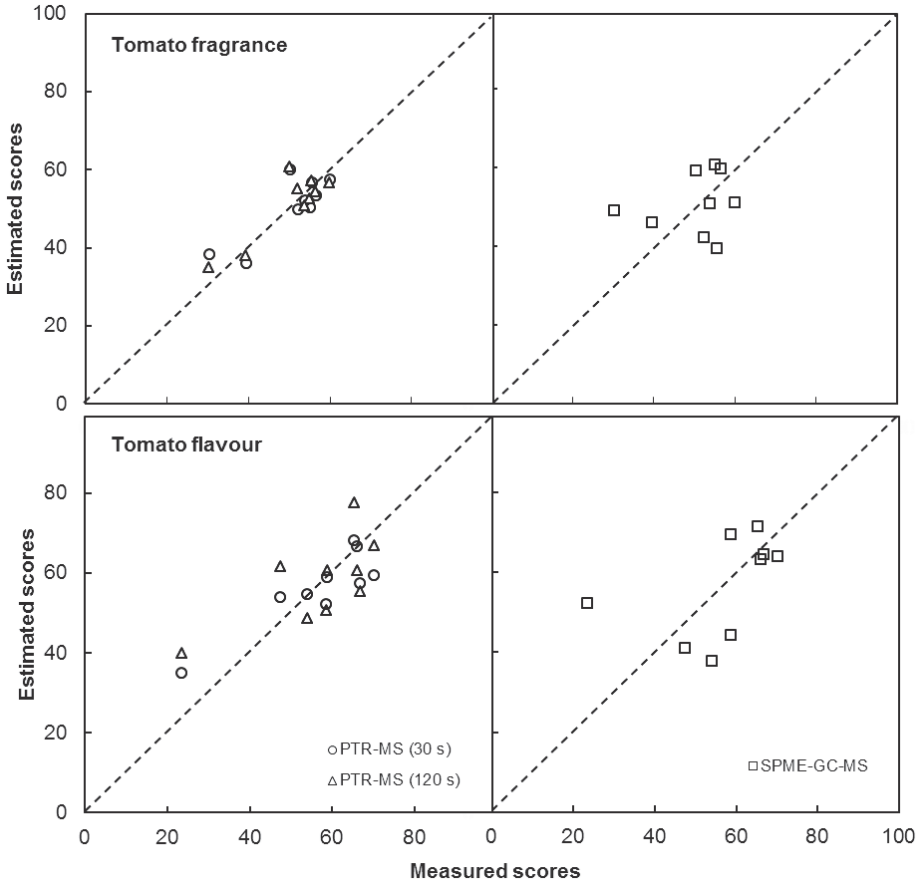

Figure 7. Cross-validated estimation of the attribute "tomato fragrance" and "tomato flavour" based on SPME-GC-MS data and on PTR-MS data obtained 30 and 120 seconds after artificial mastication.

The overall prediction of the sensory attributes, based on the 30 seconds PTR-MS data, is graphically presented in figure 8 by using spider diagrams. Considering the relatively limited set of samples, the cross-validated prediction described the aroma-flavour profiles made by trained panellists sufficiently well.

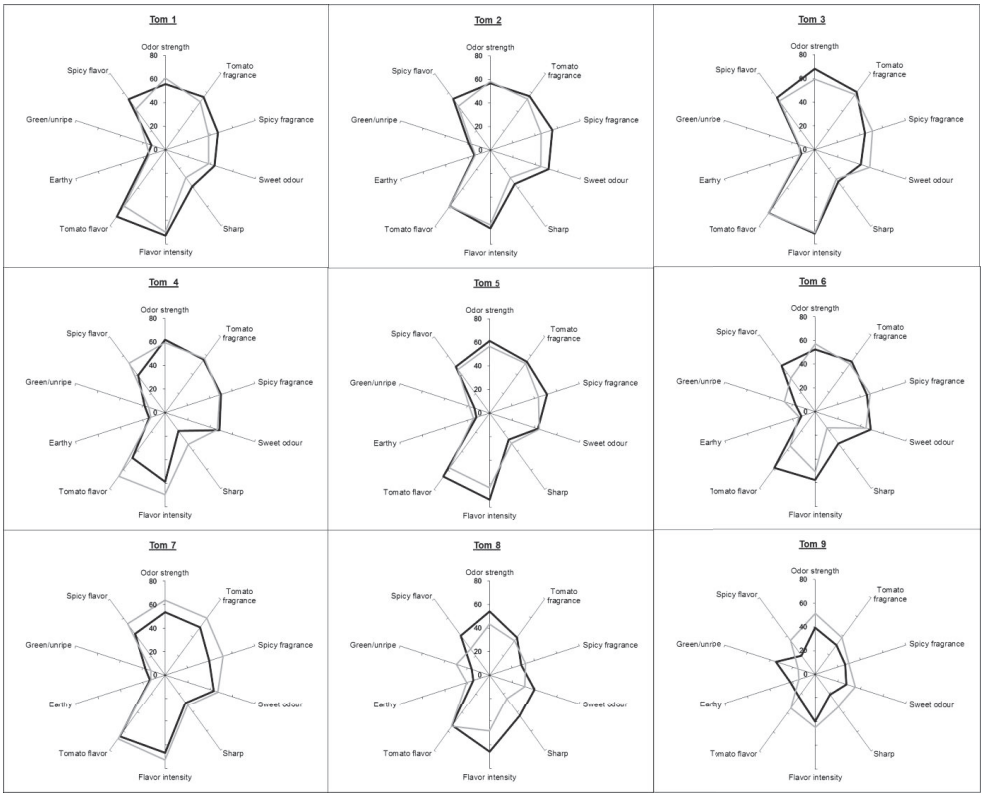

Figure 8 Spider graphs of cross-validated estimation of tomato flavour-aroma attributes based on PTR-MS data (grey line) and measured by QDA analysis (black line).

4. Discussion

The aroma profile of tomato fruits produced after in vitro mastication using a chewing device was both qualitatively and quantitatively comparable to in vivo (human chewing) analysis. Taking into account the physical proprieties of the analytical system used in this research, such as the inlet PTR-MS flow, the length of the connectors and the jar volume, measurements at about 30 seconds after the artificial mastication most closely approached the volatile pattern and abundance following human chewing.

In dry food analyses such as soya beans or bread often artificial saliva is added before mastication (van Ruth & Roozen, 2000; van Ruth et al., 2004; Poinot et al., 2004). In preliminary experiments, we did not find any effect of adding artificial saliva to the tomato during chewing. This lack of effect may be due to the high water content of tomato fruits and the absence of starch at the full ripening stage (Centeno et al., 2011). In further experiments, no saliva was added to the chewed samples.

Describing the release kinetics of volatile compounds while the food matrix is being chewed, may be as important as other commonly considered parameters such the odor perception thresholds and the Log odour unit values. The faster and more abundant the volatile emission, the more quickly and intensely the compound can reach the olfactory receptors and the greater will be the human perception. We assume that a volatile compound already produced during the fruit ripening and accumulated inside the fruit will be very effective in final aroma perception compared to a compound only produced after fruit maceration like C6 volatiles. In a recent work Tieman et al. (2012), investigating the role of volatiles in tomato aroma perception, suggested that the impact of aldehydes is less important than it was previously considered. The metabolic suppression of the lipoxygenase activity in transgenic tomato fruit, by the silencing of the LoxC gene, blocked the production of C6 volatiles without significant altering the overall final consumer preference (Tieman et al., 2012). This result indicates that there are not always clear correlations between volatile odour units and consumer flavour perception (Klee and Tieman, 2013) and confirms our hypothesis to consider the "release ratio" as an additional important factor for describing the aroma perception. As described here, C6 volatiles, as produced enzymatically after chewing, have a "release ratio" close to 1. Emission of these compounds from the chewed fruit increased in a gradual linear way.

PTR-MS allows to make a 3D volatile fingerprint of a product during maceration, by monitoring rapid concentration changes over time of aroma compounds at trace levels. This makes the instrument very suitable to characterize aroma features of different fruit types and to investigate the effect of growing and postharvest conditions. GC-MS analysis is, in this respect, much more elaborate and time consuming. However, the combination of these two analytical methods can provide an even more comprehensive qualitative and quantitative investigation on the volatile compounds involved in the aroma formation. The possibility of comparing the rapid PTR-MS characterization of large sample sets with GC-based analyses of a few selected samples can give valuable information to identify PTR-MS peaks and subsequent data interpretation. Two main limitation of PTR-MS analysis are the lack of proper identification of the measured compounds and the requirement to preselect some characteristic masses before the on line analysis in order not to decrease the analytic resolution. These disadvantages will be solved in future studies by using a PTR-TOF-MS. Without losing the performance of the quadrupole version, the PTR- TOF-MS provides improved mass resolution, better mass accuracy and shorter acquisition time (Soukolis et al., 2012).

This research partially confirmed results of Farneti et al. (2012): GC-MS analysis of tomato volatiles extract by using CAR-PDMS-SPME does not entirely cover the tomato volatiles profile. As a consequence, fingerprinting separation of the tomato lines investigated in this research based on multivariate statistical analysis of GC-MS data does not wholly correspond with the outcome of the sensory analysis. On the contrary, multivariate statistical results of PTR-MS data, obtained 30 and 120 seconds after the artificial mastication of the fruit, showed more close similarities with consumer sensory perception. Compounds that usually are not considered fundamental for the aroma characterization, such as acetone, ethanol and acetaldehyde, dominated the PTR-MS fingerprint and these may therefore also be important in flavour, either as direct aroma compound or in combination with other volatiles.

For a better understanding of the effects of volatile compounds on the flavour perception, aroma compounds should not be considered based solely on their individual

concentration since they do not interact in a linear way. More attention should be paid to the interaction dynamics between the molecule and human receptors. Combinations of different volatiles can act synergistically or antagonistically, changing the levels of detection for the individual components in the mix (Rospars et al., 2008, Ferreira, 2012).

5. Conclusion

The aim of this work was to develop an analytical system, based on PTR-MS analysis, to study the aroma profile perceived by consumers during eating of tomatoes that mimics, as close as possible, the release of volatiles during chewing in the human mouth. The great advance of such analytical system is the possibility to study the release kinetics of volatiles during eating and to adjust the method in such a way that the patterns and abundances closely match the in vivo condition. Moreover, the use of a solvent and incubation free methodology for real time analysis of volatiles excludes the possible formation of volatiles in product-unrelated chemical reactions.

For the overall understanding of the aroma profile all the volatiles may result essential, even those that are generally considered less important based on their odour perception threshold and odour unit values.

To our opinion this analytical methodology is ideal for fast and easy screening of breeding material based on VOCs profile, applicable not only for the tomato fruit, but also for other valuable fruit species. Moreover, the possibility to monitor at high sensitivity the emission of a large number of volatiles, connected to consumer perceived quality and also to ripening/senescence processes, make this technique suitable for physiological investigations related to VOCs production of several agro-industrial products.

6. Acknowledgments

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7. Supplementary material

Table S1. PTR-MS mass abundances (ppb) at 30 seconds after mastication of different tomato genotypes. Results are presented as the average of four independent samples.

m/z	Tom 1	Tom 2	Tom 3	Tom 4	Tom 5	Tom 6	Tom 7	Tom 8	Tom 9
41	32	18	17	16	29	11	15	25	12
42	13	7	8	6	9	5	6	8	4
43	56	38	42	31	47	29	45	49	42
45	192	120	114	115	144	77	125	252	274
47	11	8	9	7	10	8	10	12	13
49	20	12	21	10	23	11	13	14	5
51	8	4	7	5	6	5	7	4	10
57	44	29	35	29	30	23	36	39	24
59	316	241	293	275	243	150	307	159	128
69	17	9	9	9	11	6	10	15	7
81	80	50	84	68	44	54	87	85	42
83	36	22	40	21	33	12	47	24	19
84	56	17	16	12	33	9	15	29	9
85	20	8	11	10	18	7	10	15	10
87	22	17	12	10	18	8	11	17	11
95	8	4	9	5	7	8	7	6	5
99	13	10	14	9	10	8	14	13	9
101	7	5	8	6	7	5	6	6	6
110	3	5	4	4	4	5	4	5	3
TOTAL	*953*	*623*	*751*	*647*	*725*	*439*	*777*	*775*	*633*

Table S2. PTR-MS mass abundances (ppb) at 120 seconds after mastication of different tomato genotypes. Results are presented as the average of four independent samples.

m/z	Tom 1	Tom 2	Tom 3	Tom 4	Tom 5	Tom 6	Tom 7	Tom 8	Tom 9
41	73	45	35	34	65	30	49	66	22
42	20	12	9	9	14	7	11	15	7
43	134	97	87	70	108	72	124	131	69
45	378	280	217	214	327	178	321	541	515
47	20	14	15	12	18	13	21	22	16
49	48	32	40	22	53	31	33	39	10
51	20	13	16	11	17	12	24	19	15
57	132	102	92	75	106	80	141	142	56
59	466	379	362	358	353	229	469	282	177
69	39	22	17	16	27	15	26	37	14
81	389	288	275	222	264	231	446	454	140
83	119	73	82	46	98	59	146	86	47
84	101	33	23	19	58	16	32	56	15
85	44	26	22	20	40	17	33	44	18
87	38	32	18	18	36	15	25	40	18
95	11	8	15	9	10	13	9	11	6
99	46	31	30	21	28	24	54	48	20
101	17	12	10	8	10	11	18	14	10
110	4	4	4	4	5	4	3	5	3
TOTAL	*2100*	*1502*	*1370*	*1188*	*1638*	*1056*	*1983*	*2052*	*1177*

Table S3. Abundance of aroma volatile compounds in headspace of frozen and ground red tomato samples using SPME/GC-MS analysis. Results are presented as the average of four independent samples.

Compounds	Tom 1	Tom 2	Tom 3	Tom 4	Tom 5	Tom 6	Tom 7	Tom 8	Tom 9
3-Methyl-Butanal	0.28	0.09	0.15	0.07	0.32	0.06	0.07	0.43	0.08
Pentanal	0.63	0.68	0.62	0.67	0.43	0.68	0.76	0.51	0.62
1-Penten-3-one	3.22	3.09	3.04	3.06	2.51	2.86	2.90	3.12	2.68
Hexanal	28.07	29.54	33.10	26.14	33.16	31.04	34.12	30.38	30.03
Cis-3-Hexenal	11.23	9.79	7.84	6.40	12.25	13.21	8.40	5.90	9.79
(E)-2-Hexenal	44.80	47.08	45.33	38.59	41.17	36.14	43.49	36.93	34.92
(Z)-2-Heptenal	2.11	2.58	2.38	2.03	2.20	1.87	2.37	1.75	1.91
1-Nitro-Pentane	0.71	0.30	0.89	0.82	0.87	0.57	0.82	1.22	0.23
6-Methyl-5-Hepten-2-one	1.90	2.09	2.07	8.98	1.31	5.22	2.39	6.91	8.12
2-Isobutylthiazole	1.09	0.05	0.35	0.15	0.30	0.12	0.22	0.72	0.21
5-ethyl-2(5H)-Furanone	2.80	2.13	1.80	1.30	3.42	2.38	1.83	1.39	1.60
Hexanoic acid	0.56	0.52	0.65	0.73	0.54	0.65	0.50	0.98	0.81
Geranyl acetone	1.91	1.40	1.00	9.97	0.84	4.37	1.50	8.77	8.00
Beta Ionone	0.40	0.40	0.47	0.50	0.35	0.44	0.35	0.51	0.51
2-Cyclohexene-1,4-dione	0.30	0.24	0.31	0.59	0.32	0.38	0.27	0.48	0.48

Data are expressed in % of the total amount per tomato line

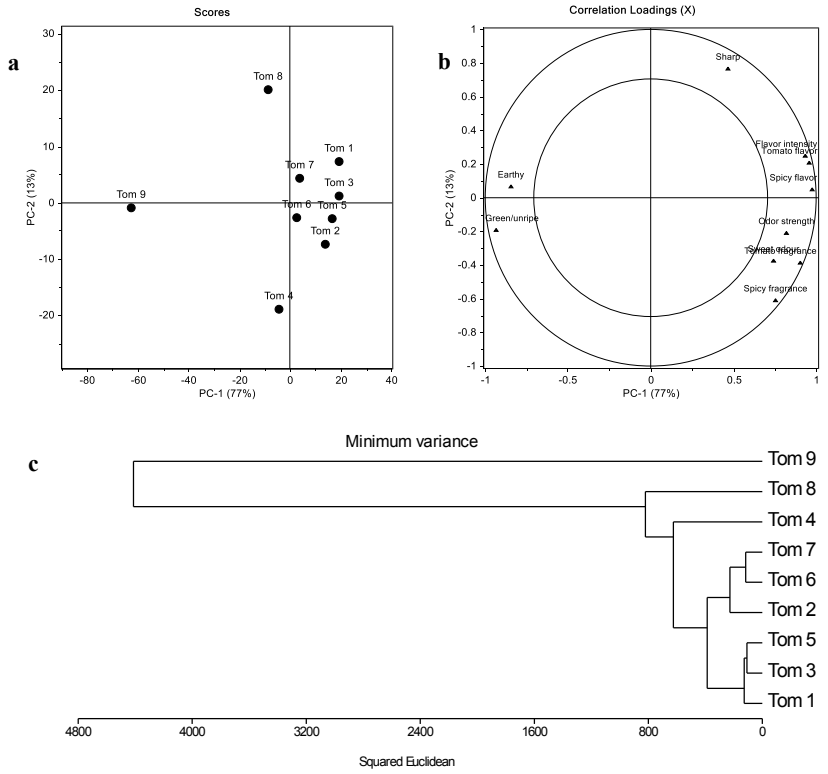

Figure S1. Sensory analysis of tomato genotypes. PCA score (a), loading plots (b) and HCA dendrogram (c) of tomato quantitative descriptive analysis (QDA) attributes. Each point is an average of 10 independent samples.

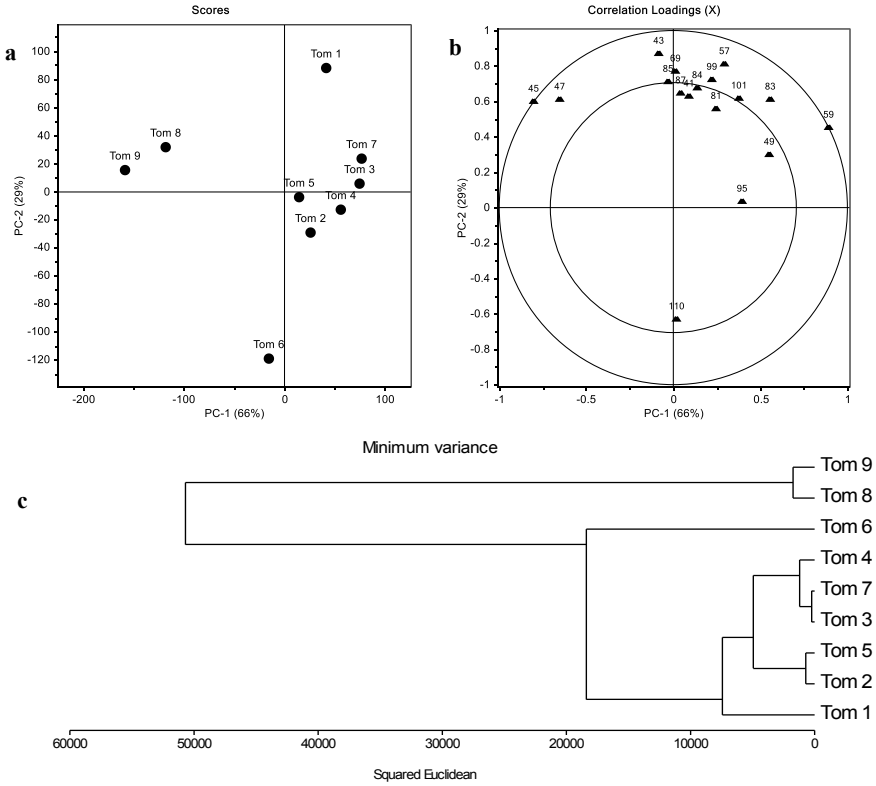

Figure S2. PTR-MS mass fingerprinting 30 seconds after chewing. PCA score (a), loading plots (b) and HCA dendrogram (c) of tomato volatiles measured by using PTR-MS 30 seconds after fruit mastication by the chewing device. Each point is an average of four independent samples.

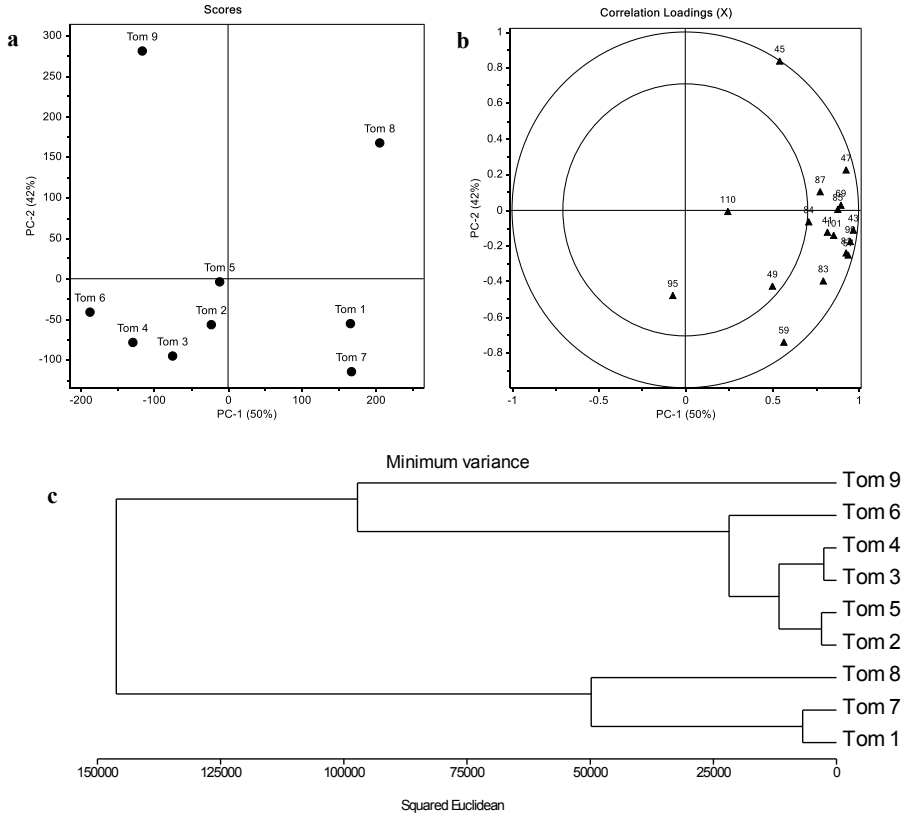

Figure S3. PTR-MS mass fingerprinting 120 seconds after chewing. PCA score (a), loading plots (b) and HCA dendrogram (c) of tomato volatiles measured by using PTR-MS 120 seconds after fruit mastication by the chewing device. Each point is an average of four independent samples.

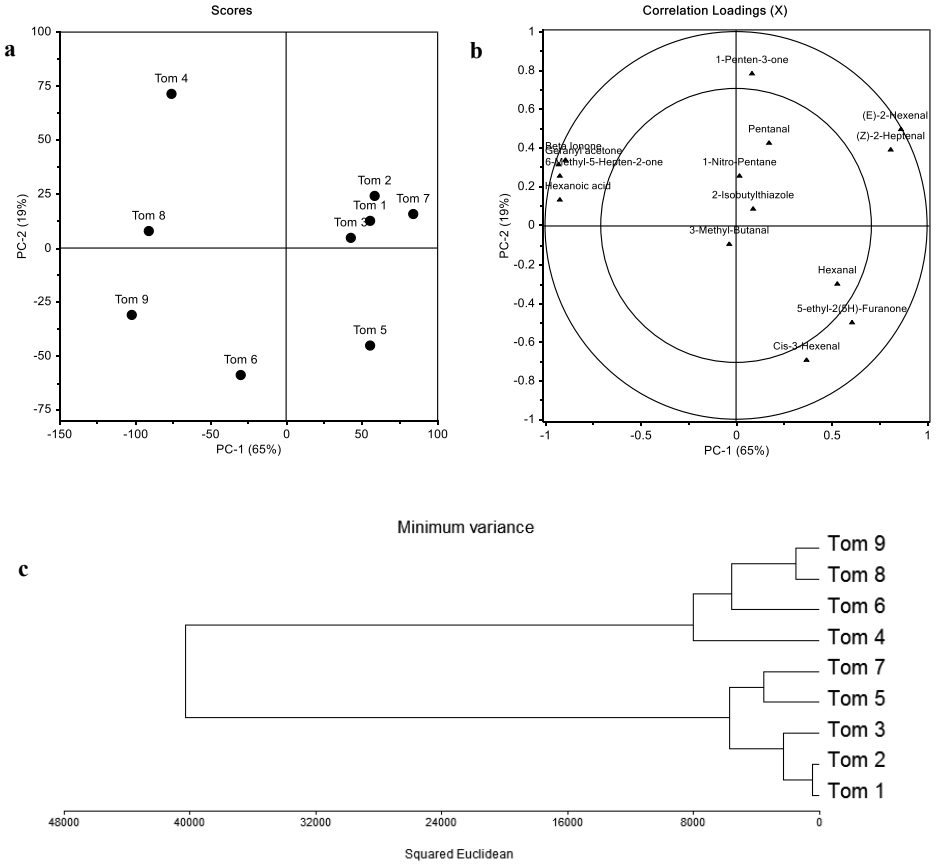

Figure S4. GC-MS analysis of volatiles. PCA score (a), loading plots (b) and HCA dendrogram (c) of tomato volatiles measured by using SPME-GC-MS. Each point is an average of four independent samples.

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Chapter 5

Chilling-induced changes in aroma volatile profiles in tomato

Abstract

Fruit and vegetables are regularly stored by consumers in the refrigerator at temperatures that may be well below the recommended storage temperatures for e.g. tomato, bell pepper and cucumber. Also in the distribution chain these products may (accidentally) be exposed to chilling temperatures. Apart from causing visible symptoms such as watery, sunken areas on the skin, chilling may also induce changes in e.g. fruit textural properties and flavor. The aim of this research was to investigate the effect of low temperature storage on tomato flavor and off-flavor production. To more closely mimic the real consumer aroma perception while eating, in addition to the standard Solid-phase Microextraction Gas Chromatography-Mass Spectrometry (SPME/GC-MS) analysis, volatiles were also measured using a chewing device connected to a Proton Transfer Reaction– Mass Spectrometer (PTR-MS). Aroma volatiles were assessed in red ripe tomatoes of the cvs Cappricia RZ (round truss) and Amoroso RZ (cocktail truss) stored at refrigerator temperature (4°C) and at room temperature (16°C and 22°C) for 20 days. The dynamics of aroma production when the fruit was brought from room to refrigerator temperature and vice versa was also measured.

After bringing the fruit from room to refrigerator temperature, the abundance of most volatiles was greatly reduced within 3 to 5h, closely following the decrease in fruit temperature. When temperature was restored to room temperature following varying times of cold storage, the abundance of most volatiles increased again but with a slight delay. With shorter cold storage times, the volatile abundance generally was restored to the same level as in the non-cold stored (control) fruit; at longer storage times the levels of main lipid and carotenoid-derived volatiles did not increase to the level of the control fruit while other volatiles (e.g. acetaldehyde, ethanol) reached levels well above levels in the control fruit. Overall the effects of low temperature storage on the decrease in volatile abundance was more pronounced in cv Cappricia RZ than in cv Amoroso RZ. On the contrary, the production of off-flavours following cold storage was more pronounced in cv Amoroso. Apart from changes in the overall abundance of the volatiles, marked changes in the volatile profile were observed in fruit stored

for longer times in the cold and this may at least in part explain the negative effect of cold storage on overall tomato flavor.

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1. Introduction

Providing more tasty fruit and vegetables is likely to increase their consumption. More effort and attention should be paid to improve and optimize flavour upon delivery to the consumer and flavour should be considered as a central trait to determine fruit postharvest quality. The end of flavour life, due to changes in sugar, acids and aroma volatiles and the development of off-flavours (mainly caused by fermentative metabolism) often precedes the end of shelf life as determined by visual and textural features (Kader, 2003; Kader, 2008). Fruit volatile compounds can be considered as the end products of fruit metabolic processes. Changes in fruit metabolism may cause unpredicted alteration of the fruit volatile composition during storage (Zhang et al., 2008). Metabolic pathways for volatiles biosynthesis, including those derived from amino acids, fatty acids and carotenoids, are diverse and often highly integrated with other portions of both primary and secondary metabolism (Mathieu et al. 2009; Dudareva and Klempien, 2013; Goff and Klee, 2006). Postharvest abuses, such as harvesting too immature fruit, mechanical injury during sorting and packing, extreme atmospheres, and improper temperature management have been related to altered aroma volatile profiles and altered flavour perception (McDonld et al. 1996; Sargent et al., 1997; Moretti et al., 1998; Maul et al. 1998; Maul et al., 2000; Tasdelen and Bayindirli, 1998; Fellman et al. 2003; Forney, 2008; Boukobza and Taylor, 2002; Mejia-Torres et al. 2009).

The influence of postharvest strategies to maintain quality for long-distance shipping and long-term storage on quality of red-ripe tomato were intensively studied over the last years (Beckles, 2012; Suslow and Cantwell, 2009). Exposure to storage temperatures below 13 °C may induce significant chilling injury (CI) in tomato fruit (King and Ludford, 1983; Saltveit, 2002; Saltveit, 2005; Farneti et al., 2012a). One of the negative effects of chilling injury on quality is aroma degradation. As determined by quantitative descriptive analysis as well as GC-MS analytical quantification tomatoes subjected to low storage temperatures have lower levels of (ripe) aroma, lower levels of tomato flavour and higher levels of off- flavour compounds when compared to tomatoes undergoing non-chilling temperature treatments (Bai et al., 2011; Boukobza and Taylor 2002; Krumbein et al., 2004; Maul et al., 2000; Zhang, et al., 2008). Off-flavor compounds are thought to influence the perception of other aroma compounds: for instance, ethanol and methanol at higher concentrations have been found to suppress perception of certain tomato aroma volatiles, such as hexanal, 3-methylbutanol and phenylethanol, while enhancing perception of other volatiles, such as trans-2-hexenal, hexanol, and 3-methylbutanal (Tandon et al., 2000; Abegaz et al., 2004).

Avoiding incorrect storage practices along all the distribution chain may not be sufficient to prevent fruit quality reduction, since home refrigerator storage of a variety of chilling sensitive commodities such as tomato, cucumber, bell pepper is still common practice (Farneti et al., 2012a). Among evident quality deterioration, such as fruit discoloration, lycopene degradation, softening and skin pitting (King and Ludford, 1983; Saltveit 2002; Saltveit 2005; Farneti et al., 2012a), home refrigerator storage at around 4- 6°C may cause a severe alteration of the tomato aroma profile and it could be considered as one of the most contributing factors to consumer complaints about inferior tomato flavour (Maul et al., 2000). According to Bai et al. (2011) tomato placed at room temperature after a period of cold storage should recover aroma volatiles until some point without reaching the control level. However

those results were limited to six-carbon (C6) aldehydes, such as hexanal, Z-3- hexenal and E-2-hexenal and corresponding alcohols.

The aim of this research was to investigate the effects of low temperature storage on tomato aroma compounds with particular attention on the degree of aroma reversibility and the release of off-flavour volatiles after rewarming.

2. Materials and methods

2.1. Fruit sampling and storage treatments

Tomatoes of the cvs. Cappricia RZ (round truss) and Amoroso RZ (cocktail truss) from breeding company RijkZwaan BV, The Netherlands, were obtained in two production seasons (2010 and 2012) from a commercial greenhouse operation in the south east of the Netherlands. Both cultivars were grown in the same greenhouse compartment under identical growing conditions. Homogenous batches of red ripe tomatoes from each cultivar were selected on the base of fruit size and colour as measured by a hand-held photodiode array spectrophotometer (Pigment Analyzer PA1101, CP, Germany) (Farneti et al., 2012a).

Tomato fruit of the first growing season (2010) were stored for 20 days at 4 ± 0.5 °C and at 16 ± 0.5°C in the dark. Storage temperature was recorded hourly by a data logger (KeyTag KTL-108, Comtest, South Africa). At five days intervals (at 5, 10 and 15 days) a tomato batch prior stored at 4°C was placed at 16°C. Samples for volatile analysis by Solid-phase Microextraction Gas Chromatography-Mass Spectrometry (SPME/GC-MS) were taken at the starting time of the experiment (T0), every five days for the storage at 4°C and 16ºC and after 1, 3 and 7 days after fruit were switched from 4°C to 16°C storage.

Tomato from the second growing season (2012) were stored for 12 days at 4 ± 0.5 °C and 22 ± 1°C in the dark. Tomato samples stored at 4°C and 22°C were each day analysed for volatile production by using a chewing device coupled to a Proton Transfer Reaction – Mass Spectrometer (PTR-MS). The frequency of analysis was increased to 5 measurements a day, with a time interval of about 3 hours, during the first 2 days after the fruit were brought from 22 to 4°C and after the fruit were brought from 4°C to 22°C (after 6 and 12 days at 4°C). Air storage temperature and tomato temperature were recorded by using thermo couples (Pico Tech., TC-08). For fruit temperature analysis thermo couples were placed both on the skin and approximately 1 cm inside two tomatoes of each cultivar.

2.2 SPME/GC-MS analysis

Samples of fresh tomatoes (five fruits per cultivar for each temperature treatment) were quickly cut into quarters and immediately frozen in liquid nitrogen. The samples were stored at -80°C and ground in liquid nitrogen in a metal electric grinder prior to analysis. The profiling of volatiles was performed using the headspace SPME/GC-MS method described by Tikunov et al. (2005) with slight modification (Farneti et al., 2012b). Frozen fruit powder (1 g fresh weight) was weighed into a 20 mL crimp cap vial; the vial was closed and incubated at 30°C for 10 min. The closed vials were then sonificated for 5 min. Thereafter the samples were incubated at 60°C with agitation for 30 min and the headspace volatiles were extracted from the vial headspace and injected into the GC-MS apparatus (Trace GC Ultra, Thermo, IL, USA) equipped with a

TriPlus SPME autosampler (Thermo, IL, USA). The extraction was done by inserting a Carboxen/PDMS SPME fiber (Supelco, Zwijndrecht, Netherlands) to the vial headspace during the last 20 min of the incubation under continuous agitation and heating at 60°C. The fiber was desorbed for 15 min at 250°C in the injection port of the GC in splitless mode. Chromatography was performed on RTX-WAX (Restek, Bellefonte, PA) capillary column (60 m length x 0.32 mm i.d., 0.25 μm film thickness) with helium as a carrier gas. The GC interface and MS (DSQ II, Thermo, IL, USA) source temperature were both 250°C. The GC temperature program started at 40°C (5 min), was then raised to 240°C at a rate of 5°C per min, and finally was held at 240°C for 10 min. The total run time including oven cooling was 60 min. Compound identification was based on mass spectra matching in the standard NIST library and retention time of authentic reference standards.

2.3 PTR-MS analysis

For tomato volatile analysis by PTR-MS, one intact tomato was placed into the 800 ml glass cuvette of the chewing device (Farneti et al., 2013) that was flushed with 1 L/h of clean air. Before crushing the fruit, the headspace VOCs concentration of the intact fruit was measured for 140 seconds to ensure reaching the equilibrium. The chewing was done through manually pressing the notched plunger 5 times within 10 seconds. VOCs analysis continued for about 3 minutes following mastication. Each measurement was done in 3 replications. The head space was drawn from the chewing device at 0.5 L/h by a vacuum pump for on line analysis into the PTR as described by Farneti et al. (2012b). The PTR-MS apparatus consists of: (i) an ion source in which H3O+ ions are produced, (ii) a drift tube where the trace gases are ionized by proton-transfer reaction with H3O+ ions, (iii) a collision dissociation chamber, (iv) a quadruple mass filter and (v) a secondary electron multiplier. (For details, see Holger et al., 2012). The drift tube operated at a pressure of 2.08 mbar and was heated at about 55°C. The mass number of the detected ion is given by the molecular mass of the substance plus the mass of the single proton mH (mRH+ in atomic mass units).

Based on preliminary experiments with tomato fruits and based on earlier reported data (Farneti et al., 2012b) we selected and monitored 20 predominant masses from the overall tomato volatile spectrum (m/z 33, 41, 42, 43, 45, 47, 49, 51, 57, 59, 69, 81, 83, 84, 85, 87, 95, 99, 101,110). Mass spectrometric data were collected using a dwell time of 0.2 s per mass.

2.4 Data analysis

The multivariate statistical software Canoco 4.5 (Biometris-Plant Research International, Wageningen) was used for principal component analysis (PCA): both GC-MS and PTR-MS data were LOG transformed prior to the analysis.

3. Results and discussion

3.1. VOCs composition of round and cocktail truss tomato

In order to assess the effect of storage temperature on red ripe tomato volatile emission, tomatoes were first selected based on size and colour.

The volatile compound profile of round truss (cv Cappricia RZ) and cocktail truss tomato (cv Amoroso RZ) as analyzed by SPME/GC-MS is shown in table 1. The analysis allowed for the identification and quantification of 15 main compounds considered essential for the overall tomato aroma perception (Farneti et al,. 2012b; Buttery et al., 1987; Buttery et al., 1989; Krumbein and Auerswald, 1998; Krumbein et al., 2004; Tandon et al., 2000). In accordance to Farneti et al. (2012b) red ripe tomatoes of cv Amoroso RZ were characterized by a 30% higher volatile content (per FW unit) in comparison to the cv Cappricia RZ.

Table 1. Main volatile compounds of red ripe tomatoes of cvs. Amoroso RZ and Cappricia RZ assessed at harvest by SPME-GC-MS. Data are expressed in percentage of the total amount.

Volatile compound	**Amoroso (%)**	**Cappricia (%)**
Hexanal	58.05	61.36
Trans-2-Hexenal	27.26	19.19
1-Nitro-Pentane	5.08	4.55
3-Methyl-Butanal	3.24	3.26
5-ethyl-2(5H)-Furanone	1.73	1.66
Hexanoic acid	1.26	1.63
1-Penten-3-one	1	1.92
Pentanal	0.81	1.43
6-Methyl-5-Hepten-2-one	0.64	3.42
Cis-2-Heptenal	0.37	0.46
2-Cyclohexene-1,4-dione	0.29	0.33
Geranyl acetone	0.1	0.18
Cis-3-hexenal	0.07	0.1
2-Isobutylthiazole	0.07	0.47
β-Ionone	0.03	0.05
Total concentration (µg/g)	**314.8**	**209.6**

It was confirmed that aldehydes are the most abundant volatiles in tomato, especially the ones derived from lipoxygenase activity: hexanal and trans-2-hexenal amount to more than 80% of the total volatile headspace content of tomato. In addition, results confirmed that the ratio between hexanal and hexenals (trans-2-hexenal and cis-3-hexenal) varies in different tomato types with round truss tomato characterized by a higher value (3.2 for cv Cappricia RZ)

than cocktail truss tomato (2.1 for cv Amoroso RZ). Despite their high levels and relatively low odor threshold concentrations, the importance of aldehydes for tomato liking has recently been questioned (Tieman et al., 2012, Farneti et al., 2013). Other compounds considered more important for the characteristic tomato flavor and liking are the compounds derived from carotenoids metabolism, such as geranyl acetone, β-ionone and 6-methyl-5-hepten-2-one (Goff and Klee, 2006) and, the ones derived from the amino acids leucine, isoleucine and phenylalanine such as methyl butanal (Klee and Tieman, 2013; Tieman et al., 2006; Buttery and Ling, 1993). Among these classes of VOCs, 6-methyl-5-hepten-2-one, turned out to be a distinctive compound to discriminate the two tomato cultivars in this investigation; the relative headspace concentration of 6-methyl-5-hepten-2-one was much higher in cv Cappricia RZ (3,42 %) than in cv Amoroso RZ (0,64 %). For all the other compounds no substantial differences (> 2 times) in relative amount were observed (table 1).

Volatiles measured using a SPME fiber do not necessarily fully reflect the actual head space composition of the tomato samples, due to discrimination of volatiles on basis of partition coefficients and adsorption kinetics (Berna et al., 2004). For instance, some relevant compounds such as methanol, ethanol and acetaldehyde that are highly present in tomato fruits were not detected (Farneti et al., 2012).

3.2 VOCs changes during storage assessed by SPME/GC-MS

Tomato volatile profiles of both cvs Amoroso RZ and Cappricia RZ were significantly affected by the storage condition. Figure 1 shows the PCA biplots of the volatiles of tomatoes during storage measured by SPME/GC-MS. Variation of volatile content during storage is explained by the first two components for 64.1 % and 79.2% for cvs Amoroso RZ and Cappricia RZ, respectively. According to these results, red ripe fruit of cv Amoroso RZ exhibit a more stable volatile profile during the 20 days of storage at 16°C in comparison to cv Cappricia RZ. This may be related to the sustained ripening during the storage period of cv Cappricia RZ tomatoes that were not fully ripe at harvest as indicated by an increase in red coloration during storage (data not shown). From the loading plots of the PCA analysis (data not shown) tomatoes of cv Cappricia RZ during the 16°C storage were described by an increase in the concentrations of carotenoid-derived volatiles such as geranyl acetone and β-Ionone whereas this was not observed in cv Amoroso RZ.

The VOC profile of 4°C stored fruits significantly differed from 16°C stored fruit. For both cultivars the effect of 4°C storage was similar: the longer the period of cold storage the greater the distance between the 4°C samples and the control in the PCA plot. These differences are mainly caused by a decrease in abundance of almost all the volatile compounds.

In accordance with Bai et al. (2011) and Boukobza et al. (2002), VOC profiling of fruit brought from 4 to 16 °C shows that the flavour recovery is negatively affected by the length of the period of cold storage: the longer the cold storage period, the less the recovery of the VOCs. This was more evident for cv Cappricia RZ than for cv Amoroso RZ. After 5 days at 4°C fruit of both cultivars show an almost complete recovery of all volatiles whereas after 10 and 15 days at 4°C the recovery was only partial.

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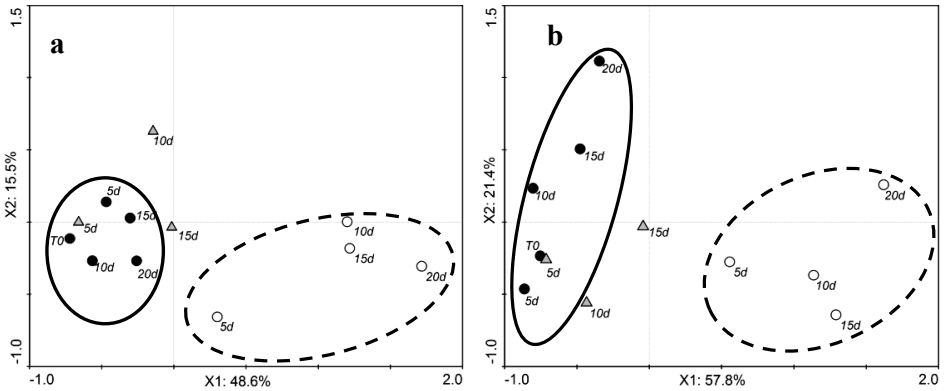

Figure 1. PCA scores of tomato volatiles assessed by SPME-GC-MS of the cvs Amoroso RZ (a) and Cappricia RZ (b). Measurements were done during storage for up to 20 days at 4°C (○) and 16°C (●) and after one day of restoration at16°C following a storage period of 5, 10 and 15 days at 4°C (▲); the number next to each point indicates the number of days of storage. Each point is the average of 3 measurements of samples from a batch of 5 fruit. The data belonging to 16 and 4°C have been circled with a solid and dash line, respectively.

3.2.1 VOCs derived from lipid metabolism

Dynamics of selected lipid-derived volatiles content during storage are shown in figure 2. Values, measured by SPME/GC-MS, are reported as a percentage relative to the initial concentration of the volatile at harvest (=100%). Tomato fruit of both cultivars, stored at 16°C, were characterized by a rather stable level of hexanal, being the most abundant volatile compound (about 60% of total volatile concentration). Conversely, content of trans-2-hexenal, the other main aldehyde of the tomato VOC profile, steadily decreased during the 16°C storage period. After 20 days of storage the concentration of trans-2-hexenal was only 70 and 50% of the initial value in cvs Amoroso RZ and Cappricia RZ, respectively. On the contrary, the other lipid derived VOCs, namely cis-3-hexenal and 1-penten-3-one, were rather stable during the 16°C storage. These results confirmed that tomato storage for long periods may reduce aldehydes content and consequently also the "freshness" flavor perception provided by these compounds (Bai et al. 2011; Krumbein et al., 2004; Maul et al., 2000).

Depletion of aldehydes was generally more severe during the storage at chilling temperature (4°C), showing different patterns in fruit of the two tomato cultivars. A decrease in the two main aldehydes was observed; this decrease was more sudden and more drastic in cv Cappricia RZ than in cv Amoroso RZ. In fruit of cv Amoroso RZ the aldehyde content (hexanal and hexenals) decreased in a gradual and linear way during the 20 days of cold storage while in fruit of the cv Cappricia RZ aldehyde content rapidly decreased within 5 days of storage. This effect of low temperature storage on volatile concentration was less evident for cis-3-hexenal and 1-penten-3-one.

Upon switching from 4 to 16°C, production of volatiles increased and was stable within one day. Hexanal levels were generally not restored to original levels but, especially after a

longer period at 4°C, were much reduced. Levels of trans-2-hexenal and its isomeric form cis-3-hexenal were restored to levels similar to those of 16°C stored fruit; levels of 1-penten-3-one, in both cultivars, showed an increase over the level in fruit at 16°C.

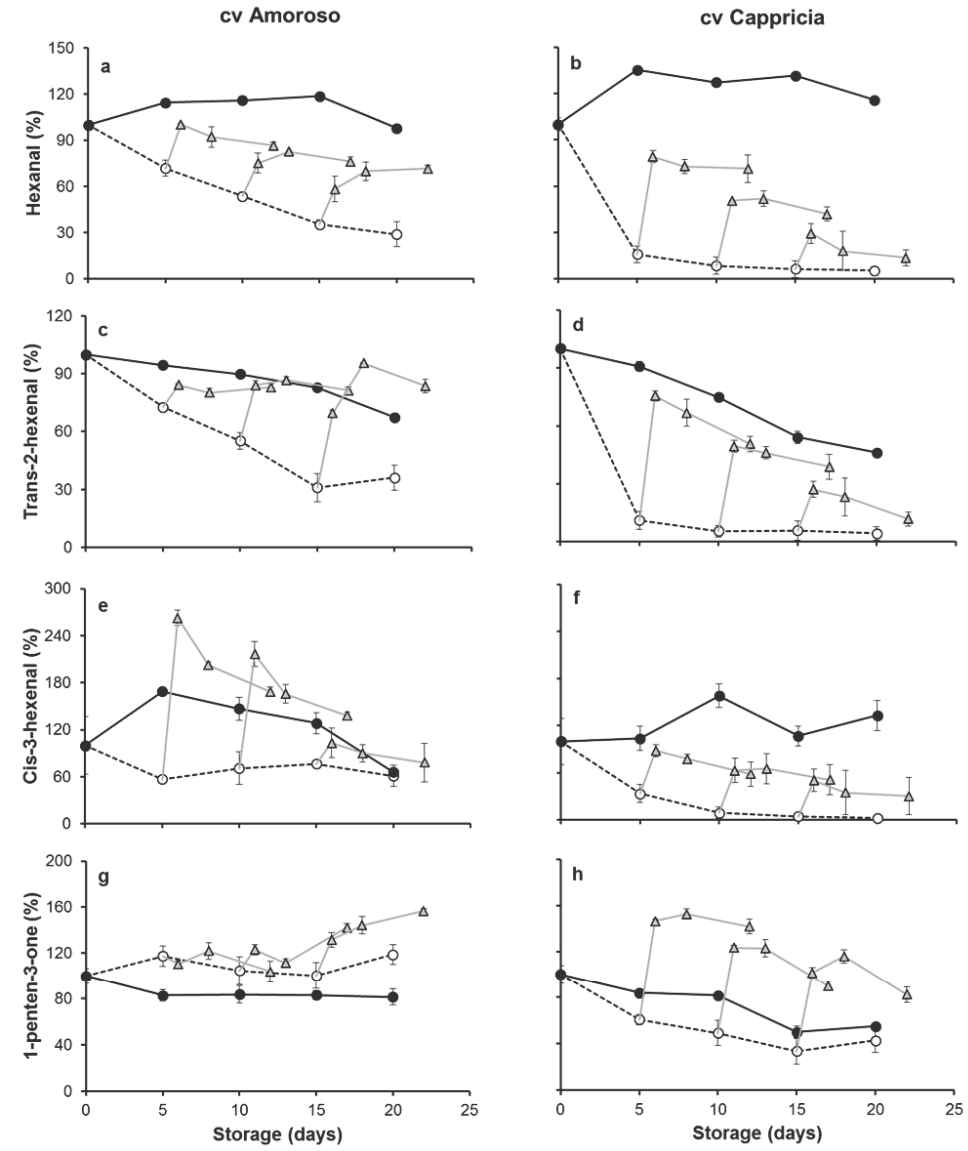

Figure 2. Volatiles derived from lipid metabolism assessed by SPME-GC-MS analysis of cvs Amoroso RZ (left column a, c, e, g) and Cappricia RZ (right column b, d, f, h). Measurements were done during storage for up to 20 days at 4°C (○) and16°C (●) and after restoration at16°C following a storage period of 5, 10 and 15 days at 4°C (▲).Each point is the average of 3 measurements plus standard deviation of samples from a batch of 5 fruit. Volatile abundance at t=0 was set at 100%.

In accordance with Bai et al. (2011) fruit of both cultivars resumed the production/emission of lipid derived volatiles within 24 hours when transferred from the storage at 4°C to 16°C, nevertheless without reaching the same level of the fruit stored continuously at 16°C. The more prolonged was the cold storage, less abundant was the resumed content of aldehydes, particularly of hexanal. Comparing the aldehyde volatile dynamics of cvs Amoroso RZ and Cappricia RZ tomatoes, cv Cappricia RZ appeared to be generally more sensitive to the low temperature storage; volatile production shows a more dramatic change after bringing the fruit to 4°C as well as after bringing the fruit back to 16°C. In cv Cappricia RZ fruit, that has been stored for some time at 4°C and thereafter brought to 16°C, the levels of hexanal and hexenals are much lower and the level of 1- penten-3-one is much higher than in fruit continuously stored at 16°C. From this observation it may be expected that the fruit will have a less intense tomato flavour and the flavour may be less pleasant since 1-penten-3- one is commonly considered as a tomato off-flavor, due to the unpleasant organoleptic characteristics (Baldwin et al., 1998).

3.2.2 VOCs derived from carotenoid metabolism

Effects of storage temperature on volatiles derived from carotenoid precursors, namely geranyl acetone, β-ionone and 6-methyl-5-hepten-2-one, are shown in figure 3. Compared to the lipid-derived volatiles, the response of carotenoid-derived aroma compounds to cold storage temperature was more cultivar dependent and less drastic. Geranylacetone and β-ionone content (figure 3 a, b, c, d) were not noticeably affected by cold storage in the cv. Amoroso RZ whereas fruit of cv Cappricia RZ showed already after 5 days of storage at 4°C a reduction in the levels of these volatiles of over 50%. Differences in volatile production between fruit stored at 4° and 16°C increased throughout the 20 days of storage period in cv Cappricia RZ due to an continuous increase at 16°C and a steady decline of the levels at 4°C. As a consequence of the sustained postharvest ripening of cv Cappricia RZ as reflected in the continued postharvest coloration of these fruit (data not shown), the concentration of volatile compounds derived from the carotenoids lycopene and β-carotene, may have increased. On the contrary, "Amoroso" fruit, evidently harvested at fully ripe stage, did not show any significant increase in both red colour and carotenoids-derived volatiles. The production of 6-methyl-5-hepten-2-one (figure 3 e,f) after bringing the fruit to 4°C showed a completely different pattern in the two cultivars. In cv Amoroso RZ the production was about 3 times higher at 4°C than at 16°C while in cv Cappricia RZ no clear effect of cold storage was observed.

When restored to 16°C after a period of cold storage (4°C) production of geranyl acetone and β-ionone increased in fruit of cv Cappricia RZ (figure 3 b,d) but not to the levels of the fruit stored continuously at 16°C. The longer the storage duration, the less restoration of volatile production was observed. In cv Amoroso RZ (figure 3 a,c) no clear effect of the temperature change was seen.

A different trend was observed in 6-methyl-5-hepten-2-one following the temperature change from 4 to 16°C (figure 3 e, f). In cv Amoroso RZ fruit the production decreased at 4°C and was restored to the original levels within 2 days at 16°C; in cv Cappricia RZ the production also decreased at 4°C but, as it had not increased after bringing the fruit to 4°C, it now felt well below the original level

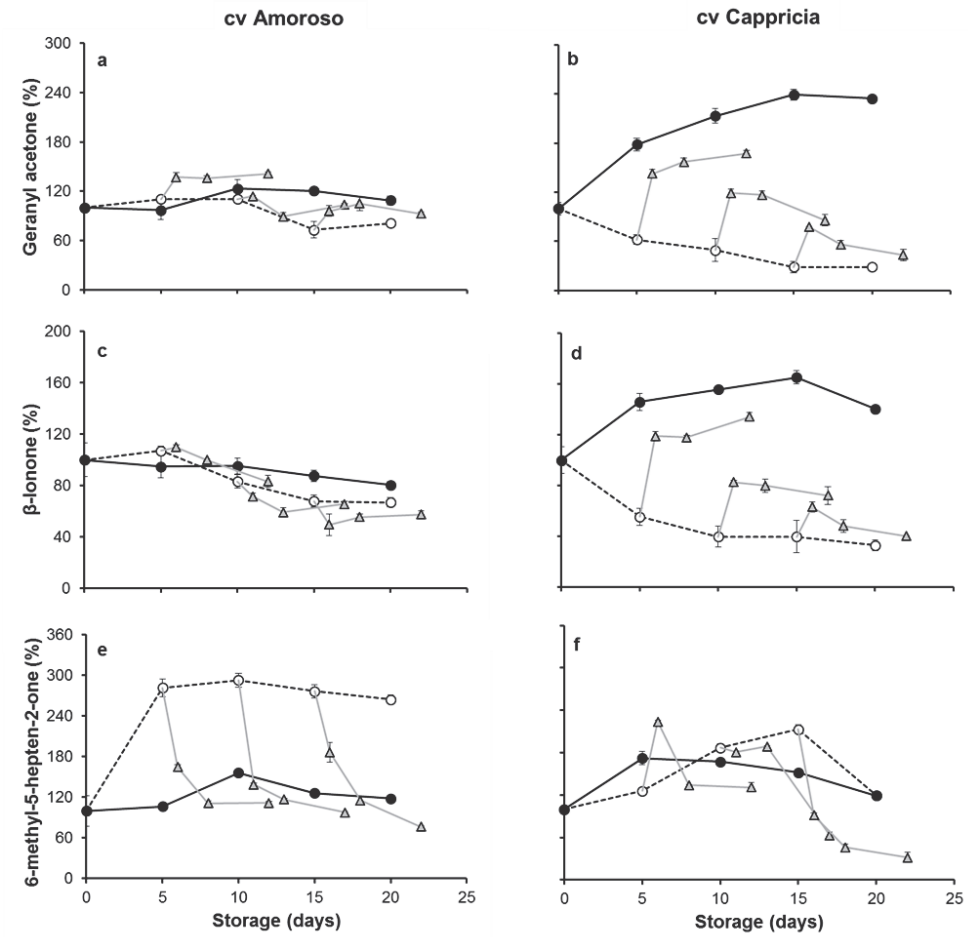

Figure 3. Volatiles derived by carotenoid metabolism assessed by SPME-GC-MS analysis of cvs Amoroso RZ (left column a, c, e) and Cappricia RZ (right column b, d, f). Measurements were done during storage for 5, 10 and 15 days at 4°C (○) and 16°C (●) and after restoration at16°C following a storage period of 5, 10 and 15 days at 4°C (▲). Each point is the average of 3 measurements plus standard deviation of samples from a batch of 5 fruit. Volatile abundance at t=0 was set at 100%.

These results are consistent with the research of Farneti et al. (2012a) on the effect of low storage temperature (below 10°C) on carotenoid synthesis and degradation in cocktail and round truss tomato (cv Amoroso RZ and Cappricia RZ). Following prolonged storage at chilling temperature a decrease in lycopene content was observed due to a decreased synthesis and/or an increased breakdown (Farneti et al. 2012a). Carotenoid-derived volatiles can be considered as degradation products of carotenoids or of carotenoid precursors. The reactions responsible for these conversions presumably take place in the chloroplasts and chromoplasts (in red fruit). At present the exact biosynthetic pathways are not entirely known (Mathieu et al.2009, Simkin et al. 2004). Geranylacetone is most likely derived from the compounds between phytoene and

ζ-carotene (including ζ-carotene); 6-methyl-5-hepten-2-one from the compounds between ζ-carotene (excluding ζ-carotene) and α-carotene, including lycopene; β-ionone is presumably derived from β–carotene (figure 4).

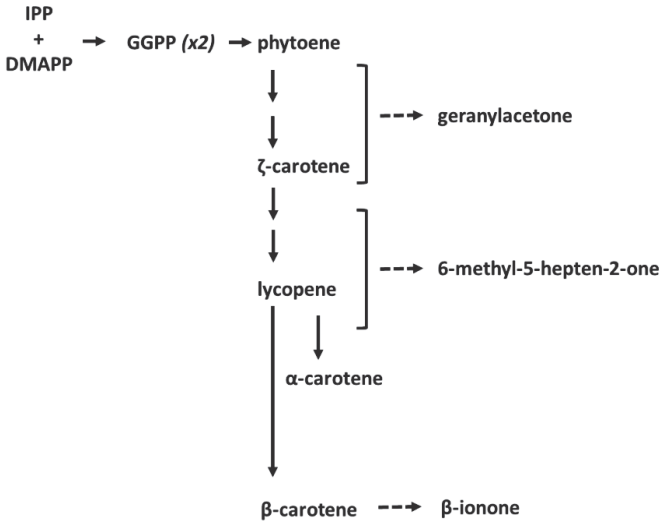

Figure 4. Scheme outlining the putative steps in carotenoid metabolism. Carotenoid biosynthesis begins with a C5-compound, isopentenyl-diphosphate (IPP), and its isomer dimethylally-diphosphate (DMAPP). Chain elongation leads to the formation of the C20-compound geranylgeranyl-diphosphate (GGPP). The head-to-head condensation of two GGPP molecules produces the first, colourless carotene, phytoene. A series of desaturation reactions lead to the coloured chromophore of lycopene, and subsequent cyclization reactions produce the β-carotene ((adapted from Simkin et al., 2004 and Lewinsohn et al., 2005).

Particularly 6-methyl-5-hepten-2-one production was shown to be correlated with phytoene synthase activity, a key enzyme in the synthesis pathway of lycopene. Indeed, this molecule can be synthetized from lycopene precursors such as prolycopene, δ-carotene, and neurosporene (Mathieu et al., 2009; Tieman et al., 2006, Baldwin et al., 2000, Lewinsohn et al., 2005; Simkin et al., 2004) as well as from lycopene oxidation.

In the present experiments, at room temperature, the levels of the three apocarotenoid volatiles were stable over time in Amoroso RZ. As Amoroso RZ fruit did not show any further colour development at room temp we may assume that the carotenoid pathway was operating at a low steady state level. On the contrary, Cappricia RZ tomatoes were harvested red but still showed further increase in lycopene during storage at room temp. At room temperature a significant increase in geranylacetone and β-ionone production was observed, with only a minor increase in 6-methyl-5-hepten-2-one concentration (figure 4). This may indicate a higher activity of the carotenoid pathway in Cappricia RZ compared to Amoroso RZ. Based on

absolute levels (in mg/g), the production of 6-methyl-5-hepten-2-one is about 4 time higher in Cappricia RZ compared to Amoroso RZ, (table 1).

During storage at low temperature, the production of 6-methyl-5-hepten-2-one considerably increased in Amoroso RZ fruit whereas the other carotenoid volatiles were not much affected (figure. 3 a, c, e) This indicates that in Amoroso RZ the cold storage may specifically affect enzymes involved in conversion of carotenoid precursors to 6-methyl-5-hepten-2-one. This increased synthesis may alternatively be due to the accumulation of lycopene precursors. The latter may be caused by an arrest of one of the last steps of lycopene synthesis. When a tomato fruit is restored to room temperature the last steps of lycopene synthesis may resume causing a decreasing of 6-methyl-5-hepten-2-one in favour of lycopene building up. As the β-ionone concentration was not much affected by the low temperature storage in Amoroso RZ this may indicate that the conversion of lycopene to β-carotene was not significantly affected. A diminished amount of lycopene or of its precursors and a continued breakdown of lycopene will lead to gradual loss of lycopene and of red colour. Following a switch from cold to room temperature, original levels of volatiles are restored, indicating that effects of low temp on carotenoid enzymes is reversible and that no permanent damage is done.

When Cappricia RZ is stored at low temp, a decrease in geranylacetone and β-ionone was observed but 6-methyl-5-hepten-2-one was not changed. This indicates a decreased activity of the carotenoid pathway. Considering the decrease in geranylacetone and β-ionone the unchanged level of 6-methyl-5-hepten-2-one may be viewed as a relative "increase". This may indicate that, as in Amoroso RZ, also in Cappricia RZ the increased conversion of lycopene or its precursors to 6-methyl-5-hepten-2-one may at least partly explain the decreased lycopene levels in the cold. Following a switch from cold to room temperature, the levels of volatiles are not restored to original levels but stay lower than the original (also 6-methyl-5-hepten-2-one). This indicates that carotenoid pathway in Cappricia RZ may be more sensitive to cold than in Amoroso RZ as permanent loss of activity occurs.

3.3 VOCs changes during storage assessed by PTR-MS and chewing device.

Volatile profiling assessed by PTR-MS combined with a chewing device may give a more reliable reflection of real flavor perception during eating than the GC-MS measurements on frozen samples. The maceration procedure mimics the tissue breakdown that occurs in vivo during mastication while the PTR-MS is monitoring the development of flavor profile in a time span comparable to the food residence time in the mouth during chewing. Fruit volatile profile of cv. Amoroso RZ and Cappricia RZ, stored at 4°C and 22°C, were analyzed by PTR-MS combined with a chewing device. The rapidity of this technique, not only allowed more measurements along the storage period, but also a more accurate monitoring of the VOC profile variations when storage temperature was changed from 4°C to 22°C.

Total volatiles emitted from tomato matrix during mastication, as affected by fruit temperature, is shown in figure 5. The internal temperature of the fruit was influenced, as expected, by the volume/mass of the fruit with fruit of cv Amoroso RZ (around 30 gr of weight) characterized by a slightly faster change in temperature than cv Cappricia RZ (around 80 gr of weight). Decrease in the emission of volatiles followed with slight delay the decrease in fruit

temperature in both cvs (figure 5 a, c). Within 5 to 6 h following the change from 22 to 4°C, both the temperature and the volatile production were at a stable level. Following the change to 22°C after 6 days at 4°C the increased production of volatiles followed the temperature change, but with a somewhat more pronounced delay (figure 5 b,d). Down-regulation of volatile emission by low temperature can be explained by an activity decline of the enzymes involved on volatile production (Bai et al. 2011) as well as a lower rate of volatilization (Henry's law).

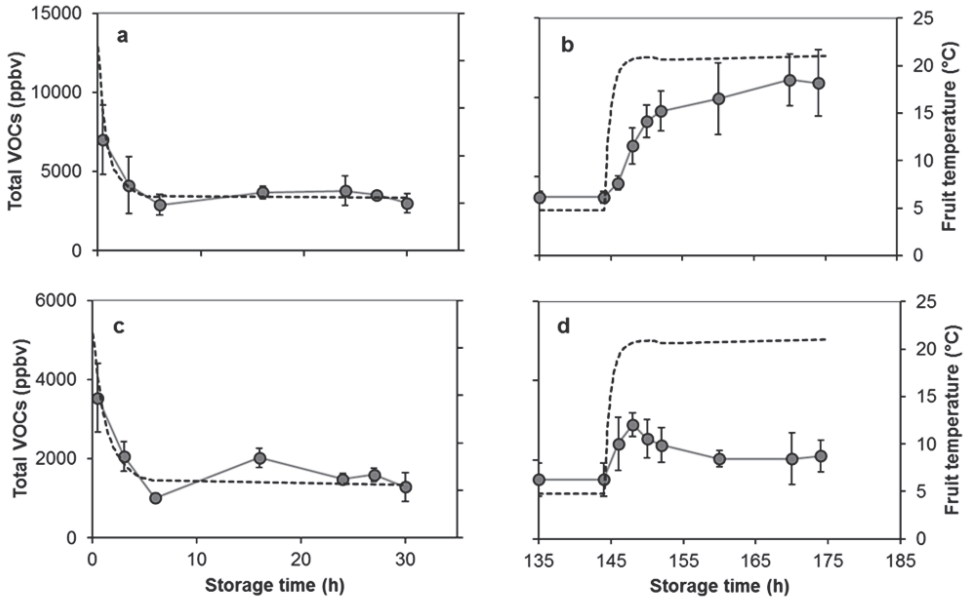

Figure 5. Comparison of internal fruit temperature (black dot line) and the changes of total volatile production (grey circles) assessed by PTR-MS coupled with a chewing device of tomato fruits of cv Amoroso (a,b) and Cappricia (c,d). Each point is the average plus standard deviation of measurements of 3 tomatoes measured 30 seconds after the artificial mastication.

The volatile profiles of tomatoes of cvs Amoroso RZ and Cappricia RZ following storage for different times (3, 6, 9, 12 days) at 22 and 4°C and measured at 22°C after 6 and 12 days of storage at 4°C by PTR-MS coupled with the chewing device, are shown in the PCA biplots (figure 6). Variations in volatile content during storage are explained by the first two components for 73.9 % and 77.7% for Amoroso RZ and Cappricia RZ, respectively. Similar to the result obtained by SPME/GC-MS analysis (figure 1), it is noticeable a separation of the tomato volatile profiles in two main clusters, one for each storage temperature (4°C and 22°C). In addition, similarly to SPME/GC-MS results (figure 1), tomato of cv Amoroso RZ showed a more stable volatile profile during the twelve days of storage at 22°C than cv Cappricia RZ. This may be a result of the more homogeneous and complete ripening stage of cv Amoroso RZ at harvest. The longer the storage time at 4°C, the greater the distance to the control (t0) in the biplot for both cultivars.

Fruit kept at 4°C for 6 and 12 days and then restored at 22°C were characterized by a different volatile profile, not determined only by overall lower compound concentration but also by an increased production of off-flavors (figure 6).

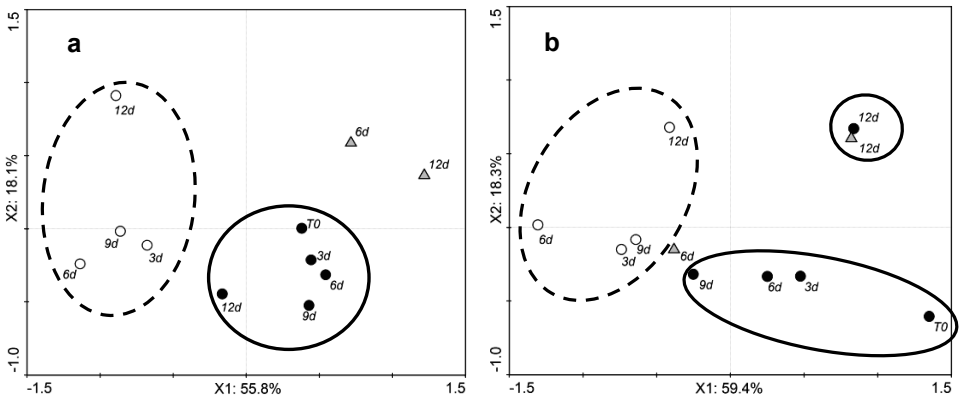

Figure 6. PCA scores of tomato volatiles assessed by PTR-MS (coupled with the chewing device) of the cvs. Amoroso RZ (a) and Cappricia RZ (b). Measured were done during 12 days of storage at 4°C (○) and 22°C (●) and after one day of restoration at 22°C following a storage period of 6 and 12 days at 4°C (▲); the number next to each point indicates the number of days of storage. Each point is the average of the measurements of 3 individual tomatoes measured 30 seconds after the artificial mastication. The data belonging to 22 and 4°C have been circled with a solid and dash line, respectively.

Volatile patterns in fruit stored for 6 and 12 days at 4°C and thereafter brought to 22°C show a different behavior in cv Amoroso RZ compared to cv Cappricia RZ (figure 6). According to the PCA biplots of the volatiles of the two cultivars, differences between these samples and the control were mainly explained by variation of the second principle component. According to loadings plots (not shown), the variation explained by the second principle component is mainly correlated with the concentration of compounds commonly associated with tomato off flavor, namely methanol, acetaldehyde, ethanol and acetone, masses 33, 45, 47 and 59, respectively. More detailed information on the concentration of these off-flavour compounds is presented in figures 7 and 8. The four off flavor compounds had similar behavior related to the storage temperature as the overall volatile patterns (figure 5): a decrease and increase along with the temperature changes. Concentration of VOCs is drastically reduced already after 6 hours of cold storage and thereafter it stabilizes, at a low level for the remaining cold storage period. Immediately after restoration to 22 °C the production and release of all four off -flavor compounds restarted, more evidently for the fruit of cv Amoroso RZ than cv Cappricia RZ (figure 7, 8). The longer the storage period at 4°C the greater the off flavors production after the restoration at 22°C. After 12 days of cold storage, production of acetaldehyde and ethanol in fruit of cv Amoroso RZ was almost doubled in comparison to the fruit stored constantly at 22°C. Fruit of cv Cappricia RZ appeared more stable with less fluctuation with respect to off flavor production related to temperature and storage period (figure 8).

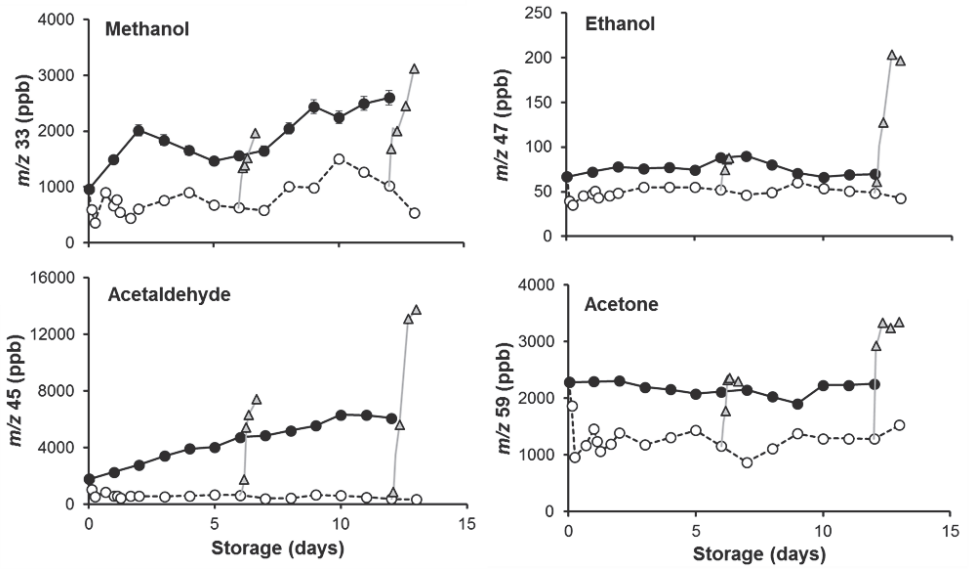

Figure 7. Off-flavor volatiles assessed by PTR-MS (coupled with the chewing device) of cv. Amoroso RZ. Measurements were done during 13 days of storage at 4°C (○) and 22°C (●) and immediately after temperature restoration to 22°C following a storage period of 6 and 12 days at 4°C (▲). Each point is the average of the measurements of 3 individual tomatoes plus standard deviation measured 30 seconds after the artificial mastication.

Ethanolic fermentation is a two-step process in which pyruvate is first decarboxylated to acetaldehyde by pyruvate decarboxylase, and acetaldehyde is subsequently converted to ethanol by alcohol dehydrogenase (Tadege et al., 1999). Acetaldehyde and ethanol are commonly considered as anaerobic fermentation products but the ethanolic fermentation process can also take part in aerobic situation as a stress-signal response, especially under abiotic stress such as dehydration or chilling. (Tadege et al., 1999).

Methanol can be considered as well as an indirect stress response molecule since it can be produced from the hydrolysis of methyl ester groups in pectins: one of the most evident symptoms of senescence or induced stress in fruit, especially in tomato, is an increased rate of softening caused mostly by an increase of the pectin methyl esterase activity (Micheli, 2001; Frenkel et al.,1998; Anthon and Barrett, 2010). Tomatoes of the cv. Amoroso RZ were characterized by a higher rate of softening than cv Cappricia RZ (data not shown) at room temperature as well as at 4°C, that matches with the higher methanol production (figure 7).

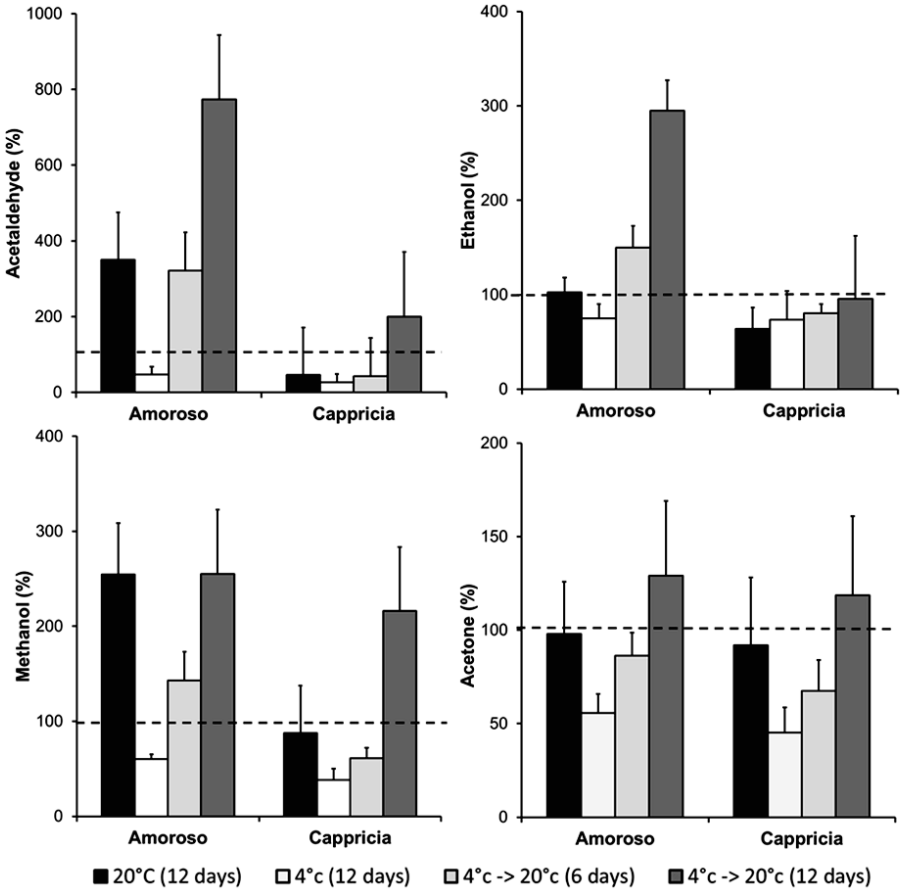

Figure 8. Off- flavour volatiles measured by PTR-MS (coupled with the chewing device) expressed as percentage of the initial value assessed at harvest (t0=100%; dotted line). Each point is the average plus standard deviation of measurements of 3 individual tomatoes measured at 30 seconds after the artificial mastication. Prior low temperature stored samples were assessed one day after restoration to room temperature.

4. Conclusion

A combination of sampling methods, including SPME-GC-MS and PTR-MS coupled with a chewing device, was used to study the volatile composition of cocktail and round truss tomato during cold storage and following restoration to room temperature. The aim of this research was to investigate the role of low storage temperature on tomato flavor and possible off-flavors production. Home refrigerator storage is still a common practice for the consumer to prolong the shelf life of fruit and, temperature abuse may also occur in the distribution chain. The emission of volatiles was related to the fruit temperature, with much lower total volatile emissions at 4°C compared to room temperature suggesting that down and up-regulation of

volatile emission by temperature can be explained either by a direct control of the enzymes activity involved on volatile production or by a control in the rate of volatilization.

Following restoration of temperature, volatile production generally went up again, but may reach lower levels than in fruit continuously stored at room temp. In addition, the volatile profile of prior cold stored fruit was different from fruits continuously stored at room temperature, with a relatively higher abundance of volatiles considered off-flavors such as methanol, ethanol and acetaldehyde.

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Chapter 6

General discussion

The quality of fruits and vegetables has to be considered as a central trait to optimise fruit chain management (Mowat and Collins, 2000; Benner et al., 2003; Costa et al., 2001). In order to satisfy consumer's demands more effort and attention have to be devoted, from a scientific and practical background, to improve and optimize quality upon delivery to the consumers. The component attributes of quality vary with context. The choice of what to measure, how to measure and knowing what values are acceptable depends on the intended use of the product and the available measurement technology (Abbott 1999). Defining and quantifying quality proprieties of fruit and vegetables, in relation with distinct segments of the production chain, needs comprehensive investigations. It implies a synergy of analytical approaches from different branches of knowledge. The basic approach of this work was mainly based on a quantitative analysis of still unrevealed quality traits of tomato fruit.

The work reported in this thesis aims to enhance the knowledge and understanding of some major pre- and post-harvest factors affecting tomato quality. This work focuses in particular on production chain factors that, up to date, were still poorly or far from completely studied such as:

- Effect of storage temperature on tomato quality parameters: pigment compounds (chapters 2.1 and 2.2) and on aroma compounds (chapters 5)
- Effect of greenhouse climate management on tomato postharvest quality (chapter 3)
- Development of analytical techniques for volatile analysis (chapters 4.1 and 4.2)

1. Effect of storage temperature on tomato quality

Temperature is the most important environmental factor in the post-harvest life of tomato fruit and it can severely affect the final nutrient and quality composition of the fruit as it influences ripening and senescence (Hertog et al.,2007; Tijskens and Evelo, 1994; Maul et al., 2000; Stern et al., 1994; Javanmardi and Kubota, 2006)). Fruit ripening is a complex, genetically programmed process that culminates in dramatic changes in colour, texture, flavour, and chemical compositions (Campbell et al., 1990). Ripening of tomato has been widely studied, from both a physiological and genetic point of view, with the main objective to extend tomato texture, colour and shelf life. One way to prolong the shelf life of tomatoes is to harvest them at an immature stage. Harvesting immature fruit can adversely affect the flavour and the overall quality. Storage of tomatoes at temperatures above or below the optimal biological temperature (around 16°C) will decrease the acceptability of the fruit (King and Ludford, 1983; Saltveit 2002; Saltveit 2005). Storage of tomatoes and other subtropical fruits at temperature between 0 and 12 °C induces chilling injury and flavor decay (Bai et al., 2011; Boukobza and Taylor 2002; Krumbein et al., 2004; Maul et al., 2000; Zhang, et al., 2008). The major chilling injury symptoms in tomatoes appear in mature-green and breaker tomato. These symptoms include a failure to ripen normally, surface pitting and increased fungal decay.

In Chapter 2.1 and 2.2 we show that chilling injury also affects red ripe tomato causing a visible discoloration and lycopene content depletion. The decrease of the lycopene content induced by low temperature storage may be caused by lycopene fragmentation. Lycopene quenches highly reactive singlet oxygen (O_2^-) and traps peroxyl radicals ($ROO^{\cdot}$) that results in break down products like acetone (chapter 5), 6-methyl-5-heptan-2-one (Chapter 5), leavulinic aldehyde and glyoxal (Conn et al., 1991; Palozza, 1998). Lycopene degradation during storage not only reduces the nutritional and presumed health promotional value of the product but also affect the visual quality since the colour is one of the main quality attribute considered by a consumer. Based on the results presented in this thesis (chapters 2.1, 2.2, 5) and on still unpublished data low temperature lycopene breakdown in tomato has a genetic component since there are differences between genotypes grown at the same growing conditions. On the other hand, also growing conditions play a role as tomato of the same cultivar, harvested in different seasons or periods of the year, were differently affected by this abiotic physiopathy.

Avoiding incorrect storage practices along all the distribution chain may be not enough to prevent fruit quality reduction since storage in the home refrigerator is still a common practice for the consumer (chapter 2.1). Next to discoloration (chapters 2.1 and 2.2), home refrigerator storage also causes an alteration of the tomato aroma profile (chapter 4.1 and 5), specifically lower flavour compound content and higher off-flavour production. Based on this it might be that home refrigerator storage is one of the most contributing factors to consumer complaints about low tomato quality. In order to enhance or maintain quality of tomato fruit consumers must be better informed on optimal storage conditions, for example with clear information on the package. Plant physiologist, breeders and refrigerator unit producers might be able to create and market refrigerators with different temperature controlled compartments, each one optimal for the conservation of one type of fruits and vegetables.

2. Effect of greenhouse climate management on tomato postharvest quality

The current quality of a fruit is very much determined by the initial (harvest) quality. Products generally become unacceptable if a certain threshold for an important quality parameter (e.g. texture, colour and off-flavours) has been reached.

It has been found that growing conditions may have a significant effect on tomato postharvest ripening behaviour and presumably also on the final fruit quality after storage (Schouten et al., 2007). To date greenhouse climate control strategies have been used to optimise crop characteristics and yield but not much attention has been paid to the effects on fruit quality. How greenhouse climate management affects tomato quality during postharvest storage and handling is still unclear.

The concept of closed or semi closed greenhouses has recently been introduced in protect cultivation to improve the energy efficiency and the crop yield (Heuvelink and Bakker, 2008). They combine new ways of temperature, air humidity and carbon dioxide management with closure of the greenhouse cover. Mostly, since there are limited ventilation losses, the CO_2 concentration remains higher than in conventional greenhouses, and the temperature is, instead, more efficiently controlled by cooling (heath exchanger) systems. On the other hand cooling system may generate a vertical temperature gradient when cooling is applied in the lower part of the greenhouse (Opdam et al., 2005; Heuvelink and Bakker, 2008; De Gelder et al., 2012) with possible consequences to the physiological processes of the plant and/or of the fruit.

Several studies revealed the effect of climate factors on growth rate and final fruit size of tomato (Adams, 2001, Bertin, 2005; Qian et al., 2011) but, to date, there is a lack of knowledge about the effect of the combination of these environmental factors on quality attributes. In chapter 3 we investigated how different semi-closed greenhouse climate management may affect the postharvest quality of tomatoes during the production season. Compared to the open greenhouse, semi-closed greenhouses produced heavier and firmer fruit at the commercial harvesting stage based on colour while sugar and acid levels resulted hardly affected. Fruit maturity, based on the firmness assessment, could be linked to the vertical temperature gradient, which produces a lower temperature environment during the last phases of fruit development, and to the CO_2 levels in the different greenhouses. It was assumed that red ripe tomatoes are already predominantly losing firmness when still attached to the plant. A lower temperature in the last growing phase, caused by the air temperature gradient (chapter 3), could have slowed down the process of firmness decay causing a different synchronization with the lycopene synthesis. A reduction of the storage temperature from 24 to 16°C reduces the rate of lycopene synthesis (chapter 2.2) not as drastically as for the firmness decay rate (figure 1, unpublished data). Assuming that fruits attached or detached from the plant have a comparable physiological behaviour during the last phases of ripening (from mature green to red ripe), we can elucidate that the altered colour/firmness synchronization between tomatoes of greenhouses with different climate management is mainly controlled by temperature. This seems to confirm the hypothesis that a low temperature in the last growing phase slows the process of firmness decay. In addition, also CO_2 might affect the synchronisation between colour and firmness maturity as Islam et al. (1996) demonstrated that CO_2 enrichment may increase tomato colouring.

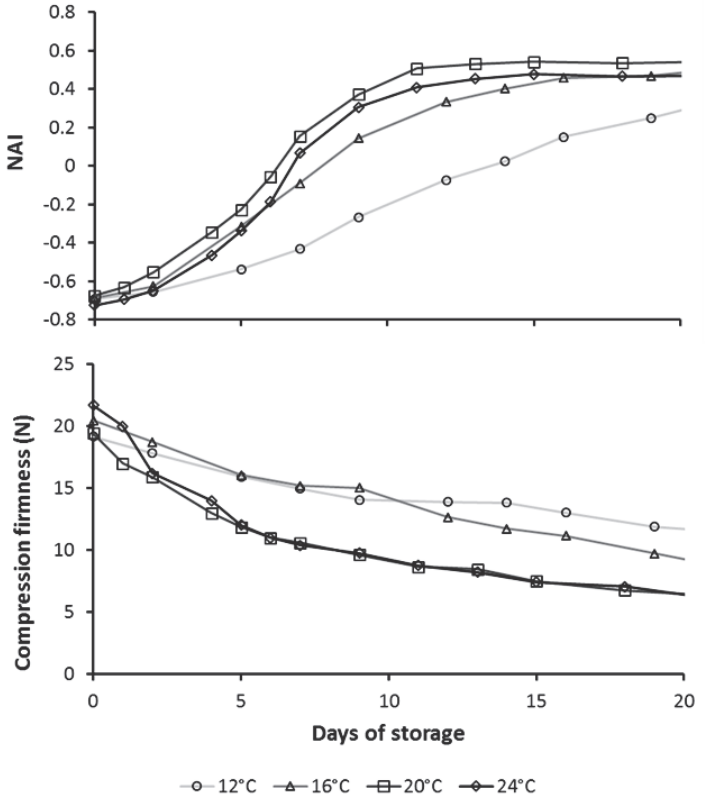

Figure 1. Colour development, expressed as NAI, and firmness (N) decay of tomatoes (cv Cappricia RZ) harvested at mature green stage and stored at 12, 16, 20, and 24 °C.

Additional to greenhouse effects also resolute effects of the harvest month were observed for both firmness and sugar to acid ratio. This monthly variation in the maximum firmness (F_{max}) showed an alike trend as found for the monthly initial sugar levels, suggesting a plausible direct relationship between sugar content and the part of firmness generated by cell turgor.

These results depicted how important is to connect postharvest quality analysis and growth management in order to optimize the acceptability of a fruit product. Moreover combining non-destructive firmness measurements with kinetic modelling may improve the amount of information we can gain on storability and fruit physiology in connection with growth condition and season variability. In this case postharvest storage experiments result useful not only to improve the storability of fruit and vegetables but also to better comprehend the biological variability of a batch produced by the morphological and physiological alterations caused by environmental growth condition. In fruit and vegetable products, biological variation is often the largest source of variation if compared with random and systematic errors related to data gathering (Tijskens and Schouten, 2009).

3. Analytical techniques to evaluate quality

Analytical techniques are needed to ensure that products have the appropriate physicochemical properties (Abbott 1999). The physiochemical properties of fruit (optical, rheological, flavour, and stability) ultimately determine their perceived quality, sensory attributes and behaviour during production, storage and consumption.

Box 1. Physiochemical properties of fruit and vegetables

Optical properties are determined by the way electromagnetic radiation in the visible region of the spectrum interacts with the product by a specific combination of absorption, scattering, transmission and reflection.

The rheological properties are determined by the way that the shape of the product changes in response to some applied force.

The flavour of a food is determined by the way that certain molecules in the food interact with receptors in the mouth (taste) and nose (smell) of human beings. The perceived flavor of fruit depends on the type and concentration of flavour constituents within it, the nature of the food matrix, as well as how quickly the flavour molecules can move from the food to the receptors in the mouth and nose.

The stability is a measure of its ability to resist changes in its properties over time. These changes may be chemical, physical or biological in origin.

The quality and desirability of a food product is determined by its interaction with the sensory organs of human beings, e.g., vision, taste, smell, feel and hearing. Individuals' perceptions of sensory attributes are often fairly subjective, being influenced by such factors as trends, nutritional education, climate, age, health, and social, cultural and religious patterns (Deliza and MacFie, 1996; Shewfelt, 1999). Although sensory analysis is often the ultimate test for the acceptance or rejection of a particular food product, there are a number of disadvantages: it is time consuming and expensive to carry out and tests are subjective. Furthermore, it cannot be used to provide information about the safety, composition or nutritional value of a food (Shewfelt, 1999). For these reasons objective analytical tests, which can be performed in a laboratory using standardized equipment and procedures, are often preferred for testing quality properties.

In order to define perceived quality, techniques are needed that simulates human perception as closely as possible. The question is whether it is needed to have measurement techniques with high accuracy and sensitivity that often imply a total modification of the food matrix by chemical extraction and long incubation time, or if it is better to use techniques with a lower sensitivity and accuracy but online detection.

Box 2. Criteria to characterise analytic techniques

Precision: a measure of the ability to reproduce a response performed by the same scientist (or group of scientists) under unchanged conditions.

Reproducibility: a measure of the ability to reproduce a response by scientists using the same experimental approach and plant material but changed measurement conditions.

Accuracy: a measure of how close one can actually measure the true value of the variable being measured.

Simplicity of operation: a measure of the ease with which relatively unskilled workers may carry out the analysis.

Speed: the time needed to complete the analysis of a single sample or the number of samples that can be analysed in a given time.

Sensitivity: a measure of the lowest concentration of a component that can be detected by a given procedure.

Specificity: a measure of the ability to detect and quantify specific components within a food material, even in the presence of other similar components.

Safety: many reagents and procedures used in food analysis are potentially hazardous e.g. strong acids or bases, toxic chemicals or flammable materials.

Destructive/Non-destructive: Some analytical methods require irreversible alterations, whereas in others it remains intact.

On-line/Off-line: some analytical methods can be used to measure the properties of a food during processing, whereas others can only be used after the sample has been taken from the production line.

Cost: the total cost of the analysis, including the reagents, instrumentation and salary of personnel required to carry it out.

Next to the challenge to analytically quantify sensorial perceived quality traits, is the difficulty of working with the large variation in quality properties of fruit within a batch (Tijskens et al., 2003; Hertog et al., 2004). For this reason it is feasible to decrease the analytical power of measurement, mainly accuracy and sensitivity, and increase the speed of analysis as to measure all samples in a batch within a certain unit of time. Examples of analytical techniques suitable for quality assessments that are able to quickly measure samples in a batch but that sacrifice some of accuracy and sensitivity considered in this thesis, are:

- Nondestructive assessment of color pigments, lycopene and chlorophyll, made by VIS spectroscopy (Pigment Analyzer) (Chapters 2.1, 2.2, 3).
- Nondestructive firmness evaluation by materials testing machine (Zwick Z2.5/TS1S) and acoustical firmness sensor (AWETA) (Chapter 3).
- PTR-MS combined with a chewing simulator (Chapters 4.1, 4.2, 5).

3.1. Colour and pigment assessment

Colour is the first quality attribute of a food product to stimulate a consumer's desirability (Costa et al., 2011; Grossman and Wisenblit, 1999; Crisosto et al., 2003; Leon et al., 2006; Nisha et al., 2011; Bayarri et al., 2001; Francis, 1995). Colour is the first indication of the perceived quality when making a purchase decision. Colour determines also the optimal harvesting time or the ideal storage condition (Tijskens and Evelo, 1994; Schouten et al., 2007) and therefore also defines the management strategy of the production chain. Moreover, the colour evaluation of tomato fruit is a practical, indirect way, to estimate the antioxidant potential since the orange/red colour is mainly determined by two carotenoids (lycopene and β-carotene) presented in the tomato pericarp (Gray et al., 1994; Arias et al., 2000). In this thesis commonly used and innovative analytic techniques to compare colour and pigment compounds in tomato fruit are reported.

- *Visual matching*: describes colours of fruits and vegetables using colour charts and guides.

Pros: fast, simple, and a cheap non-destructive evaluation.
Cons: subjective, lacking in precision and reproducibility.

- *Light reflectance meter*: measures colour on the basis of the amount of light reflected from the surface of the product. Examples of commonly used techniques are Minolta tristimulus colorimeters or RGB image analysis (chapter 2.1).

Pros: objective evaluation of colour similar to human perception. The analysis is fast, simple, reproducible and non-destructive.
Cons: the measurement assesses only the peel colour. Results lack specificity in terms of the total pigment compound concentration (e.g. lycopene) (chapter 2.1). Since both colours are quantified by tristimulus colorimeters based on the same parameter (a*) (figure 2) it is impossible to separate red and green pigments.

- *Optical spectroscopy*: the measurement is based on light remittance (photodiode array spectrophotometer) through the skin, into the pericarp and again through the skin and allows assessment of all fruit pigments, such as chlorophyll, carotenoids and anthocyanins. Examples of used devices are Pigment Analyzer PA1101 (chapters 2.1, 2.2, 3) and DA-meter (Ziosi et al., 2008).

Pros: Precise, fast and user friendly portable devices. Non-destructive quantification of colour compounds (for tomato lycopene and chlorophyll) results in a high correlation with the pigment concentration as obtained by HPLC (chapters 2.1 and 2.2). Possibility to separate red and green pigments when they are simultaneously present in the tissue (figure 2). Robustness calibration.
Cons: results may be cultivar dependent. Necessity to make a calibration per cultivar. Easy to reach the saturation point of analysis; this limit can be overcome by changing settings of the instrument, for instance increasing the light emission power or the photodiode capability.

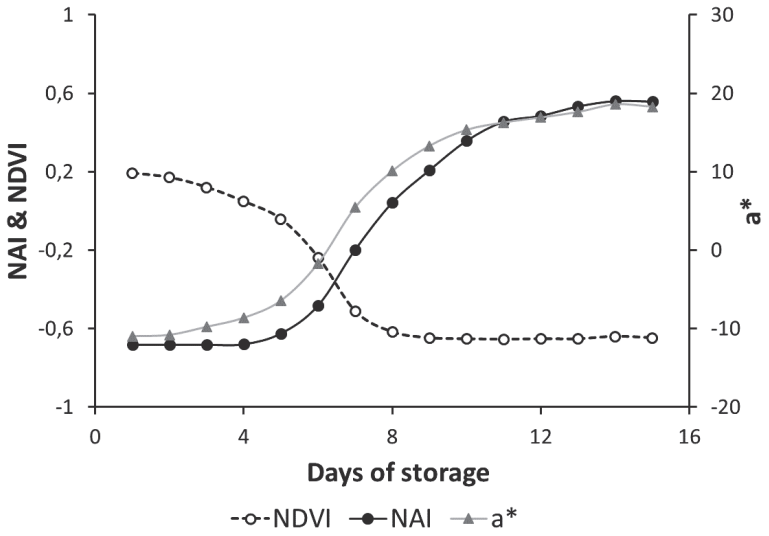

Figure 2. Comparison of tomato colour measurements done respectively by Pigment Analyzer (NAI and NDVI) and Minolta tristimulus colorimeter (a*). Colour of intact mature green tomatoes (cv Cappricia RZ) was assessed non-destructively during 15 days of storage at 20°C till fruits had reached the red ripe.

Results presented in chapters 2.1 and 2.2 revealed that remittance VIS spectroscopy is a very accurate and fast method to non-destructively assess colour pigment levels (mainly chlorophyll and lycopene) in pericarp tomato tissue, although there is a slight cultivar specific interaction. This might be related to the thickness of the pericarp, as this differs per tomato type, or to different physical properties of the peel. Remittance VIS spectroscopy measurements, in combination with tristimulus colorimeter (chapter 2.1) and mathematical kinetic modelling (chapter 2.2), may allow a precise colour phenotyping, necessary for screening existing and new tomato genotypes. Quantifying the colour performance would allow, for instance, to classify genotypes that i) can be harvested early due to the high amount of lycopene precursors at harvest, ii) can ripen irrespective of the temperature in the logistic chain, or iii) can be less sensitive for lycopene breakdown at low temperature storage.

3.2. Texture assessment

Texture is a quality attribute that combines a wide range of structural and mechanical properties of a food perceived in the hand or in the mouth of the consumer (Pascua et al., 2013; Szczesniak, 2002; Chaib et al., 2007; Costa et al., 2012). Texture of fruit and vegetables can be judged by a sensory panel or by market experts. Horticulturists use firmness to describe the mechanical properties of the fruit tissue particularly when measured as the force required to push a cylinder probe to a predetermined depth into the fruit flesh (Harker et al., 1997).Fruit firmness can be estimated by different techniques including the measurement of force-deformation curves, the analysis of impact forces, the measurement of acoustic responses to vibrations and impacts, the

measurement of optical properties, and nuclear magnetic resonance (Ruiz-Altisent et al., 2010). Commonly used techniques to assess fruit texture can be classified in puncture, compression, and acoustic response tests.

• *Puncture test* measures the force required for a probe to penetrate a pre-specified depth into a sample. The test involves both compression and shearing and simulates biting of a food. It is the most common way to assess firmness and it is usually carried out by using a hand-operated penetrometer or, more precisely and accurately, by a materials testing machine (e.g. Zwick or Instron).
Pros: easy and practical technique to assess firmness. The outcome of analysis, expressed as an amount of force (N), is generally well correlated with the hardness perceived during a food consumption. It is possible to assess acoustic parameters related to texture, such as crispiness or crunchiness if a high resolution microphone is combined with to the material testing machine
Cons: It is an invasive and destructive methodology. Analysis done by using a hand-operated penetrometer may result in operator dependent results that lack precision and reproducibility.

• *Compression tests* are often conducted under uniaxial loading between two plates, or between a plate and a probe, and the sample is allowed to expand freely in the other two directions. This technique is somewhat imitative of the tactile sensation when a fruit is in the consumer hands. Analysis are assessed by using a materials testing machine (e.g. Zwick or Instron) (chapter 3) or the Durofel.
Pros: at limited compression ranges it is a non-destructive methodology to assess firmness, mainly related with the elasticity of the product. In several fruit, such as tomato, kiwifruit or mango, the outcome of the analysis resulted well correlated with fruit flesh hardness. In tomato, repeated recordings of firmness during time using the same fruit is possible without causing damages (chapter 3).
Cons: In several typology of fruit this measurement may not correlate with the hardness of fruit flesh.

• *Acoustic response tests* are used to estimate the overall firmness of the fruit. The response of fruit and vegetables to vibrations depends on their modulus of elasticity, their mass and their shape. Different types of vibrations can be used, commonly acoustic or mechanical (which in some cases are very similar). Using a microphone or a piezoelectric sensor, acoustic methods measure the signal issued by the fruit after making it vibrate by means of a small impact. The acoustic signal captured is Fourier transformed and the main frequency is calculated and expressed as stiffness (Schotte et al., 1999).
Pros: it is a fast, easy to use and non-destructive. Analysis done on tomato fruits showed a good correlation with compression firmness assessed using Zwick testing machine (figure 3).
Cons: The acoustic stiffness parameter depends on fruit shape and dimension or by the skin proprieties.

Combining non-destructive firmness measurements with kinetic modelling enhances the amount of information we can gain on storability and fruit physiology (Schouten et al., 2007). In chapter 3 we reported on results of tomato firmness as influenced by greenhouse climate

management and harvest period of the year. Firmness measurements over the season, assessed non-destructively with the Zwick testing machine, allowed an evaluation of the effects of growing conditions. Results showed that the differences in firmness behaviour between greenhouse types in one month can be mainly allocated to fruit maturity differences. This is a vivid example of how predictions on firmness behaviour based on a single firmness evaluation can be incorrect.

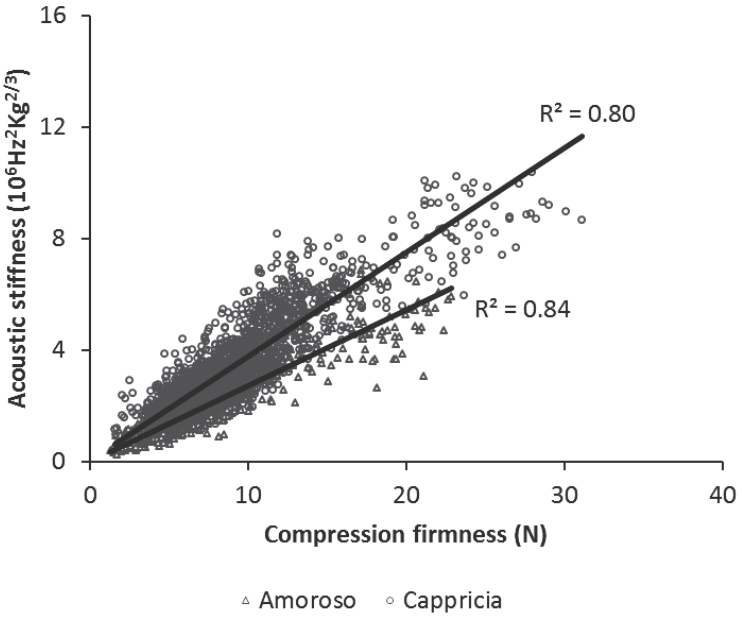

Figure 3. Correlation between limited compression firmness measured by ZWICK and stiffness measured by AWETA. Measurements were carried out on tomato fruits of cv. "Amoroso" and "Cappricia" during three weeks of storage at 16, 20, 24 °C.

3.3. VOCs assessment

Monitoring of VOCs from fruit and vegetables needs analytical techniques that are capable of dealing with challenging issues: first, the need of separating and quantifying VOCs in complex gas mixtures; second, the need to detect concentrations that may span a large range, from trace levels to parts per million or more; and third, the need to track concentrations that rapidly change over time (Biasioli et al., 2011). Because of these experimental constraints, the ideal methodology for VOC monitoring should be highly selective, with high sensitivity and dynamic range, and with high time resolution. The benchmark analytical method for VOC identification and quantification is currently *gas chromatography–mass spectrometry* (GC-MS). Although very valuable and, in many cases, indispensable, they are not designed to examine the dynamic changes of VOCs (Dewulf et al., 2002). Overcoming such problems means employing

techniques without chromatographic separation. Different methods have been proposed, such as arrays of solid-state gas sensors (*E-Noses*), and direct injection mass spectrometry (DI-MS) (e.g. *PTR-MS*) (Biasioli et al., 2011).

• *GC-MS* is the most important tool for the identification and quantification of volatile and semi-volatile organic compounds in complex mixtures (Dewulf et al., 2002). It is an analytical method that combines the features of gas-liquid chromatography and mass spectrometry to identify different substances within a test sample. The difference in the chemical properties between different molecules in a mixture and their relative affinity for the stationary phase of the capillary column in the gas chromatograph will promote separation of the molecules. The molecules are retained by the column and then eluted at different times. The mass spectrometer captures, ionizes, and identifies these molecules separately.
Pros: GC-MS systems have high precision and, if coupled with suitable pre-treatment and pre-concentration stages, for instance by using SPME fibres, can reach detection limits as low as 0.1 parts per trillion.
Cons: GC-MS suffers from a relatively low time resolution and risk of artefacts. Many compounds can be analysed only if they are chemically derivatives. The low time resolution makes it impossible to examine the dynamic changes of VOCs in fast processes.

• *E-Noses* are instruments which comprise an array of electronic chemical sensors with partial specificity for a select number of VOCs and a pattern recognition system, capable of recognizing odours. The output of a series of VOC unspecific sensors, based on different technologies (chemiresistors, optical sensors or mass sensors), can be used to build models to classify products by means of chemometrics or data mining (Gardner and Barlett, 1993). The name of the technology, E-nose, indicates that it mimics the behaviour of human olfaction and this is why its main fields of application are food and flavour sciences, where the substitution of proper sensory analysis, difficult and time consuming with instrumental methods, is a long hoped-for objective (Schaller et al., 1998).
Pros: volatile analysis by e-nose is fast and it does not need skilled personnel to operate.
Cons: e-noses suffer from several drawbacks such as sensor poisoning, high sensitivity to moisture, poor linearity and poor reproducibility on different instruments and sensor drift.

• *PTR-MS*: Proton transfer reaction—mass spectrometry (PTR-MS) is one of the most established DI-MS methods (Lindinger et al., 1998). A PTR-MS instrument consists of an ion source that is directly connected to a drift tube and an analysing system (quadrupole mass analyser or time-of-flight mass spectrometer).
Pros: the PTR-MS technique has the advantage of allowing real-time monitoring of most VOCs with very low detection limits is fast and has a high sensitivity and non-invasive sensor for volatile compounds. In comparison to other DI-MS techniques only a small amount of energy is transferred during the ionization process, therefore molecule fragmentation is reduced and the obtained mass spectra are easily interpretable. In principle with PTR-MS it is possible to determine VOC concentration without need of calibrating the instrument (Cappellin et al., 2013). Possibility to enhance analytical information by combining PTR-MS with a Time-of-flight (ToF) mass analysers, build upon the observation that heavier ions fly more slowly than

lighter ions having the same kinetic energy. Beside mass resolution, ToF detectors provide better time resolution and mass range than quadrupole mass analysers (Cappellin et al., 2012). *Cons*: Difficult to have a precise identification of volatile compounds with PTR-MS. The low specificity of the analysis does not permit a precise detection and of compounds with similar chemical structure. Because only molecules with a proton affinity higher than water can be detected by PTR-MS, proton transfer from H_3O^+ is not suitable for all fields of application. This limit has been recently reduced by the introduction of other reagent ions such as O_2^+, NO^+, Kr^+, Xe^+ (Cappellin et al., 2014).

For a better understanding of the effects of volatile compounds on flavour perception, aroma compounds should not only be considered based on concentration, but also on the interaction between the VOC and human receptors (chapter 4.2). Combinations of different volatiles can act synergistically or antagonistically, changing the levels of detection for the individual components in the mix.

In order to describe the release kinetics of VOCs while the food matrix is being chewed, we developed an analytical system based on an artificial chewing device coupled with the PTR-MS, as showed in chapter 4.2 and 5. Evaluation of these release kinetics appeared as important as other commonly considered parameters such the odour perception thresholds and the Log odour unit values. The faster and more abundant the volatile emission, the more quickly and more intensely the compound can reach the olfactory receptors and the larger the human perception. Our hypothesis to consider the "release ratio" as an additional important factor for describing the aroma perception was indirectly confirmed by Tieman (2012) and Klee and Tieman (2013). They found that C6 volatile compounds of tomato, mainly hexanal and hexenal, do not clearly affect consumer aroma perception; their discovery fits with our hypothesis since we found that tomato C6 compounds are characterized by a low "release ratio" close to 1 (chapter 4.2) that suggests a weak interaction with human odour receptors and a consequent low impact on the perceived aroma. Based on this we can assume that a volatile compound produced during fruit ripening and accumulated inside the fruit will be more effective in final aroma perception compared to a compound produced after fruit maceration, like C6 volatiles (Figure 4).

GC-MS analysis does not allow this hypothesis to be tested since long incubation times, necessary to increase the VOC concentration in the headspace, masks the "release ratio" effect. A compound that is produced enzymatically after cutting is likely by a low release ratio (chapter 4.2), will be more present than other VOCs after the incubation period (figure 2). Most of the tomato VOC studies, including chapters 4.1, 4.2, and 5, reported aldehydes as the most abundant VOC family in tomato fruit. These results obtained by GC-MS analysis are not confirmed when tomato volatile is profiled by PTR-MS coupled with the chewing device (chapters 4.2 and 5). According to the results obtained in this thesis it is clear that for a comprehensive understanding of the aroma profile all the volatiles be essential; even those compounds that are generally considered less important based only on their odour perception threshold and odour unit values have to be considered.

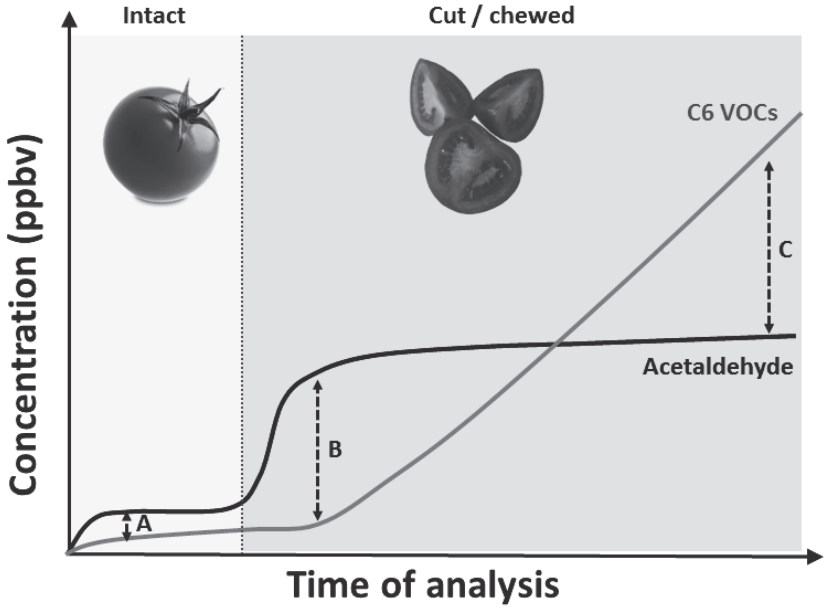

Figure 4. Graphical representation of VOC release kinetics based on results presented in chapter 3. In the figure the typical behaviour of compounds synthetized by the fruit during ripening (e.g acetaldehyde) and the ones produced enzymatically mainly after cutting (e.g C6 VOCs) are presented. A, B and C are representing the difference in concentration between the two classes of VOC when the fruit is intact (A), immediately after the fruit cutting (B) and after a long incubation (C), respectively.

4. Quality analysis for breeding

Most of the fruit quality traits are strongly influenced by environmental conditions. The genetic variation of such traits is attributed to the joint action of many genes (QTLs, for Quantitative Trait Loci), which can be mapped on the genome with genetic markers (Causse et al., 2002). QTL analysis is commonly used to localize genomic regions controlling quality traits of fruit and vegetables. Mapping the genetic linkage between fruit quality traits presents a challenge for conventional breeding methods. The use of QTL mapping to find major genes and functional markers and improve the ability to control quantitative traits is an effective way to solve these problems (Kuan-Hung et al., 2010), since conventional breeding methods provide little information on the chromosomal regions controlling these complex quality traits. Selection by conventional breeding methods, based only on visual observations, is extremely difficult when genotype-environment interactions are substantial. One approach to facilitate the selection and breeding of complex quality traits is to identify genetic markers linked to the traits of interest (Sun et al. 2012; da Costa et al., 2013; Sacco et al. 2013). Tomato is one of the first plant species in which researchers have begun to map QTL traits of agronomic importance using molecular markers (Cagas et al., 2008). In order to analyse the genetic control of quality traits in tomatoes, the genetic variation of physical, chemical and sensory attributes of fruit were studied in several segregating populations derived from interspecific crosses (Paterson et al., 1988; Goldman et

al., 1995; Eshed and Zamir, 1996; Tanksley et al., 1996; Bernacchi et al., 1998; Chen et al., 1999). Numerous relationships among sensory traits, instrumental or compositional traits and QTLs have been shown. While flavour traits (sweetness and sourness) are well described by the sugar content and titratable acidity, the prediction of aroma and texture traits seem much more uncertain because of the lack of precision in the instrumental measures and the interactions among traits (Mathieu et al., 2009; Tieman et al., 2006). Breeders need selection criteria both efficient and easy to assess for organoleptic quality breeding. Physical and chemical traits could be an alternative approach for routinely measuring some of the quality traits, but molecular markers will provide a much more efficient tool for creating improved genotypes. The genetic dissection of these complex processes will permit a more systematic approach to plant improvement than has been possible previously. An important component that has to be studied more accurately from a genetic point of view is the fruit quality deterioration during storage and the resistance of fruit at several postharvest abiotic disorders, such as chilling injury. The achievement of this goal will only be possible with a more accurate and sensory related phenotyping of quality traits, ideally combined with kinetic modelling to deeply understand the processes governing ripening and senescence.

Analytical methodologies considered in this thesis may result in tools for phenotyping quality traits and, in a later stage, to develop genetic markers that helps to screen tomato populations. This approach is detailed in box 3 and figure 5. This approach provides the opportunity to use the fast and non-destructive techniques described in this thesis to analyse a broad number of samples also for relatively unskilled personnel. Moreover, the possibility to measure non-destructively some main quality parameters such as colour, lycopene content, texture and VOCs (of intact fruit) and to evaluate data by mathematic kinetic modelling, allow the breeder to analyse batch of limited number of fruits. This may result of utmost importance for the breeding screening when a small amount of plant for genotype are available.

Quality attributes that may benefit from this approach including the analytical methodologies are:

- Lycopene content: optical spectroscopy (Pigment Analyzer)
- Texture: compression test (Zwick or Instron)
- Aroma profile: PTR-MS (preferably with ToF detector) coupled with chewing simulator
- Storability and tolerance to storage abiotic stress: compression test (Zwick or Instron), optical spectroscopy (Pigment Analyzer), PTR-ToF-MS (detection of volatiles, included ethylene on intact or cut fruit)

Box 3. Phenotyping protocol for tomato quality traits on populations

1) Select and label a small number of tomato trusses, or for round tomato, six to ten fruits, at the mature green ripening stage (NDVI of -0.1 and NAI of -0.6). (*1-2 minutes x genotype)*

2) Monitor the colour (pigment) development by using a VIS spectroscopy (using e.g. a Pigment Analyzer) every one or two days until they reach the red ripe stage (NDVI of -0.5 and NAI of + 0.5). This analysis will give an idea about the rate of carotenoids synthesis and chlorophyll degradation in the greenhouse or growth chamber. (*1-2 minutes x genotype x day of analysis*)

3) Harvest fruit at the red ripe stage and evaluate colour values (using tristimulous Lab and/or VIS spectroscopy) and firmness by limited compression (Zwick or Instron) of all fruits. (*10-15 minutes x genotype*)

4) Analyse fruit VOCs by using PTR-MS coupled with a ToF mass analyser, using an artificial chewing simulator to detect VOCs. The analysis of 3-4 tomatoes can be split in two parts: a) analysis of VOCs of intact fruit, including ethylene; b) analysis of VOCs released after the artificial mastication. (*All process will take not more than 3 minutes for sample*)

5) Collect the tomato juice produced after the artificial mastication and quantify the total soluble solids (by a digital refractometer) and titratable acidity. (*1-2 minutes x genotype*) For a more detailed analysis of carbohydrates and acids for a High-performance Anion Exchange Chromatography (HPAEC) can be used.

6) Store few trusses of red ripe tomato at.4°C for an evaluation of chilling injury and a few trusses at 16°C for 7-10 days and repeat steps 3 to 5.

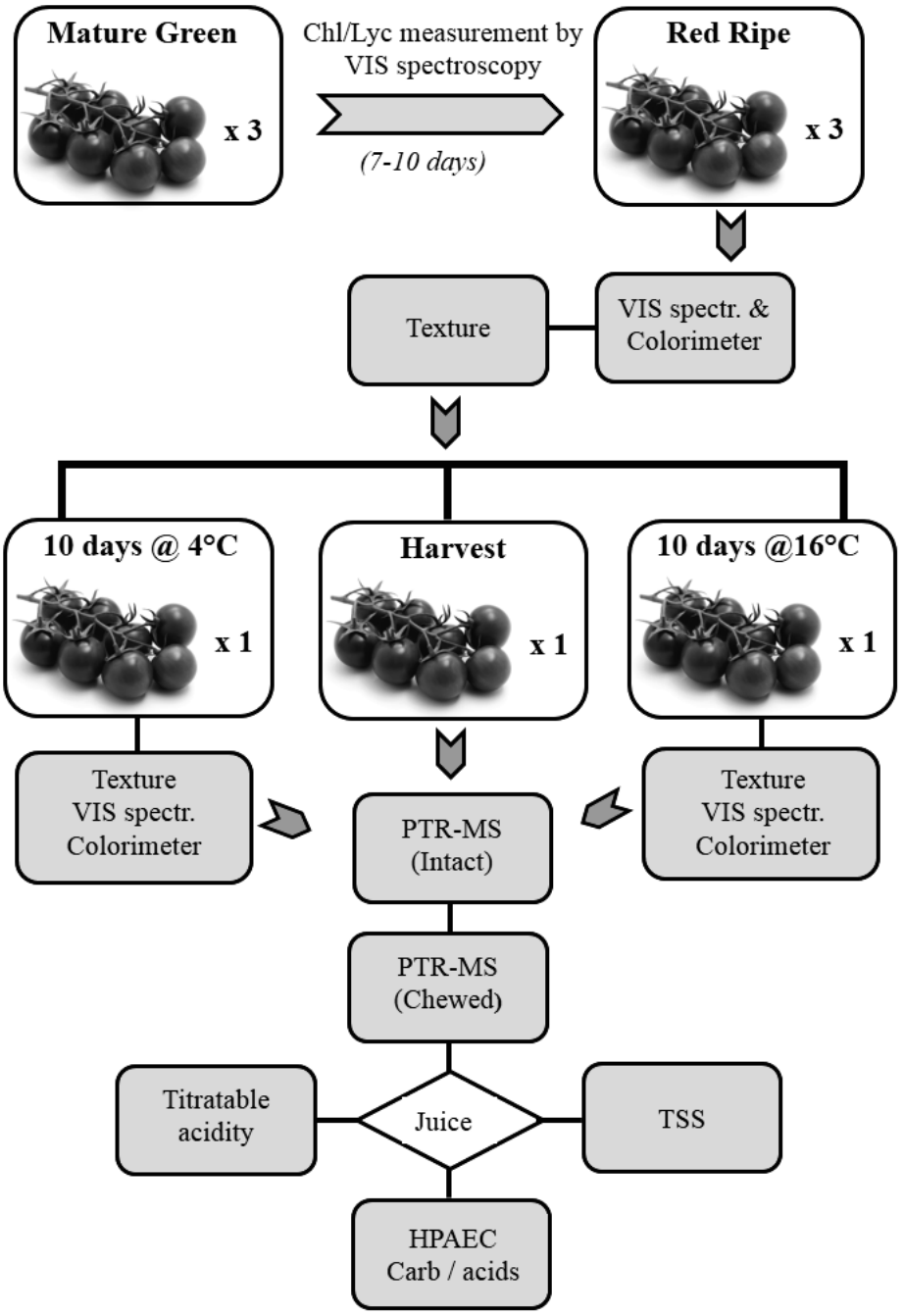

Figure 5. Practical example of phenotyping assessment of quality traits applicable for tomato breeding screening.

4. Conclusion and possibilities for further researches

This thesis provides new insights into the effect of pre and postharvest conditions on tomato fruit quality. Tomato quality traits such as colour, texture and aroma were evaluated as function of cultivar, growth and storage conditions with innovative technologies and methodologies aimed to improve the quality analysis. The main advances offered by these technologies are a non-destructive, quick, highly repeatable analysis of quality traits. These features, combined with the appropriate process-oriented modelling, provide the possibility to inspect fruit and vegetables quality throughout the production chain, from breeding to the consumer.

With the introduction of improved greenhouses management regarding environmental conditions combining researches on pre- and postharvest management as demonstrated in chapter 3 will become increasingly feasible. Combing pre- and postharvest information allowed us to improve the understanding how tomato texture and colour are controlled during fruit growth and maturation as a function of greenhouse management and seasonal variation. From our perspective further investigations are needed in order to have a more comprehensive explanation on the effect of growth condition on tomato quality. The quality development during growth has to be studied also on fruit that are still attached to the plant which needs non-destructive techniques in order to avoid the effects on growth and development of the fruit. For this approach we suggest the use of a VIS spectroscopy to monitor colour transformation, and to develop a portable version of an acoustic firmness sensor, based on the principle of AWETA, to understand the texture development. Moreover, also production of volatiles during ripening may be differently synchronised with colour development, by changing the environmental temperature.

Another challenging quality investigation will be to connect the environmental factors, mainly temperature, light and CO_2, with fruit susceptibility to chilling injury. Since tomato lycopene depletion during cold storage is season dependent (chapter 2.1 and 2.2) and since treatments to alleviate the injury did not work such as hot water treatment (chapter 2.1), antioxidant application (unpublished data) and ethylene control (unpublished data) we propose that solely environmental factors control fruit chilling injury susceptibility.

Further research should also be directed to i) the genetic connection between quality traits and QTLs and extracting genetic markers, ii) focus on understanding tomato chilling injury susceptibility, iii) increase the knowledge on the effects of incorrect storage of fruit on quality traits and finally, iv) apply the analytical tools to study quality, developed in this thesis, on other fruit types.

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Summary

The quality of fresh products has to be maintained or even enhanced during storage and marketing and it has to be considered as a central trait in fruit chain management. Improving product quality management can improve the performance of the whole fruit and vegetables production chain. This needs a detailed quantification of quality attributes of fruit and vegetables in distinct segments of the production chain. It implies synergy of approaches from different branches of knowledge, such as genetic, physiology, analytical chemistry and sensorial analysis to improve and optimize quality upon delivery to the consumers. Analytical techniques to evaluate the quality of food are needed to ensure the appropriate physicochemical properties of a product.

The aim of this study is to enhance the knowledge and understanding of some major pre- and post-harvest factors affecting tomato (*Solanum lycopersicum*) quality as perceived by the consumer. We focused on several part of the production chain that, up to our knowledge, were still poorly or far from completely studied that affect the consumer assigned quality.

Chapter 2 focuses on the effect of postharvest management, mainly storage temperature, on tomato colour changes. Colour was assessed by regularly used techniques, such as tristimulus colour measurements and RGB image analysis, and also with remittance VIS spectroscopy that permitted a non-destructive quantification of the main tomato colour pigments. In **chapter 2.1** we focused on the discoloration of red-ripe tomato fruit due to storage at chilling temperatures. Along the production chain tomatoes may be exposed to chilling temperatures e.g. when they are transported together with other perishable products. Also, consumers often store tomatoes in the refrigerator at temperatures well below optimal as was found by us in a survey among consumers. The effects of storage at chilling temperatures on quality aspects of tomatoes was investigated. The colour and lycopene content of red ripe tomatoes of two cultivars (cocktail and round type) was evaluated during twenty days of storage at 4, 8, 12 and 16 °C. Colour was repeatedly measured over time by tristimulus colour measurements, RGB image analysis and colour intensity was scored by eye using a consumer panel. Lycopene content was repeatedly assessed by following the NAI index over time. This index, obtained from remittance VIS spectroscopy, was found to relate closely to the lycopene level as measured by HPLC measurements of pericarp tissue. Temperatures below 12°C resulted in lycopene loss in ripe-red tomatoes and substantial color loss when assessed by visual evaluation. Colour measurement using tristimulus colour measurements and RGB image analysis did not correlate well with lycopene content. Prior hot water treatment did not prevent lycopene loss at chilling temperatures.

Furthermore VIS remittance spectroscopy was used to assess the the dynamics and synchronization of chlorophyll breakdown and lycopene synthesis in tomato fruits of a cocktail

and a round cultivar. In **chapter 2.2** we describe the tomato colour transformation process expressed as changes in lycopene and chlorophyll levels, as influenced by temperature, using a kinetic modelling approach. The aim of this study was to increase the understanding of how chlorophyll breakdown and lycopene synthesis are synchronised in tomato fruit on a quantitative level. Tomatoes were stored at either constant temperatures between 4 and 24 °C or first stored at 4 °C and then switched to 16 °C after varying times of storage. Chlorophyll and lycopene levels were repeatedly measured during storage and used to calibrate the kinetic model that describes how an autocatalytic, ethylene related, enzyme system links both chlorophyll breakdown and lycopene synthesis, including lycopene precursor breakdown and lycopene breakdown. The proposed model is able to accurately describe the changes in chlorophyll and lycopene levels over broad temperatures ranges. Based on obtained results, remittance VIS spectroscopy in combination with a model parameter estimation tool, allow to screen tomato genotypes based on colour transformation performances.

In **chapter 3** we describe how different greenhouse climate management systems may affect the postharvest quality of tomatoes during the whole production season. In this study important quality properties such as firmness, sugar and acid levels were analysed of tomatoes harvested from three greenhouses during a five month period and stored at 16 °C for over twenty days. Tomatoes were harvested from three identical, neighbouring, greenhouses which were either conventionally ventilated (open greenhouse) or mechanically cooled (semi-closed greenhouses). Sugar and acids levels were hardly affected by greenhouse type. Compared to the open greenhouse, semi-closed greenhouses produced heavier and less mature (firmer) fruit at the commercial harvesting stage as based on colour. Fruit maturity differences could be linked to the vertical temperature gradient and to CO_2 levels in the different greenhouses. This indicates that CO_2 levels and temperature affect the synchronisation between colour and firmness maturity at harvest. The acceptance period, i.e. the time period both tomato colour and firmness are considered acceptable to consumers, will likely be positively affected when growers switch from conventionally ventilated to semi-closed production systems. Additional to greenhouse effects, also effects of the harvest month were observed. The sugar to acid ratio was highest and glucose to fructose ratio was lowest in July, the month with the highest irradiance, irrespective of greenhouse type. The estimated value for the maximum firmness (Fmax) varied from 17.9 N in August to 31.2 N in June. This monthly variation in Fmax explains an important part of the variation found in the postharvest behaviour of tomatoes. Interestingly, the monthly variation in Fmax showed the same trend as found for the monthly initial sugar levels. It is hypothesized that the monthly variation in glucose and fructose levels causes variation in that part of firmness that is generated by cell turgor.

Chapter 4 describes the development of a rapid and accurate method, based on proton-transfer reaction-mass spectrometry (PTR-MS), suitable to dynamically monitor the emission of volatiles defining the tomato aromatic profile. The availability of rapid and accurate methods to assess fruit flavour is of utmost importance to support quality control especially in the breeding phase. Breeders need more information and analytical tools to facilitate selection for complex multigenic traits such as flavour quality. In **chapter 4.1** we described the analytical method based on PTR-MS, already used in other investigations related to food quality, to tomato VOC profiling. The method was developed by evaluating several tomato varieties and cultivars at different ripening stages with both PTR-MS and solid-phase microextraction-

gaschromatography- mass spectroscopy (SPME/GC-MS). In this study, it is shown that PTR-MS is a suitable method to monitor at high sensitivity the emission of volatiles determining the tomato aromatic profile such as hexanal, hexenals, methanol, ethanol and acetaldehyde. Multivariate statistics (PCA and Cluster analysis) of the PTR-MS results allow an unambiguous separation between varieties, especially with a clear fingerprinting separation between the different tomato types: round truss, cocktail and cherry tomatoes. PTR-MS was also successfully used to monitor the changes in volatile profiles during postharvest ripening and storage.

In **chapter 4.2** we describe a further development of the methodology to better mimic the release of volatiles during chewing in the human mouth and the consequent retronasal olfaction perception. VOC profiles of 9 tomato lines, selected based on flavour characteristics by a sensory panel, were acquired by both a PTR-MS system following artificial chewing and by SPME/GC-MS and compared to the quantitative descriptive analysis (QDA) measured by the trained sensory panel. Based on multivariate statistical analysis, data obtained by the PTR-MS system showed a better correlation to the outcome of the QDA than SPME/GC-MS, especially for the descriptive parameters “tomato fragrance” and “tomato flavour”.

The great advantage of such an analytical system was the possibility to study the release kinetics of volatiles during eating and the possibility to consider volatile concentration similar to in vivo condition resulting in an improved characterization of the aroma profile .We developed an analytical system to study the tomato aroma profile that mimics, as close as possible, the release of volatiles during chewing in the human mouth and the consequent retronasal olfaction perception.

In **chapter 5** we demonstrate how a storage management of tomato fruit at low temperatures can affect the overall production of flavour and off-flavour compounds. To better understand the real consumer aroma perception while eating, in addition to the more ordinary SPME-GC-MS analysis, volatiles were also assessed by using a chewing device connected with the PTR-MS. Tomatoes of two cultivars, a round and cocktail type, were harvested at red-ripe stage and stored at refrigerator temperature (4°C) and at room temperature (16°C and 22°C) for up to 20 days. Particular attention was paid to study the dynamics of aroma production when the fruit was brought to room temperature after a period of cold storage. Volatile emission closely followed the internal fruit temperature: reduction of fruit temperature from 22 to 4 °C, occurred in about 3 hours and caused for most volatiles a steep decline in emission that reached the minimum after 6 hours. Rewarming the fruit after different periods of cold storage generally caused a rapid increase in volatile emission up to the level of the non-cold stored fruit (control) after short storage times. After longer storage times, emission of most volatiles did not increase to the level of the control fruit and some volatiles (e.g. acetaldehyde, ethanol) reached levels well above level in the control fruit. Down-regulation of volatile emission by low temperature can be explained by an activity decline of the enzymes involved on volatile production as well as a lower rate of volatilization. Combined results obtained by SPME-GC-MS and PTR-MS analyses, showed that the damaging effect of chilling on the volatile profile was evident after the fruit was restored to high temperature: the longer the exposition at low temperature, the lower the regeneration of the major tomato flavour compounds and the higher the production of off flavours, such as methanol, ethanol and acetaldehyde.

In **chapter 6** we discuss the new insights into the effect of pre and postharvest conditions on tomato fruit quality as outlined in chapters 2-5. Attention is given to the main advances offered by new technologies used in this thesis to assess quality traits, mainly colour, texture, and volatile compounds. The properties of these tools are compared with the ones of commonly adopted quality methods. The features of these analytical tools, combined with the appropriate process-oriented modelling, resulted ideal to inspect fruit and vegetables quality in all phases of the production chain from breeding to the final consumer consumption. The chapter is concluded with a practical example of phenotyping of tomato postharvest quality traits feasible for breeders and with suggestions for future quality related researches.

Samenvatting

Het behoud van product kwaliteit in de afzet keten is van groot belang voor het uiteindelijke financiële resultaat van alle betrokkenen en voor consumenten tevredenheid. Verbetering van product kwaliteit management vereist inzicht in de verschillende aspecten van productkwaliteit en vereist beschikbaarheid van technieken om kwaliteitsaspecten op een gemakkelijke en objectieve manier te meten.

Het doel van mijn onderzoek was meer inzicht te verkrijgen in het effect van zowel voor- als naoogst factoren op de kwaliteit van tomaten zoals door de consument ervaren.

In **hoofdstuk 2** wordt het effect van naoogst omstandigheden (voornamelijk bewaar temperatuur) op roodkleuring van tomaten beschreven. De rode kleur werd gemeten met conventionele technieken (Minolta tristimulus kleur meting, rood-groen-blauw image analysis) en met een relatief nieuwe method (remittance VIS spectroscopie) waarmee de belangrijkste tomaten kleurstof, lycopeen, niet destructief gemeten kan worden.

In **hoofdstuk 2.1** wordt de ontkleuring van rode tomaten beschreven als gevolg van opslag bij te lage temperatuur. Dit kan gebeuren als de vruchten gezamenlijk met andere bederfelijke producten vervoerd worden. Lycopeen gehalten blijken af te nemen bij temperaturen on 12°C. Lycopeen is een belangrijke antioxidant met vermoedelijke gezondheid bevorderende waarde. Uit een enquête onder consumenten bleek dat de meerderheid na aankoop de tomaten in de koelkast bewaard. Deze lage temperatuur kan leiden tot lycopeen afbraak en daarmee wordt de vrucht visueel minder aantrekkelijk en neemt de gezondheidswaarde af. De nieuwe meetmethode was beter instaat de lycopeen gehalten te meten dan de conventionele methoden. Een kort durende heet water behandeling bleek geen effect op de latere koude-geïnduceerde lycopeen afbraak te hebben.

In **hoofdstuk 2.2** wordt het kleuringsprocess in tomaat, en het effect van bewaartemperatuur hier op, in meer detail onderzocht met behulp van Remittance VIS spectroscopie. Kinetische modellering is toegepast om de transitie van groen naar rood te beschrijven als functie van bewaartemperatuur. Hieruit blijkt dat een auto katalytisch, ethyleen gerelateerd, enzym systeem betrokken is bij zowel de chlorofyl (groen) afbraak als de lycopeen (rood) synthese. Het enzymsysteem zou gerelateerd kunnen zijn aan eerdere beschreven STAY-GREEN eiwitten. De modellen kunnen gebruikt worden om het verkleuringsgedrag van verschillende genotypes beter te karakteriseren.

In **hoofdstuk 3** wordt het effect van verschillend kasklimaat management op de kwaliteit van tomaat in de keten beschreven. Het verloop van stevigheid, kleur, suiker en zuurgehalten tijdens de naoogst fase is bestudeerd van tomaten uit verschillende teeltsystemen gedurende een heel teeltseizoen. Het betrof een conventioneel teeltsysteem (open kas) en twee alternatieve teeltsystemen (half-gesloten kassen) waarin gebruik gemaakt wordt van warmte uitwisseling met diepere waterlagen in de bodem. Doordat deze kassen meer gesloten zijn kan

er een hogere kooldioxide concentratie gehandhaafd worden. Afhankelijk van de plaatsing van de koude lucht voorziening kunnen er sterke verticale temperatuur gradiënten ontstaan. De half-gesloten kassen produceerden grotere en stevigere (minder rijpe) vruchten op het moment van commerciële oogst (gebaseerd op kleur) dan de open kas. Dit is een gevolg van zowel de hogere kooldioxide concentratie en van de temperatuur gradiënt. Er waren weinig verschillen in suiker en zuurgehalten van de vruchten uit de verschillende teeltsystemen. De hogere stevigheid van de vruchten uit half-gesloten kassen leidt tot een langere houdbaarheid van de vruchten omdat het stevigheidsverlies na de oogst eenzelfde snelheid vertoont als van de vruchten uit de open kas. Over het teeltseizoen waren er grote verschillen in beginstevigheid van de vruchten en dit is waarschijnlijk één van de redenen voor de verschillen in houdbaarheid. Begin stevigheid in juni was bv bijna 2x zo graat als in augustus. Er was een goede correlatie tussen stevigheid en suikergehalten hetgeen suggereert dat de suikers via verhoogde cel turgor to meer vrucht stevigheid leiden.

In **hoofdstuk 4** wordt het gebruik van een snelle methode voor het meten van aromaprofiel van vruchten beschreven. De methode is gebaseerd op Proton Transfer Reaction – Mass Spectroscopy (PTR-MS) en levert binnen enkele seconden een vingerafdruk van de geproduceerde aromastoffen.

In **hoofdstuk 4.1** wordt de methode gebruikt om het aroma profiel van verschillende tomaten genotypes en cultivars en van verschillende rijping stadia te karakteriseren. De methode wordt vergeleken met standaard gaschromatografie-mass spectroscopie (GC-MS). Hier tonen we aan dat PTR-MS een snelle en goede techniek is om bv. genotypes te onderscheiden en om rijping stadium en de verandering in aromaprofiel door bewaring te karakteriseren. De methode is vergelijkbaar met conventionele GC-MS, maar veel sneller en gemakkelijker uit te voeren.

In **hoofdstuk 4.2** wordt een uitbreiding op de PTR-MS technologie beschreven. Om beter de situatie tijdens eten van de vruchten te benaderen is een kunstmatig kauw apparaat gemaakt. Het vrijkomen van aromastoffen wordt nu gemeten met PTR-MS tijdens het kauwen. Dit geeft de mogelijkheid om vooral te concentreren op aromastoffen die snel (binnen bv. 10 seconden) vrijkomen omdat deze waarschijnlijk de meeste impact hebben op de smaak/aroma perceptie. Er is gewerkt met 9 verschillende tomaat genotypen. De PTR-MS aromaprofielen zijn vergleken met GC-MS analyses en met smaakpanel beoordelingen. De PTR-MS karakterisering van de genotypes blijkt beter te correleren met de smaakpanel beoordelingen (vooral voor de attributen “tomato fragrance” en “tomato flavour”) dan de GC-MS analyse.

In **hoofdstuk 5** wordt het effect van koude bewaring (4°C) op de productie van aroma stoffen en van “off-flavours” beschreven. Hier wordt gebruik gemaakt van zowel PTR-MS als van GC-MS. Zodra de vruchten koud worden opgeslagen neemt de aroma productie af. Als de vruchten weer terug bij kamertemperatuur worden geplaatst neemt ook de aroma productie weer toe. Echter, vooral na langere tijd koude bewaring komt de aroma productie niet meer op het oude nivo terug. Tevens worden er verhoogde hoeveelheden “off flavours” zoals ethanol en acetaldehyde geproduceerd. De effecten van koude bewaring op de productie van carotenoid-gerelateerde aromastoffen hangt mogelijk samen met het effect van koude bewaring op de synthese en afbraak van lycopeen zoals beschreven in hoofdstuk 2.

In **hoofdstuk 6** wordt het gebruik van nieuwe methoden voor kwaliteitsbepaling van vruchten in combinatie met het gebruik van kwaliteitsmodellering voor kwaliteitsmanagement in de distributieketen en voor naoogst fenotypring bediscussieerd.

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Curriculum vitae

Brian Farneti was born on 29th April 1983 in Forlimpopoli, Italy. After completing high school in his home town in 2002, he undertook his higher education studies at the Agricultural University of Bologna (Italy). In July 2005 he received his Bachelor degree with honours in "Technology of plant production" with the thesis entitled "Evaluation of the maturation trend of fruits of different varieties of Actinidia". In October 2007 he received his Master degree with honours in "International master in horticultural science" with the thesis entitled "Characterization of secondary metabolites of different Actinidia spp. Varieties with respect of their antioxidant capacity". During his Master degree he spent one year at the Technische Universität München (TUM) in Freising (Germany) where he attended courses and finalized his Master thesis. From February 2008 until April 2012 he was employed as PhD student at the Horticultural Supply Chains Group at WUR, and conducted research on factors affecting quality of tomato. The results of this work are presented in this thesis. From May 2012 until now he is employed as a Postdoc at the Department of Agricultural Sciences (Bologna University, Italy) on an Italian project about apple quality ("AGER melo"). During his Postdoc activity he is collaborating with the Edmund Mach Foundation (FEM) in Trento (Italy).

List of pubblications

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Farneti, B., Khomenko, I., Cappellin, L.,Ting, V., Romano, A., Costa, G., Biasioli, F., Costa, F. Comprehensive VOC profiling of apple germplasm by PTR-ToF-MS. *(submitted)*

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PE&RC Training and Education Statement

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (5 ECTS)
- Tomato flavour and health: the factors that influence the quality (2009)

Writing of project proposal (3 ECTS)
- Tomato flavour and health: interactions between genotype, cultivation and postharvest conditions

Post-graduate courses (3.6 ECTS)
- Basic statistics; PE&RC (2008)
- Introduction to R for statistical analysis; PE&RC (2008)
- Multivariate analysis; PE&RC (2009)

Laboratory training and working visits (4.5 ECTS)
- VOCs analysis by PTR-MS; Radboud University, Nijmegen (2009-2012)

Deficiency, refresh, brush-up courses (1.5 ECTS)
- Product quality and post-harvest physiology; only lectures; WUR

Competence strengthening / skills courses (3.1 ECTS)
- PhD Competence assessment; WGS (2008)
- Personal efficacy; WGS (2008)
- Supervising and teaching thesis students; NWO (2009)
- Techniques for writing and presenting a scientific paper; WGS (2009)

PE&RC annual meetings, seminars and the PE&RC weekend
- PE&RC Day (2008-2010)
- ALW Meeting Experimental Plant Sciences; Lunteren (2008)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)
- Frontier Literature in Plant Physiology (2008-2012)
- Workshop Trace Gas Facility; Nijmegen (2011)

International symposia, workshops and conferences (8.1 ECTS)
- Postharvest Unlimited; Berlin (2008)
- Eucarpia meeting; Wageningen (2008)
- 6th International Postharvest Symposium; Antalya, Turkey (2009)
- The 10th Controlled & Modified Atmosphere Research Conference; Antalya, Turkey (2009)
- Gordon Research Conference on Postharvest Physiology; Tilton, United States (2010)
- 43rd International Symposium on Essential Oils; Lisbon, Portugal (2012)

Lecturing / supervision of practical's /tutorials (3 ECTS)
- Postharvest physiology (2009-2010)
- Physiology and development of plants in horticulture (2010)
- Research methodology in plant sciences (2011)
- Hortonomy (2011)

Supervision of MSc Students
- Evaluate the quality and shelf life of new tomato cultivars by prediction analysis
- Influence of storage temperature on quality characteristics of new tomato varieties
- The effect of storage temperature on quality attributes of two tomato varieties
- Developing a method for measuring juiciness in tomatoes
- Investigation on the cause (s) of tomato fruit discoloration and damage under chilling condition
- Tomato color, firmness and volatile compounds affected when the storage temperature is changed
- Influencing tomato aroma by investigating volatile organic compound (VOCs) behavior
- Sequence of ripening processes in mango cultivars 'Keitt' and 'Kent'

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Rijk Zwaan is worldwide vegetable breeding company that focuses on the development of high-quality vegetable varieties for professional growers in food-producing horticulture.
The Greenery is a leading international fruit and vegetable trading company.

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