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Abstract

Effects of storage conditions on tomato flavor has been of recent interest in terms of flavor and post-harvest handling. This study aims to establish an analytical procedure to analyze volatile organic compounds (VOCs) from tomato fruit, analyze how post-harvest storage conditions affect important tomato fruit quality parameters, and to evaluate how the post-harvest chain affect VOC composition in tomatoes. Post-harvest chain was simulated to typical chain in Jæren, Norway, including harvest day (18°C for one day, in darkness), packaging and transport (12°C for 3 days, in darkness), retail (18°C for 2 days, with light) and consumer storage in either refrigerator (stored at 20°C for 4 days, with light) or kitchen counter (stored at 4°C for 4 days, in darkness).

Volatile composition was analyzed using HS-SPME/GC-MC. Fruit quality parameters included sugars, titratable acidity, dry matter content, total soluble solids, firmness and pigments. The results showed no severe effect of cold storage on fruit quality and volatile profile. The most significant change was decrease in firmness during storage. Composition of VOCs was more different from fresh fruit when stored at 20°C as compared with 4°C storage, mainly due to the fatty acid derived volatiles 1-hexanol, (E)-2-heptenal and hexanal. These compounds were generally higher after 20°C storage as compared to 4°C storage. Perceived overall tomato taste generally decreased after storage. Tomato flavor and quality was more dependent on variety then storage conditions when tomato fruits are harvested ripe.

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Abbreviations

ANOVA	Analysis of variance
B	Briosso
DMC	Dry matter content
DW	Dry weight
FC	Fold change
FID	Flame ionization detector
FL	Flavance
GC	Gas chromatography
HS	Headspace
LOX	Lipoxygenase
MEP	Methyl-erythritol-phosphate
MS	Mass spectrometry
MVA	Mevalonic acid
P	Piccolo
PCA	Principal component analysis
PDMS	Polydimethylsiloxane
SE	Sweetelle
SO	Sweeterno
SPME	Solid-phase microextraction
TA	Titratable acidity
TSI	Total sweetness index
TSS	Total soluble solids
VOC	Volatile organic compound

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Introduction

1.1 Volatile organic compounds in plant research

1.1.1 Definition of volatiles

Volatile organic compounds (VOCs) are lipophilic liquids with low molecular weight and high vapor pressure at room temperature (Dudareva et al., 2013). VOCs is a part of plants secondary metabolites, and plants produce thousands of these structurally diverse volatile compounds (Buettner, 2017). These compounds are mainly terpenoids, phenylpropanoids/benzenoids, fatty acids and amino acid derivatives (Buettner, 2017; Dudareva et al., 2006). Because of their physical properties, they can freely cross cellular membranes and evaporate, and they have important roles in protecting plants from stress and to interact with other plants and organisms (Materić et al., 2015). By humans they may be perceived as fruit or vegetable aroma (Buettner, 2017). There are constituents of many different biosynthetic pathways that contribute to the diversity of plant volatiles (Baldwin, 2010). Firstly, the building blocks of these secondary metabolites are generated by fundamental processes like glycolysis and Krebs cycle. Pathways including lipoxygenase (LOX) pathway, mevalonic acid (MVA) pathway, shikimic acid pathway and methyl-erythritol-phosphate (MEP) pathway generate the more structurally diverse volatile compounds from these building blocks (Buettner, 2017; Dudareva et al., 2006).

1.1.2 Why and when plants emit VOCs

Due to plants immobility, they have evolved a mechanism of producing volatiles to interact with the environment (Dudareva et al., 2006). Plants can use their anabolic process to produce VOCs, which they use as protection against stress, and as within- and between-plant signals (I. T. Baldwin, 2010). Every plant species will synthesize set of secondary metabolites unique to their species, depending on under which environmental factors the plants are growing. Plants will generally produce and release more VOCs during the early stage of development (Ninkovic et al., 2019).

Various abiotic and biotic factors will constantly alter the physiological activity of plants and VOCs are the most common indications that reflects the current status (Ninkovic et al., 2019). Some of these include the stress factors high temperature, high light intensity or herbivore attack. Herbivore-induced plant volatiles are beneficial as to stimulating neighbors in the environment to adjust their defense. Another example of interaction with the environment are cues about the presence of specific neighbors, which in turn will induce growth responses that increase competitiveness (Ninkovic et al., 2019). These signal compounds are also used to attract pollinating insects with typically floral scents, and seed dispersing animals with fruity scents indicating a source of carbohydrate energy. Some also function as defense substances against fungi, bacteria and viruses (Buettner, 2017).

In summary, VOCs can give detailed information about the identity and physiological condition of the plants.

1.1.3 VOCs in greenhouse

The health status of plants in a greenhouse is primarily assessed by humans. As greenhouses rapidly increase in scale, this task will gradually be taken over by automation (Jansen et al., 2010). It can be easier to manage and control the health status of crops by detecting emerging health problems at an early stage with help of technological developments. An approach which has attracted much interest is the measurement of VOCs emitted by unhealthy plants (Jansen et al., 2010).

The emission of plant volatiles from crop plants grown in greenhouses is affected by several factors divided into the two categories stressors and non-stressors, where stressors is defined as those factors that adversely affect crop productivity (Jansen et al., 2010). Stressors can be divided into biotic and abiotic stressors, where biotic stressors are caused by biological sources while abiotic stressors are caused by non-biological, environmental forces. Crops can be affected by numerous stressors, however due to monoculture and environmental control in greenhouse, the number of stressors challenging the crop growth is limited (Jansen et al., 2010). Non-stressors does not show a correlation with plant health, but does affect the emission of volatiles

from plants. Temperature and light are considered non-stressors as they can be avoided in modern greenhouses due to climate control. Other non-stressors include alterations in CO_2 concentrations. Carbon dioxide is crucial for primary metabolism, it is the carbon source and in turn will affect the production of secondary metabolites (McCormick, 2016). These factors must be taken into account when correlating the level of volatiles to plant-health issues (Jansen et al., 2010).

1.1.4 VOCs relevant for tomato flavor

There are over 400 detected volatiles in tomatoes, however only a small set of 15 to 20 of these volatiles are present in sufficient quantities to have an impact on human perception (Quinet et al., 2019). However, even though some volatiles in tomato are present below odor threshold, they can modify other perceptions related to taste and therefore have an impact on tomato flavor (Baldwin et al., 2000). Volatile metabolites responsible for fruit flavor in tomatoes are generally biosynthesized during tomato ripening. The levels of the volatile compounds vary between varieties and cultivars, and the abundance of the different volatiles ranges from microgram per gram of fresh weight to nanograms per gram or lower (Quinet et al., 2019). Some volatiles associated with fruity/floral taste has been found to enhance perception of sweetness, while other volatiles has the characteristic fresh aroma of cut grass and would enhance perception of sourness. The complex mixture of volatiles interacts with sugars and acids present in the fruit, adding to the characteristic sweet-sour flavor of tomato (Baldwin et al., 2008). Baldwin et al. (2008) found that manipulation of sugars, acids and volatiles can direct tomato flavor towards sweet, fruity, floral tases or towards more earthy tastes.

Based on the compounds listed by Baldwin et al. (2000), the flavor related volatiles relevant for this project are: (Z)-3-hexenal, *beta*-ionone, hexanal, *beta*-Damascenone, 1-Penten-3-one, 2-methylbutanal, 3-methylbutanal, (E)-2-hexenal, 2-isobutylthiazole, 1-nitro-2-phenylethane, (E)-2-heptenal, phenylacetaldehyde, 6-methyl-5-hepten-2-one, (Z)-3-hexenol, 2-phenylethanol, 3-methylbutanol, methyl salicylate, geranyl acetone, *beta*-Cyclocitral, 1-nitro-3-methyl-butane, geranial, linalool, 1-penten-3-ol, (E)-2-pentenal, neral, pentanol, pseudo ionone, isobutyl cyanide, hexanol and epoxy-beta-ionone. These volatiles will be important in order to investigate

how environmental conditions affect the composition of volatiles in tomato in relation to consumer perception.

1.2 Background

Tomato (*Solanum lycopersicum* L.) is one of the most important fruit or vegetable crops in the world in regards to production and consumption. Over the past couple of decades, there has been an intensive breeding of tomato which has resulted in a loss of flavor and nutrients in the fruit (Klee & Tieman, 2013). Generally, cultivars which gives high yield and rapid growth have been selected over nutrient content. To ensure best transportation and storage of tomatoes, they are often harvested at the green immature stage and induction of ethylene helps the ripening process. This also has a negative effect on taste and aroma (Bennett, 2012).

Tomato flavor is a complex, sensitive quality parameter that is severely dependent on the cultivar (Kanski et al., 2020; Tieman et al., 2012). Flavor in general is the sum of primary and secondary metabolites measured by the taste and olfactory system. For tomato, flavor is influenced by the complex interactions of sugars (glucose and fructose), acids (glutamate, citrate and malate) and volatile compounds (Klee, 2010; Lee et al., 2018). Textural attributes such as firmness and juiciness also affect the perception of flavor (Martina et al., 2021; Verhagen & Engelen, 2006). In a study by Tieman et al. (2017) it was found that when comparing heirloom varieties and modern cultivars based on flavor-associated chemical compounds, the modern cultivars were not well liked. A lot of research has been conducted in recent years to understand the complexity of tomato flavor and how to recover through molecular breeding (Martina et al., 2021). Zhang et al. (2016) found that refrigeration would alter volatile composition, but did not affect sugar and acid content. They showed that RNAs encoding transcription factors that are essential for ripening are reduced in response to chilling, which in turn can cause reduction in many downstream genes during chilling such as transcripts encoding key enzymes for the synthesis of volatiles.

Post-harvest treatment and storage conditions also affect tomato flavor. There has also been a lot of research on the changes of tomato flavor during storage (Forney et al., 2018; Kanski et al.,

2020; Krumbein et al., 2004; Maul et al., 2000; Ponce-Valadez et al., 2016; Renard et al., 2013; Verheul et al., 2015). Forney et al. (2018) found that prolonged exposure to temperatures below 10°C should be avoided as it could inhibit aroma volatile synthesis. Renard et al. (2013) also found that storing tomatoes at 4°C would result in drastic loss of volatiles, however this was also prolonged storage (30 days). Maul et al. (2000) investigated different storage temperatures for 2-12 days and found that postharvest storage at temperatures lower than 20°C affected the tomato aroma negatively. However, they state that the effects of storage temperature would increase as exposure time increased and that refrigerating temperatures (3-5°C) could be one of the biggest factors for loss of tomato flavor.

Most of these studies focuses on parts of the post-harvest process. Kanski et al. (2020) investigated the whole transportation route and compared freshly harvested fruits with fruits after two different household storage conditions. They looked at the important flavor-related quality attributes sugars, acids and volatile compounds, and found no notably influence on human perception after the two different storage conditions. Verheul et al. (2015) states that even though the number of varieties grown in Norwegian greenhouses is increasing, they are all stored under the same conditions and little is known whether the different varieties is affected differently by variable storage conditions.

These experiments were done to investigate how tomato flavor and composition of volatiles were affected by the whole post-harvest chain including transport, retail and two different household storage conditions (4°C in darkness simulating refrigerator and 20°C with light simulating kitchen counter) in five different tomato varieties. Tomato quality parameters such as sugars and acids, and volatile compositions were analyzed. The varieties Flavance (FL), Sweeterno (SO), Briosso (B), Sweetelle (SE) and Piccolo (P) were chosen for this experiment.

1.3 Analysis of VOCs

Headspace analysis is an effective and universal method that involves analysis of volatile compounds in a solid or liquid sample by sampling of the vapor or gas phase. Headspace sampling can be achieved in a fully automated system such as gas chromatography (GC) for

separation and mass spectrometry (MS) for detection. Other detectors such as flame ionization detector (FID) can also be used (Sgorbini et al., 2019). This method allows the determination of VOCs in samples that cannot be analyzed by direct dosing of samples into the injector, such as viscous media, food and plants. It also simplifies identification and quantification of analytes in chromatogram as it only contains peaks from volatile compounds. However, this method has relatively high limits of detection for analytes (Rodinkov et al., 2020).

In 1990s, Prof. Pawliszyn and colleague (Arthur & Pawliszyn, 1990) introduced solid-phase microextraction (SPME) which is a more advanced extraction technique. SPME makes sampling, pretreatment, enrichment and sample introduction possible with a single step using a miniature extraction phase with no solvent (Huang et al., 2019). This method is based on analytes being sorbed in a layer of sorbing liquid or solid phase which is extended from a metal needle (Rodinkov et al., 2020). SPME can trap volatiles using one or several polymeric stationary phases coated to small fused-silica fiber (Diez-Simon et al., 2020). Figure 1 show SPME fiber device. Headspace (HS)-SPME exposes the fiber to the gas or vapor phase above liquid or solid sample. This technique can be automized to autosampler, or used to extract volatiles from samples on site, for example in greenhouse.

The applicability of the technique is defined by the selected coating. There are several different coating materials available for GC, including polydimethylsiloxane (PDMS), polydimethylsiloxane/divinylbenzene (PDMS/DVB), carboxen/polydimethylsiloxane (Car/PDMS), divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS), polyacrylate (PA) and CARBOWAX polyethylene glycol (PEG). These SPME coating materials can be found in different configurations such as fiber, thin film, in-tube and dispersed particles, where particularly fiber and thin film has been advancing recently in order to meet specific needs of many research fields (Reyes-Garcés et al., 2018). For analysis of VOCs in a complex matrix, fiber is more appropriate (Rodinkov et al., 2020). Several studies has found that 50/30μm DVB/CAR/PDMS fiber coating was most appropriate for extracting flavor volatiles when considering sensitivity and repeatability (Cortina et al., 2017; Xiao et al., 2017).

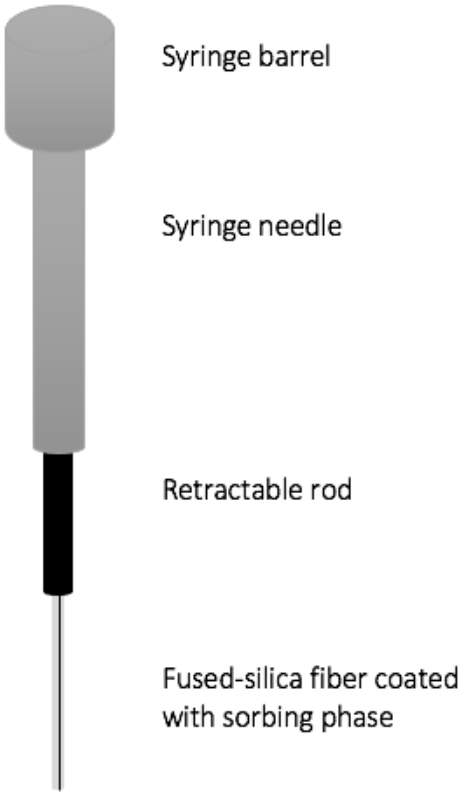

Figure 1. SPME fiber device.

Diez-Simon et al. (2020) writes that the extraction method has a significant impact on the volatile profile obtained. HS-SPME has many advantages, including the possibility of conveniently coupling the mechanism to a variety of instrumentations, and monitoring of biological changes in a living system without disturbing or cause irreversible damage to the system. The detection limit for analytes is relatively low and it eliminates contact with the matrix which might be detrimental for the fiber. HS-SPME also allows analysis of solid sample, which is important for food analysis. However, some studies have found that SPME was not able to extract complete panel of compounds present in the foodstuff that was analyzed. This may be due to the selectivity and limitations of the coating material used for the SPME (Li et al., 2020). Different sample processing methods produce characteristic volatile profiles (Diez-Simon et al., 2020) and this is important to consider when comparing results from other sources (Rambla, Alfaro, et al., 2015).

1.4 Methodology

1.4.1 Basic principles of GC

Chromatography methods are powerful separation methods used in all branches of science, including research, pharmaceutical industry, clinical analysis and petroleum. It allows for separation, identification and determination of components in complex samples. Generally for chromatographic methods, a sample is dissolved in a mobile phase which is then forced through an immiscible stationary phase. The components of the sample will be distributed between the mobile and stationary phase in varying degrees, where components will be strongly or weakly retained by the stationary phase and create different migration rates. Components with similar retention to the stationary phase will have similar migration rates (Skoog et al., 2017).

Gas chromatography (GC) is a separation method where the components of a vaporized sample are separated by being dissolved in a gaseous mobile phase. There are two types of GC, Gas-Liquid Chromatography (GLC) and Gas-Solid Chromatography (GSC). These types differ in the stationary phase, where GLC stationary phase is a liquid adsorbed or bonded to a solid surface, and GSC stationary phase is a solid. The mobile phase in GC needs to be a chemically inert gas, often called carrier gas. The most common is helium, however, argon, nitrogen and hydrogen can also be used (Skoog et al., 2017).

Compared to High Performance Liquid Chromatography (HPLC), GC has a higher efficiency (resolves more compounds per unit time), is more universal, generally simpler to use and less expensive. Analytes must vaporize in the injection port, and they must be thermally stable. Derivatization can help with increasing volatility. However, GC requires analytes to have a significant vapor pressure between about 30 and 300°C (suitable for volatiles). GC is often coupled with a temperature program which can maximize the separation and minimize time of analysis. Compared to other chromatography methods, the mobile phase of GC only functions to transport the analyte through the column and does not interact with the molecules (Skoog et al., 2017).

Retention times and volume are used for qualitative identification while peak heights and areas give quantitative information. Combining the separation capabilities of GC with identification properties of other instruments such as mass spectrometers can be done to enhance qualitative identification (Skoog et al., 2017).

1.4.2 Basic principles of MS

Mass spectrometry (MS) is a powerful detector that can be coupled with GC. It is an analytical tool that can be used to collect different types of information including the structures of inorganic, organic and biological molecules, the qualitative and quantitative composition of complex mixtures and isotopic ratios of atoms in a sample. The main components of MS include ion source, mass analyzer and detector. Simply put, MS forms ions from analyte molecules and measures mass-to-charge ratio (m/z) of ions produced.

The ion source of GC-MS system is most commonly electron ionization (EI) and chemical ionization (CI). EI uses energetic electrons to produce ions, while CI uses reagent gaseous ions. For EI, the sample is ionized by a beam of energetic electrons bombarding the sample resulting in extensive fragmentation. This gives a large number of positive ions with different masses, most often less than the molecular ion. In some cases the fragmentation ion can be greater than molecular ion due to collisions (Skoog et al., 2017).

There are different types of mass analyzers that separate ions with different mass-to-charge ratio, and the most commonly used are quadrupoles and ion-traps. The ions formed will be separated and formed into an electric signal by an electron multiplier. This results in a mass spectrum and can, along with retention time based on separation from GC, be used to identify compounds by comparing to mass spectrum from libraries (Skoog et al., 2017).

1.5 Objectives

A. Establish methodology for analysis of VOCs from tomato fruit

One objective of these experiments were to establish an analytical procedure to analyze of VOCs from tomato fruit. This includes finding a suitable analytical method and fiber, choose tomato varieties, find strategy for identifying VOCs and post-harvest conditions corresponding to the post-harvest chain in Jæren, Norway.

B. Evaluate how post-harvest storage conditions affect tomato fruit quality

Another objective was to analyze how post-harvest storage conditions affect important tomato fruit quality parameters. This includes analyzing sugars, titratable acidity, dry matter content, firmness and pigments, as it is known that these parameters can affect the perception of tomato flavor.

C. Evaluate how post-harvest storage conditions affect VOC composition of tomatoes

Final objective of these experiments was to evaluate how post-harvest storage conditions affect VOC composition of tomatoes. It is known that some specific volatiles have an impact on human perception, so these will be the main focus.

Materials and methods

2.1 Experimental design

The five different tomato varieties FL, SO, B, SE and P were used in this experiment. The tomatoes were harvested and stored in simulation to the post-harvest chain in Jæren, Norway. Tomatoes were harvested and kept at 18°C for one day simulating harvest day, then kept at 12°C for 3 days simulating packaging and transportation, followed by 18°C for 2 days with artificial light simulating retail before being kept at either 20°C for 4 days with artificial light simulating kitchen counter or 4°C for 4 days in darkness simulating refrigerator for consumer (Figure 2). Fruit quality parameters, volatile composition and sensory analysis was done for both treatments, as well as fresh fruit directly after harvest. Analysis was conducted at NIBIO (Norwegian Institute of Bioeconomy Research) research station at Særheim (Norway) and University of Stavanger (Norway).

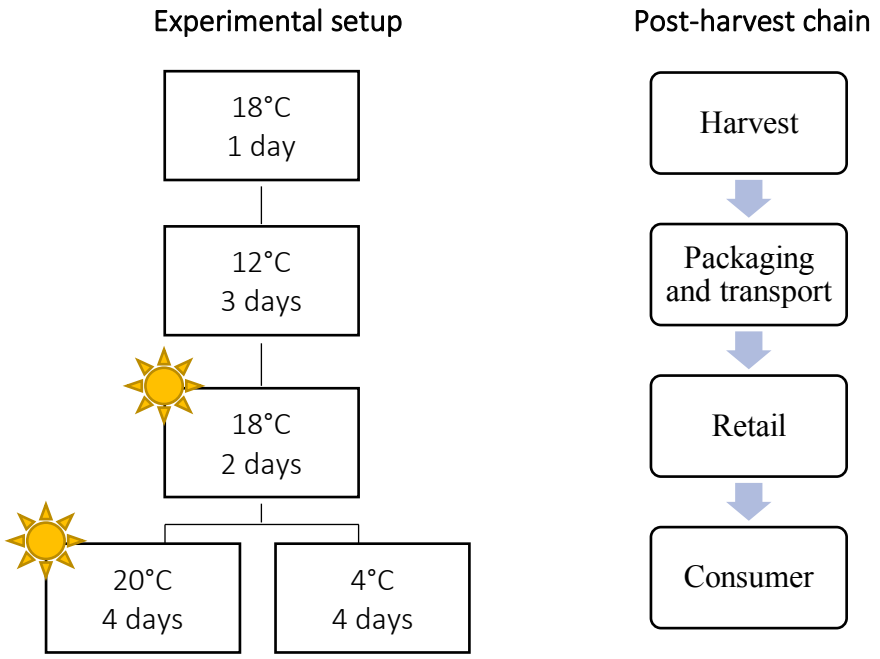

Figure 2. Experimental design simulating the post-harvest chain from harvest to consumer.

2.2 Plant material and growth conditions

The tomatoes were grown using standard climatic and nutritional conditions with artificial lightning. Ripe fruits were harvested on February 1st (FL, SO and B) and January 29th (SE and P). The samples were divided into equal amounts for fresh fruit (F), stored at 20°C with artificial light (20°C) or stored at 4°C in darkness (4°C).

The samples were stored for 1 day at 18°C (30% humidity). They were subsequently stored for 3 more days at 12°C (80% humidity) in darkness, then 2 days at 18°C (70% humidity) with artificial light for 17 hours per day. The samples were then separated to be stored either 4 days at 20°C (55% humidity) with artificial light for 10 hours per day or 4 days at 4°C (80% humidity) in darkness. Artificial light was set to 50 x10^{-6} Em^{-2} s^{-1} using Quantum Photo/Radiometer with LP 9021 PAR quantum-radiometric probe (Nie – Co – Products, Holland). Analyses were performed on all fruits, directly after harvest and after the two storage regimens 20°C and 4°C.

2.3 Establishment of analytical procedure

In order to find a suitable analytical method and fiber to analyze VOCs from tomato fruit, literature review, optimization for HS-SPME/GC-MS and library building was done. Literature review (Table 3) in results section show that HS-SPME/GC-MS was most commonly used and optimization was done for this analytical technique. Optimization was done by testing the column program used by Cortina et al. (2017) and comparing this column program to the same program with 10 min post-run added. Different extraction times (15 min and 35 min) was also tested. Based on literature review (Table 3) it was also necessary to find appropriate sample preparation. Five different sample preparations were tested: addition of 1 mL H_2O, addition of 1 mL ethylenedinitrilotetraacetic acid disodium salt dihydrate/sodium hydroxide (EDTA disodium salt), addition of 1 mL EDTA + 2.2 g solid $CaCl_2$, addition of 1 mL EDTA saturated with $CaCl_2$, and addition of 1 mL H_2O + 2.2 g $CaCl_2$. For all samples, 2 mL tubes with homogenized material was placed in a water bath at 35°C for 10 min. 1 g of homogenized sample was placed in a 15 mL tube. 16 µL of 1-Octanol (internal standard dissolved in ethanol at a concentration of 0.16 µM) was added. Samples were sonicated for 15 min, vortexed to mix and 1 mL processed sample was transferred to a 10 mL screw-capped vial. Samples were analyzed as described in chapter 2.6. Total height, number of identified peaks, CV and variation of internal standard was evaluated.

In order to optimize identification of compounds, library was built. Integrated compound library GCMS DB_MoNA-Volatile-KovatsRI was expanded by spectral identification using NIST MS search 2.0 (DB v. 2011) of peaks from tomato fruit samples.

2.4 Chemicals and equipment

List of chemicals, equipment and instruments used are shown in Table 1 and Table 2.

Table 1. List of chemicals used for quality parameters and volatile analysis

Chemical	CAS Number	Vendor	Product number
Sodium Hydroxide solution (32%)	1310-73-2	Merck KGaA, Darmstadt, Germany	1.5590

Methyl *tert*-butyl ether (MTBE)	1634-04-4	Merck KGaA, Darmstadt, Germany	1.01845
Methanol	67-56-1	Merck KGaA, Darmstadt, Germany	1.06035
Ribitol (Adonitol)	488-81-3	Supelco, Germany	47266
Titriplex® III for analysis (ethylenedinitrilotetraacetic acid, disodium salt dihydrate)	6381-92-6	Merck KGaA, Darmstadt, Germany	1.08418
Calcium chloride	10043-52-4	VWR, Leuven, Belgium	22328
1-Octanol, analytical standard	111-87-5	Supelco, Germany	95446
HEPES (2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulphonic acid) ≥99.5%	7365-45-9	VWR, Leuven, Belgium	441485H
Citric acid	77-92-9	VWR, Leuven, Belgium	84841.290
Sodium citrate tribasic dihydrate	6132-04-3	Sigma-Aldrich, Germany	C8532
Ethanol absolute ≥99.8%	64-17-5	VWR, Leuven, Belgium	20821
Glucose assay reagent	/	Supelco, Germany	G3293
Glucose	50-99-7	Supelco, Germany	G7021
Fructose	57-48-7	Supelco, Germany	F2793
Sucrose, analytical standard	57-50-1	Supelco, Germany	47289
Invertase from baker's yeast (*S. cerevisiae*)	9001-57-4	Sigma-Aldrich, Germany	I4505
Alkanes C8-C40	/	Supelco, Germany	40147-U

Table 2. List of equipment and instruments used for quality parameters and volatile analysis

Instrument	Type	Vendor
Quantum-radiometric probe	LP 9021 PAR	Nie-Co-Products, Holland
Quantum photo/radiometer	HD 9021	Nie-Co-Products, Holland
Firmness tester	Durofeel	Agro-technologies, France
Hand blender	4191	Braun, Germany
Ultrasonic bath	USC- TH	VWR, USA
Microplate reader	Multiscan GO	Thermo Fisher Scientific, Finland
Refractometer	Refractometer PR-101α	Atago, Japan
Titrator	794 Basic Titrino	Metrohm, Switzerland
Freeze dryer	BK-FD10S	BIOBASE, China
Incubator	Incubating mini shaker	VWR, USA
Centrifuge	Micro Star 17R	VWR, USA
Autosampler	MPS	Gerstel, Germany
Gas chromatograph	6890 GC	Agilent Technologies, USA
Mass spectrometer	5975 MSD	Agilent Technologies, USA
SPME fiber	50/30 μm DVB/CAR/PDMS	Sigma-Aldrich, Germany
Capillary column	RXi-5sil MS	Restek, USA

2.5 Quality of the tomatoes

2.5.1 Fruit selection and sample preparation

Fruits were photographed and firmness was measured using firmness tester (Agro-technologies, France) on scale from 1 (complete lack of firmness) to 100 (complete firmness). Fresh tomatoes were blended using a hand blender (Braun, Germany) until homogenized (30-60 sec). Homogenized mixture was placed in 2 mL, 15 mL and 50 mL tubes, immediately frozen in liquid nitrogen and stored at -20°C until further analysis. Homogenized sample was used for total

soluble solids, titratable acidity, dry matter content and volatile analysis. Freeze-dried material was used for soluble sugar and pigment analysis. Fresh fruits were used for sensory analysis. 1-8 fruits were used per replicate. For the bigger tomato varieties FL and SO, 1 or 2 fruits were used due to limited amount of fruit available. For the smaller tomato varieties, 7-8 fruits were used to achieve a volume required for homogenization.

2.5.2 Total soluble solids and titratable acidity

Total Soluble Solids (TSS) and Titratable Acidity (TA) was determined based on the method published by Kanski et al. (2020) with some modifications. 15 mL tubes with homogenized sample were centrifuged at 2880g for 40 min at 20°C (VWR, USA). One drop of the supernatant was placed on a refractometer (Atago, Japan) to determine TSS expressed as °Brix (percent of dissolved solids in a solution). For TA, 3 mL of the supernatant was added to 50 mL deionized water and the solution was titrated with 0.1 M NaOH solution to pH 8.1 using automatic titrator (Metrohm, Switzerland). The following equation (1) was used to calculate the titratable acidity (TA) as a percentage of citric acid, the main acid in tomato fruits:

$$TA\ (\%) = \frac{usage\ ml\ 0.1\frac{mol}{l}\ NaOH\ x\ 0.1\frac{mol}{l}x\ milliequivalent\ of\ citric\ acid\ (0.064\ mVal)\ x\ 100}{ml\ sample\ (3\ ml)} \quad (1)$$

2.5.3 Sample preparation for soluble sugars and pigments analysis, and dry matter content

Extraction and fractionation by phase separation was done based on the method by Salem et al. (2016). Tubes, 2 mL size, with homogenized material was freeze-dried for 48 hours (BIOBASE, China) and stored at -80°C until further extraction. The tubes were weighed before and after freeze-drying to calculate dry matter content (DMC). Around 20 mg of freeze-dried material was weighed into 2 mL tubes. For extraction of the samples, 1 mL of MTBE: MeOH (3:1, v/v) with internal standard ribitol (Sigma-Aldrich, Germany) was added and the sample was vortexed, incubated on an orbital shaker (VWR, USA) at 160 rpm for 45 min at 4°C, followed by sonication

15 min in an ice-cooled sonication bath (VWR, USA). For fractionation by phase separation, 650 µL H_2O: MeOH (3:1, v/v) was added to each sample and vortexed for 1 min, followed by centrifugation (VWR, USA) at 17,000 g for 15 min at 4°C. Polar and hydrophobic fractions were aliquoted into separate tubes and stored at -80°C until further analysis.

2.5.4 Soluble sugars

Soluble sugars were analyzed and calculated based on the method by Zhao et al. (2010). The major principle of the assay is that glucose is phosphorylated into glucose-6-phosphate, which in turn is oxidized to 6-phosphogluconate in the presence of oxidized nicotinamide adenine dinucleotide (NAD). An equimolar amount of NAD is reduced to nicotinamide adenine dinucleotide (NADH) during this oxidation, and the subsequent increase in absorbance at 340 nm is directly proportional to the concentration of glucose (Zhao et al., 2010).

Fractions with polar and semipolar metabolites from previous step was diluted 20 or 30 times in ethanol. 3 x 20 µL aliquots of dilution was pipetted into 96-well plate and left to dry in an oven at 50°C for 40 min to evaporate ethanol. Glucose was analyzed by adding 20 µL distilled water and 100 µL glucose assay reagent to each well, incubated at 30°C for 15 min and absorbance was measured at 340 nm using microplate reader (Thermo Fisher Scientific, Finland). Fructose was analyzed by adding 10 µL of PGI enzyme (phosphoglucose isomerase in HEPES, pH 7.8), incubated at 30°C for 15 min. This step converts fructose in the sample to glucose. Absorbance was measured at 340 nm to obtain the sum of glucose and fructose concentrations. Sucrose was analyzed by adding 10 µL of invertase solution (10 mg invertase powder/mL 0.1 M Na-citrate buffer), incubated at 30°C for 60 min and absorbance was measured at 340 nm to obtain the overall sum of glucose + fructose + sucrose equivalent concentrations as glucose. Standard solutions containing 0 to 0.5 mg/mL glucose was used to make standard curve. Total Sweetness Index (TSI) used to indicate sweetness was calculated using the following equation (2), (Magwaza & Opara, 2015):

$$TSI = [(1.00 \times sucrose) + (0.76 \times glucose) + (1.50 \times fructose)] \quad (2)$$

2.5.5 Pigments

Chlorophylls A (Chl*a*) and B (Chl*b*) and total carotenoids was determined using spectrophotometer. The upper polar phase was diluted 20 times in MeOH and 200 µL of diluted mixture was pipetted in triplicate into 96-well plate. The absorbance was measured at 470, 652 and 665 nm. The pathlength correction factors were retrieved and applied as specified by Warren (2008). The concentrations of chlorophylls and carotenoids was measured using the formula for pure MeOH retrieved from Lichtenthaler & Buschmann (2001) and calculated as µg/g DW.

2.6 Volatiles

Sample preparation and analysis of VOCs were based on the method of Cortina et al. (2017) with some modifications. Tubes, 2 mL size, with homogenized material was placed in a water bath at 35°C for 10 min. 1 g of homogenized sample was placed in a 15 mL tube. 16 µL of 1-Octanol (internal standard dissolved in ethanol at a concentration of 0.16 µM) and 1 mL ethylenedinitrilotetraacetic acid disodium salt dihydrate/sodium hydroxide (EDTA disodium salt/NaOH) solution saturated with $CaCl_2$ was added. EDTA disodium salt/NaOH solution was prepared by adjusting 100mM EDTA disodium salt to a pH = 7.5 with sodium hydroxide. The solution was then saturated with $CaCl_2$. Samples were sonicated for 15 min, vortexed to mix and 1 mL processed sample was transferred to a 10 mL screw-capped vial (magnetic cap with silicon septum). The vial was introduced in MPS autosampler (Gerstel, Germany) and incubated for 10 min at 50°C with 250 rpm shaking speed. The volatiles were extracted by headspace solid-phase micro-extraction (SPME) with a 2 cm 50/30 µm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) fiber (Sigma-Aldrich, Germany). The sample extraction time was 15 min at 50°C and 250 rpm shaking speed. Adsorbed VOCs were immediately desorbed at 250°C in the injection port for 1 min, followed by 3 min bakeout to avoid carry-over effect.

VOCs were analyzed using Agilent 6890 Gas Chromatograph coupled with Agilent 5975 Inert Mass Selective Detector (Agilent Technology, USA). For compound separation, capillary column (RXi-5sil MS, 30 m x 0.25 mm i.d. x 0.25 µm film thickness, Restek, USA) was used. Oven

temperature conditions were 35°C for 5 min, 3°C min^{-1} ramp until 45°C and 1.5°C min^{-1} ramp until 50°C, held for 1.5 min, 3°C min^{-1} ramp until 68°C, held for 2 min, 3°C min^{-1} ramp until 131°C, held for 1 min, 10°C min^{-1} ramp until 250°C, and then held at 250°C for 2.93 min, followed by 10 min post run at 35°C using helium carrier gas at 1 mL min^{-1}. Electron ionization mass spectra were recorded at frequency 6.2 scans/s with scanning range from 35 to 250 m/z. Total runtime for one single chromatographic analysis was 58 min.

Mixture of alkanes (C8-C40, Supelco, Sigma-Aldrich) was also run with the same parameters to calculate retention index. Relative fold change (FC) was calculated by normalizing samples to internal standard, and ratio between fresh fruit and corresponding peak heights in the two storage conditions in log2 was calculated.

2.7 Sensory evaluation

The sensory analysis of tomato fruits was performed with 6-10 participants. During the test, the participants received one quarter of each variety served on a plate. Fruits stored at 4°C was adjusted to room temperature before being presented and all fruits were cut right before serving. The test was divided into two parts. The first part was done at the day of harvest to determine sensory evaluation of fresh fruit. The second part was done after the two storage conditions (20°C and 7°C). The participants evaluated the fruits based on a scale from 1 (not perceptible) to 100 (highly perceptible) for the following eight attributes: green-grassy odor, tomato-typical odor, tomato-typical flavor, sweetness, sourness, juiciness, firmness of the fruit peel and aftertaste. The evaluation scale and attributes was based on the method by Kanski et al. (2020).

2.8 Data processing and statistical analysis

Data acquisition from volatile analysis was carried out using MassHunter GC/MS software (B.07.02.1938, Agilent Technologies, USA). Retention index was calculated using Kovats Index from sample with a mixture of alkanes (C8-C40, Supelco, Sigma-Aldrich). Alignment was done using MS-DIAL software version 4.38, and identification of compounds was done using

integrated compound library (GCMS DB_MoNA-Volatile-KovatsRI) and NIST MS Search 2.0 Built May 19, 2011, using both spectrum and retention index similarity (Tsugawa et al., 2015). Average peak heights present in blank samples were removed. Statistical analysis was performed using SPSS statistical software (IBM statistics Version 26.0, Armonk, NY, United States). One-way and two-way analysis of variance (ANOVA) was performed, followed by Tukey´s *Post Hoc* test ($p \leq 0.05$). Normalization (sum of peak heights of identified metabolites), data filtering (default setting for interquartile range IQR), transformation (cube root and range scaling), principal component analysis (PCA), Partial Least Squares Discriminant Analysis (PLS-DA), ANOVA table and heatmaps were performed using online software MetaboAnalyst version 5.0 (https://www.metaboanalyst.ca/)(Chong et al., 2019).

Results

3.1 Methodology

In order to establish an analytical procedure to analyze of VOCs from tomato fruit, literature review, optimization for HS-SPME/GC-MS and library building was done.

3.1.1 Literature review

Table 3 shows an overview of frequently used methods for analysis of specific VOCs in different plant species. All methods are using HS-SPME/GC-MS technique, however there is much variation in the details of the methods including sampling, column details and temperature. An increase in temperature during extraction can accelerate the generation of headspace gases and thereby reduce extraction time (Balasubramanian & Panigrahi, 2011). They also use different ionization techniques, with electron ionization being the most common. The use of 50/30 μm CAR/PDMS/DVB fiber is common for many methods because it was most appropriate for extracting flavor volatiles when considering sensitivity and repeatability.

All methods except Farneti et al. (2015) use a salt, either NaCl or $CaCl_2$, during sample preparations. Salt additions can help increase the sensitivity of headspace analytical techniques because it releases volatiles from the sample matrix into the gas phase as it changes the physio-

chemical properties. However, terpene compounds does not show this benefit (Diez-Simon et al., 2020). Another benefit to salt addition is the precipitation of proteins and changing the ionic strength of the matrices. $CaCl_2$ has shown to promote reduction of enzyme activity. This is helpful as obtaining a stable profile of VOCs can be challenging due to enzymatic and non-enzymatic reactions (Cortina et al., 2017). Some methods use EDTA dissolved in sodium hydroxide. Tikunov et al. (2005) explained the importance of this addition as it increases matrix pH and it works as a chelating agent to reduce the action of certain metalloenzymes.

Table 3. Literature overview where SPME was used to analyze plant volatile organic compounds (VOCs).

Plant species	Collection method and sample preparation	Type of VOCs	SPME type	Analytical method	Details	Analytical software	References
Tomatoes: Local Texas A&M F_1 hybrids (TAM Hot-Ty, T3, and L501-55), commercial SV8579TE, Shourouq, Seri, Mykonos and DRP-8551	Fresh fruit was sliced and blended for 30 s, and 2g samples were collected into 20ml glass vials, saturated $CaCl_2$ (2mL) and 10 μL of 2-octanone (0.025%, v/v) in ethanol were also added. Stored at -20°C. Samples were vortexed for 1 min and sonicated for 30 min at room temperature before loading onto autosampler.	Alcohols, aldehydes, esters, fatty acids, furans, hydrocarbons, ketones, sulfur compounds	Fiber, 50/30 μm CAR/PDMS/DVB	HS-SPME/GC-MS. Incubation: 2 min, 60°C, 5s/min agitation Extraction: 45 min, 60°C, 5s/min agitation Injection: 2 min, 225°C Fiber conditioning: 7 min	Column: Zebron ZB-Wax column, 100% polyethylene glycol (30m x 0.25mm, 0.25μm) Carrier gas: Helium Flow rate: 1 mL/min Mode: Splitless Oven temperature: Initial 40°C for 1 min, 10°C/min to 90°C, 3°C/min to 175°C, 35°C/min to 230°C and held for 2 min. Total run time: 38 min. Mass spectrometry conditions, EI: Ionization energy: 70 eV Scan mode: 40-450 *m/z* 11.5 scans per second	Xcalibur, SPSS and MetaboAnalysist 4.0.	(Lee et al., 2019)
Tomatoes: Tygress, Tasti-Lee, Cherokee Purple, FL 47	Fruits were divided into different tissues, rapidly immersed in liquid nitrogen, ground to a	Aldehydes, hydrocarbons, alcohols, ketones, furans, esters,	Fiber, 50/30 μm DVB/CAR/PDMS	HS-SPME/GC-MS Extraction: 40 min, at 50°C Injection: 5 min, at 250°C	Column: HP-5 column (50 m × 0.32 mm × 1.05 μm) Carrier gas: Helium Flow rate: 1 mL/min Mode: Splitless	JMP 11.2.0. and Statistical Analysis System	(Li et al., 2019)

	powder. 4.3g of tissue powdered together with 1.7 mL of saturated $CaCl_2$ solution. Stored at -80°C.	nitrogen compounds, and sulfur and nitrogen-containing heterocyclic compounds			Oven temperature: Initial 40°C for 2 min, 5°C/min to 250°C, 250°C for 2 min. Mass spectrometry conditions, EI: Ionization energy: 70 eV Scan mode: 50-500 *m/z*	Version 9.3.	
Strawberry	Freezed, homogenized into fine powder. 1g of frozen powder (incubated for 5 min at 30°C in a water bath) to 300µL of saturated NaCl solution. Used 900µL for 10 mL vial.	Not specified. Method is extensively used for determination of volatile compounds of plant species, with strawberry as example in this case.	Fiber, 60 µm DVB/PDMS	HS-SPME/GC-MS Incubation: 10 min, 50°C, 500 rpm agitation Extraction: 30 min, 50°C, 500 rpm agitation Injection: 1 min, 250°C Fiber conditioning: 5 min, 250°C	Gas chromatography conditions Column: HP-5 column (50 m × 0.32 mm × 1.05 µm) Carrier gas: Helium Flow rate: 1.2 mL/min Mode: Splitless Oven temperature: Initial 40°C for 3 min, 5°C/min to 250°C, 250°C for 5 min. Total run time with oven cooling: 60 min. Mass spectrometry conditions Ionization energy: 70 eV Scan mode: 35-250 *m/z*	SIMCA-P and MetAlign	(Rambla, López-Gresa, et al., 2015)
Tomatoes: tomato landraces (germplasm passport 4750, 3842, 565 and 557)	Chopped, frozen samples were grounded to powder, stored at -80°C. 1g tomato powder in 15mL tube was immersed in water bath at 35°C for 10 min.	Composition of VOCs in tomato landraces and commercial varieties compared.	Fiber, 50/30 µm CAR/PDMS/DVB	HS-SPME/GC-MS Incubation: 10 min, 50°C, 500 rpm agitation Extraction: 35 min, 50°C, 250 rpm agitation Injection: 1 min, 250°C	Gas chromatography conditions Column: Varian VF-5ms (30 x 0.25 mm, 0.25 µm) Carrier gas: Helium Flow rate: 1.0 mL/min Oven temperature: Initial 35°C for 5 min, 3°C/min to	Varian MS Workstation (Version 6.6) and Infostat	(Cortina et al., 2017)

	Dissolved in 15 μL of 2-methylcyclohexanone, 1 mL 100 mM EDTA/NaOH solution and $CaCl_2$ (2.2g). Samples were sonicated for 15 min and 1 mL sample was transferred to 10 mL vial.			Fiber conditioning: 3 min, 250°C	45°C, 1.5°C/min to 50°C, held for 1.5 min, 3°C/min to 68°C, held for 2 min, 3°C/min to 131°C, held for 1 min, 10°C/min to 250°C , 250°C for 2.93 min. Total run time with oven cooling: 58 min. Mass spectrometry conditions Ionization energy: 70 eV Scan mode: 33-300 *m/z* 1 scan per second		
Tomatoes: Lycopersicon esculentum L. cultivars	Fruits were reduced to a paste. 10 g of sample was mixed in a 20 mL vial with sodium chloride (10% (w/w)).	Aldehydes, higher alcohols, ketones and terpenes (95% total peak area), and fatty acids, esters, furans, sulphur compounds, aliphatic aromatics and hydrocarbons (5% total peak area)	Fiber, 50/30 μm CAR/PDMS/DVB	HS-SPME/GC-qMS Extraction: 45 min, 40°C, 800 rpm agitation Injection: 5 min, 250°C	Gas chromatography conditions Column: fused silica capillary column (30 x 0.25 mm, 0.25 μm) Carrier gas: Helium Flow rate: 1.1 mL/min Mode: Split-splitless Oven temperature: Initial 40°C for 1 min, 1.2°C/min to 80°C, held for 2 min, 3°C/min to 150°C, held for 2 min, 40°C/min to 220°C, 220°C for 10 min. Mass spectrometry conditions Ionization energy: 70 eV Scan mode: 35-300 *m/z* 7 scans per second	SPSS 17.0	(Figueira et al., 2014)

Tomatoes: F4-breeding lines (Black Cherry x Primabella and Black Cherry x Roterno F1)	50g of fruit and 89.5 mL 3.18 M NaCl-solution was homogenized for 30 s with a hand blender, and centrifuged at 4°C for 30 min at 1,690 *g*. 8 mL of the supernatant and 4 g of NaCl was added to a 20 mL glass vial, vortexed for 10 s, and stored at -20°C until analysis.	Profiling	Fiber, 100 μm PDMS	HS-SPME/GC-MS Incubation: 15 min, 35°C, constant agitation, 500rpm Extraction: 30 min, 35°C, constant agitation Injection: 1 min, 250°C (splitless mode), 9 min (split mode, ration 1:10)	Gas chromatography conditions Column: SH-Stabilwax (30 m × 0.25 mm × 0.25 μm) Carrier gas: Helium Flow rate: 1.24 mL/min Mode: Splitless/split (1:10) Oven temperature: Initial 35°C for 5 min, 5°C/min to 210°C and held for 20 min. Flame ionization detector (FID) with FID temperature: 250°C. Mass spectrometer with mass detector in electron impact ionization mode: 70 eV.	SPSS (v. 25) and R (v. 3.6.1)	(Kanski et al., 2020)
Tomato (*Lycopersicon esculentum* Mill.)	Fruit material was frozen in liquid nitrogen, grounded and stored at -80°C. 1g frozen fruit power was incubated at 30°C for 10 min, 1 mL of 100mM EDTA-NaOH and solid $CaCl_2$ was added. Vials were sonicated for 5 min and 1 mL was transferred into 10 mL vial.	Profiling of all volatiles detected by the analytical method used	Fiber, 65 μm PDMS/DVB	HS-SPME/GC-MS Extraction: 20 min, 50°C, constant agitation Injection: 1 min, 250°	Gas chromatography conditions Column: HP-5 (50 m × 0.32 mm × 1.05 μm) Carrier gas: Helium Oven temperature: Initial 45°C for 2 min, 5°C/min to 250°C and held for 5 min. Mass spectrometry conditions, EI Ionization energy: 70 eV Scan mode: 35-400 *m/z* 2.8 scan per second	Xcalibur	(Tikunov et al., 2005)

Tomatoes (Cappricia RZ and Amoroso RZ)	Fruit material was frozen in liquid nitrogen and grounded to powder. 1g frozen fruit powder was incubated at 30°C for 10 min and sonicated for 5 min.	Profiling. Aldehydes, carotenoids, amino acid derivatives.	Fiber, CAR/PD MS/DVB	HS-SPME/GC-MS Incubation: 10 min, 60°C, constant agitation Extraction: 20 min, 60°C, constant agitation Injection: 15 min, 250°C	Gas chromatography conditions Column: RTX-WAX (60 m × 0.32 mm × 0.25 µm) Carrier gas: Helium Mode: Splitless Oven temperature: Initial 40°C for 5 min, 5°C/min to 240°C and held for 10 min.	Canoco 4.5	(Farneti et al., 2015)

3.1.2 Optimization for HS-SPME/GC-MS

Optimization was done by testing the column program used by Cortina et al. (2017) and comparing this column program to the same program with 10 min post-run added. The results showed that 10 min post-run was beneficial (data not shown). Post-run gave increased throughput and allowed for sample extraction while previous sample was being analyzed. Different extraction times were also tested. 15 min extraction time compared to 35 min extraction time was not much different, so 15 min was chosen based on efficiency.

In order to establish method with good repeatability and reproducibility, five different sample preparation methods were tested. Addition of 1 mL H_2O, 1 mL EDTA, 1 mL EDTA + 2.2 g solid $CaCl_2$, 1 mL EDTA saturated with $CaCl_2$, and 1 mL H_2O + 2.2 g $CaCl_2$ were used with the same separation and detection method as described in chapter 2.6. The main criteria were to obtain relatively high total peak height, high number of peaks, low coefficient of variation (CV) of total peak height with each treatment and subjective perception of sample preparation procedure. Table 4 show that addition of 1 mL EDTA + 2.2 g solid $CaCl_2$ gives relatively high total peak height and the highest number of peaks, however CV is also relatively high. Addition of 1 mL EDTA saturated with $CaCl_2$ gave lowest variation of IS based on results viewed in MS-DIAL (data not shown). From Table 4 we see that addition of 1 mL EDTA saturated with $CaCl_2$ also gives relatively low CV. In regards to sample preparation, using solid $CaCl_2$ was time consuming and did not form a homogenized mixture for pipetting. Using solution saturated with $CaCl_2$, however,

formed a homogenous mixture and was less time consuming, and in turn increased reproducibility. In summary, solution saturated with $CaCl_2$ gave minimal variation of IS and sample preparation was better in terms of reproducibility.

Table 4. Optimization of sample preparation for HS-SPME/GC-MS analysis. n=3.

Sample preparation	Total peak height	CV (%)	Number of peaks
1 mL H_2O	4234284	19	138
1 mL EDTA	4825882	4	157
1 mL EDTA + 2.2 g solid $CaCl_2$	8131795	19	296
1 mL EDTA saturated with $CaCl_2$	6088855	12	194
1 mL H_2O + 2.2 g $CaCl_2$	9533674	21	134

3.1.3 Compound library

In order to optimize identification of VOCs in tomato fruits, a home-made compound library was built. Compound library GCMS DB_MoNA-Volatile-KovatsRI which was provided together with MS-DIAL was expanded by spectral identification using NIST MS search 2.0 (DB v. 2011) of peaks from tomato fruit samples. The integrated compound library contained information of fragmentation pattern, retention index, retention time and quantitative mass for approx. 200 VOCs. Information of approx. 200 more VOCs were added.

3.2 Fruit Quality Parameters

The five different varieties are shown in Figure 3. P is a red cherry tomato, SE is a red grape tomato, B is a red cocktail tomato, and FL and SO are red salad cultivars. In order to evaluate how post-harvest storage conditions affect important tomato fruit quality parameters, dry matter content, total soluble solids, titratable acidity, firmness, soluble sugars and pigments were measured for all varieties as explained in materials and methods section. Comparison table of fruit quality parameters with analysis of variance (ANOVA) and Tukey's test results are shown in Table 5. All varieties were stored in simulation to the post-harvest chain. In short, the

tomatoes were analyzed fresh from harvest, after 4°C storage in darkness and after 20°C storage with artificial light.

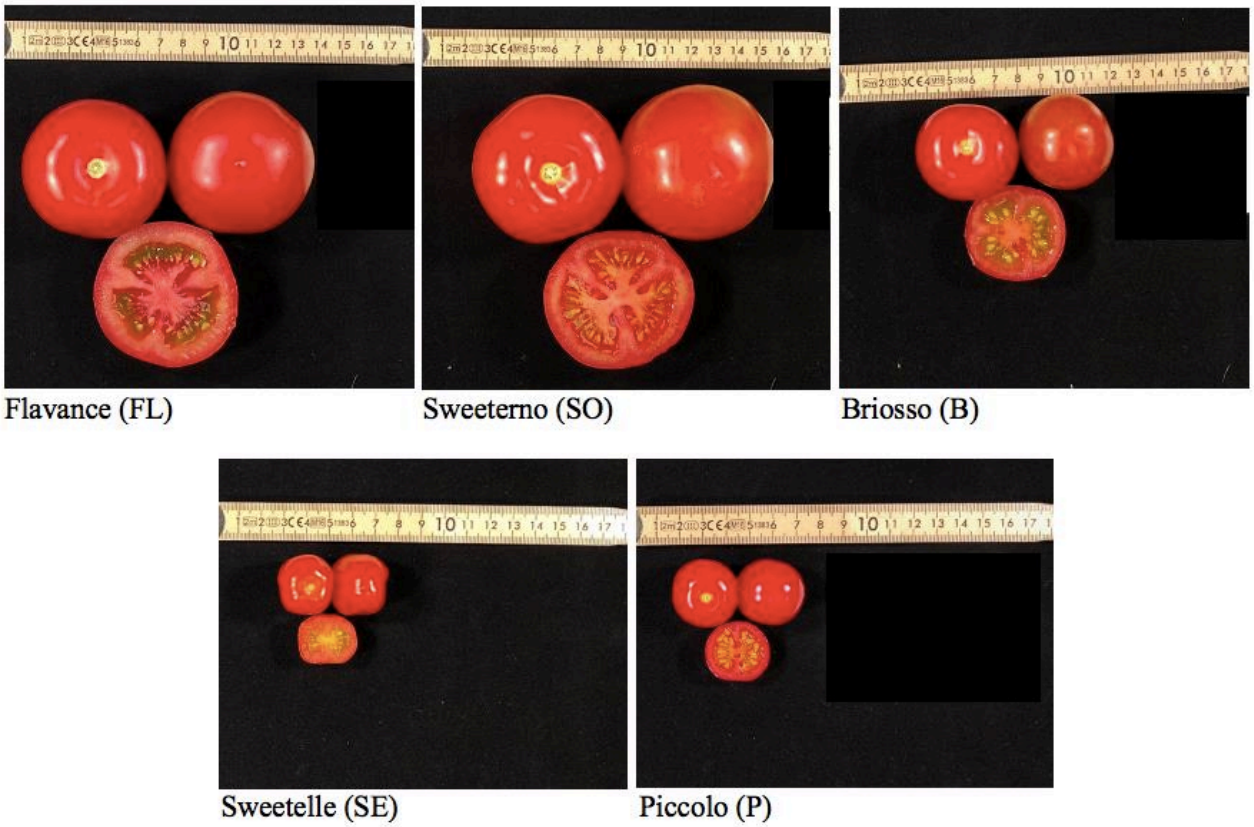

Figure 3. Tomatoes of the following varieties FL, SO, B, SE and P are shown next to the measuring scale to present their size and appearance right after harvesting.

*Table 5. Fruit quality parameters (mean ± standard deviation) for the varieties Flavance (FL), Sweeterno (SO), Briosso (B), Sweetelle (SE) and Piccolo (P) for fresh harvested fruits (fresh), after 20°C and 4°C storage, n = . Different letters indicate significant differences between fresh harvested fruits, after 20°C and 4°C storage for each variety (Tukey-Test p ≤ 0.05). A two-way ANOVA was performed as well. ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001.*

Variety	Treatment	DMC (%) n=4-5	TSS (°Brix) n=4-5	TA (%) n=5	TSS/TA ratio n=4-5	Glucose (µg/mg DW) n=4-5	Fructose (µg/mg DW) n=4-5	Sucrose (µg/mg DW) n=4-5	TSI n=5	Firmness n=5	Total carotenoids (µg/g DW) n=5	Total chlorophyll (µg/g DW) n=5
Flavance (FL)	Fresh	5.8 ± 0.4a	4.4 ± 0.3a	0.5 ± 0.0a	8.9 ± 0.2c	70.2 ± 25.7a	114.6 ± 24.6a	21.8 ± 9.5a	247.1 ± 48.7a	79.2 ± 3.1a	1.2 ± 0.1b	0.11 ± 0.07a
	4°C	6.0 ± 1.0a	4.2 ± 0.4a	0.4 ± 0.0b	10.5 ± 0.2a	65.9 ± 16.1a	122.3 ± 16.0a	34.1 ± 10.7a	267.7 ± 42.7a	71.2 ± 2.4b	1.2 ± 0.1b	0.04 ± 0.03a
	20°C	5.6 ± 0.3a	4.2 ± 0.2a	0.4 ± 0.0b	10.0 ± 0.2b	74.1 ± 11.5a	142.0 ± 19.3a	32.3 ± 12.9a	308.9 ± 45.9a	68.8 ± 3.7b	1.6 ± 0.1a	0.08 ± 0.02a
Sweeterno (SO)	Fresh	6.1 ± 0.7a	4.3 ± 0.2a	0.5 ± 0.0ab	9.1 ± 0.7a	79.4 ± 10.8a	151.9 ± 10.0a	25.0 ± 13.0a	295.4 ± 38.1a	82.4 ± 2.7a	0.8 ± 0.3b	0.01 ± 0.01a
	4°C	6.1 ± 0.3a	4.4 ± 0.2a	0.5 ± 0.0a	9.0 ± 0.7a	70.6 ± 19.5a	107.5 ± 25.3b	28.1 ± 13.7a	243.0 ± 65.2a	80.2 ± 1.5a	1.0 ± 0.1b	0.01 ± 0.02a
	20°C	5.3 ± 0.1b	4.3 ± 0.4a	0.4 ± 0.0b	10.0 ± 1.3a	53.7 ± 13.7a	103.9 ± 15.7b	27.5 ± 8.5a	224.1 ± 28.6a	73.0 ± 3.3b	1.5 ± 0.1a	0.02 ± 0.02a
Briosso (B)	Fresh	7.3 ± 0.2a	4.6 ± 0.1b	0.5 ± 0.0a	8.7 ± 0.5a	71.0 ± 12.7a	121.6 ± 29.4a	46.3 ± 13.8a	282.7 ± 51.8a	62.8 ± 1.6a	1.3 ± 0.3a	0.03 ± 0.08a
	4°C	6.8 ± 0.4a	4.8 ± 0.2ab	0.5 ± 0.0a	9.2 ± 0.9a	63.6 ± 22.3a	98.6 ± 23.7a	49.0 ± 24.1a	245.3 ± 39.3a	60.4 ± 2.6a	1.2 ± 0.2a	0.02 ± 0.02a
	20°C	7.3 ± 0.5a	5.0 ± 0.1a	0.5 ± 0.0a	9.3 ± 0.3a	74.9 ± 17.2a	90.1 ± 18.6a	50.3 ± 36.2a	242.3 ± 56.1a	53.8 ± 1.6b	1.4 ± 0.1a	0.05 ± 0.04a
Sweetelle (SE)	Fresh	10.6 ± 0.5a	7.4 ± 0.2a	0.7 ± 0.0a	10.3 ± 0.2b	70.9 ± 10.9a	89.7 ± 19.7b	38.8 ± 11.9a	227.2 ± 40.1b	65.0 ± 1.9a	0.8 ± 0.2c	0.10 ± 0.01a
	4°C	10.3 ± 0.3a	7.5 ± 0.1a	0.6 ± 0.0b	11.9 ± 0.5a	84.4 ± 18.0a	101.3 ± 21.8b	45.2 ± 7.3a	261.3 ± 45.0ab	65.2 ± 2.6a	1.0 ± 0.1b	0.05 ± 0.02b
	20°C	10.1 ± 0.4a	7.5 ± 0.2a	0.6 ± 0.0b	12.4 ± 0.3a	91.8 ± 9.3a	138.2 ± 19.8a	39.5 ± 9.4a	316.6 ± 33.9a	62.6 ± 3.5a	1.3 ± 0.1a	0.03 ± 0.02b
Piccolo (P)	Fresh	13.2 ± 0.8a	9.9 ± 0.2a	0.7 ± 0.1ab	13.7 ± 1.2a	97.8 ± 19.5a	112.5 ± 12.8a	49.0 ± 6.1b	307.8 ± 42.2a	68.6 ± 1.7a	1.0 ± 0.1b	n.d.
	4°C	12.7 ± 1.5a	8.6 ± 1.0b	0.8 ± 0.0a	11.0 ± 1.4b	86.5 ± 7.3a	106.9 ± 22.4a	44.2 ± 7.4b	258.1 ± 45.3a	63.0 ± 3.7b	1.2 ± 0.1a	n.d.
	20°C	12.4 ± 0.7a	9.3 ± 0.3ab	0.7 ± 0.1b	13.8 ± 1.5a	106.2 ± 9.6a	108.3 ± 10.5a	62.7 ± 6.0a	305.8 ± 22.4a	56.2 ± 4.0c	1.3 ± 0.2a	n.d.
Variety		***	***	***	***	***	*	***	ns	***	***	***
Treatment		ns	ns	***	***	ns	ns	ns	ns	***	***	*
Variety x Treatment		ns	***	***	***	ns	***	ns	**	**	**	ns
Flavance (FL)		5.8 ± 0.6d	4.3 ± 0.3d	0.4 ± 0.0d	9.8 ± 0.7c	69.8 ± 18.0b	126.1 ± 22.3a	29.4 ± 11.8b	274.6 ± 50.1a	73.1 ± 5.4b	1.3 ± 0.2a	0.08 ± 0.05a
Sweeterno (SO)		5.9 ± 0.6d	4.3 ± 0.3d	0.5 ± 0.0d	9.4 ± 1.0c	67.1 ± 17.9b	121.1 ± 28.2a	27.01 ± 11.0b	254.2 ± 53.3a	78.5 ± 4.8a	1.1 ± 0.4ab	0.02 ± 0.02cd
Briosso (B)		7.1 ± 0.5c	4.8 ± 0.2c	0.5 ± 0.0c	9.0 ± 0.6c	69.8 ± 17.2b	103.4 ± 26.4a	48.5 ± 24.5a	256.8 ± 49.7a	59.0 ± 4.4d	1.3 ± 0.2a	0.03 ± 0.05bc
Sweetelle (SE)		10.3 ± 0.4b	7.5 ± 0.1b	0.7 ±0.1b	11.5 ± 1.0b	82.4 ±15.2ab	109.7 ± 28.6a	41.2 ± 9.5ab	268.4 ± 53.1a	64.3 ± 2.8c	1.0 ± 0.2b	0.06 ± 0.03ab
Piccolo (P)		12.8 ± 1.1a	9.3 ± 0.8	0.7 ± 0.1a	12.8 ± 1.8a	97.6 ±14.9a	109.1 ± 14.5a	51.9 ± 10.0a	290.6 ± 42.5a	62.6 ± 6.0cd	1.2 ± 0.2ab	n.d.

3.2.1 Dry matter content

DMC of tomato fruits was significantly different between different varieties (Table 5). All varieties except B showed a decrease in average DMC after 20°C storage, however it was only significant for SO with 13% decrease. P had the highest DMC among all varieties, with 55% higher DMC than FL which was the lowest.

3.2.2 Total soluble solids

TSS showed significant storage effects only for P and B (Table 5) where B increased by 8% after 20°C storage, and TSS in P was reduced by 13% after 4°C storage. TSS showed significant differences between varieties. P (9.3 ± 0.8 °Brix) had the highest TSS among all varieties, with 54% higher TSS than FL (4.3 ± 0.3 °Brix) which was the lowest.

3.2.3 Titratable acidity

TA correlated with both treatment and variety effects (Table 5). TA decreased by 1-15% after 20°C storage for all varieties however it was only significant for FL and SE. TA decreased significantly after 4°C storage for FL and SE. TA of SO and P showed significant differences between 20°C and 4°C storage, however they were not different from fresh fruit. P had the highest TA among all varieties, with 39% higher TA than FL which was lowest. Figure 4 show that TA generally decreases during 20°C storage.

TSS/TA ratio showed both treatment and variety effects (Table 5). TSS/TA ratio was increased in all varieties after 20°C storage, however it was only significant for FL and SE. FL and SE showed significant increase in TSS/TA ratio after 4°C storage. P showed significant decrease after 4°C storage. P (12.8 ± 1.8 ratio) had the highest TSS/TA among all varieties, with 29% higher TSS/TA than B (9.0 ± 0.6 ratio) which was the lowest.

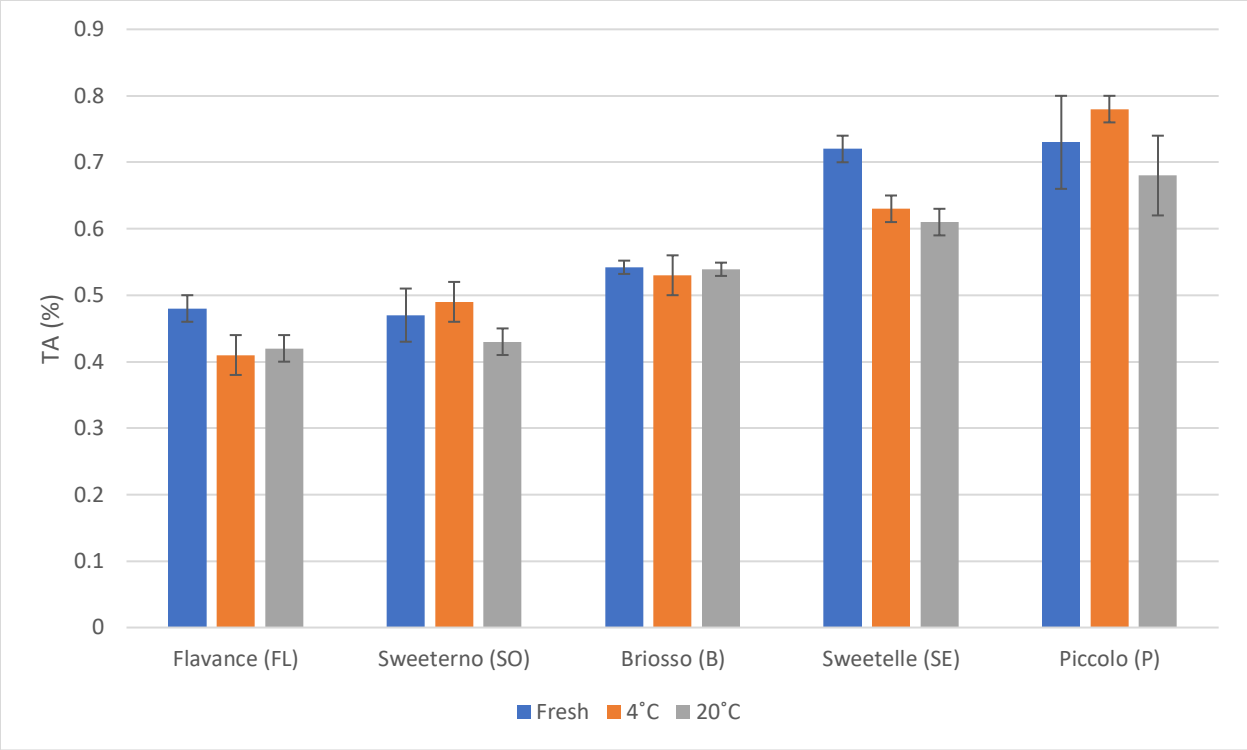

Figure 4. Titratable acidity (TA, %) for different tomato varieties for fresh harvested fruit, 4°C storage and 20°C storage. Mean values ± SD are shown. n=5.

3.2.4 Firmness

Firmness of the fruits showed significant effects of both storage and variety (Table 5). All varieties showed decrease in firmness after 20°C storage, except SE. All varieties except SE showed a decrease in firmness after 4°C storage as well, however it was only significant for FL and P. Firmness regardless of treatment was significantly different between varieties. SO (78.5 ± 4.8 Durofel units) was most firm among all varieties and B (59.0 ± 4.4 Durofel units) was lowest.

3.2.5 Soluble sugars

TSI showed no significant differences between varieties, however P (290.6 ± 42.5 TSI) showed the highest TSI, with 12.5% higher TSI than SO (254.2 ± 53.3 TSI) which was lowest (Table 5). There were no clear effect of storage on TSI.

The concentration of glucose showed no significant differences after storage, however there was a significant difference between varieties (Table 5). P was significantly higher than FL, SO and B when compared regardless of treatment.

The concentration of fructose showed significant effects of storage on SO and SE only (Table 5). SO showed a significant reduction of fructose after both 20°C and 4°C storage. SE showed a significant increase of fructose after 20°C storage. There were no significant differences between varieties when compared regardless of treatment.

The concentration of sucrose showed significant differences between varieties (Table 5). All varieties showed an increase of sucrose after 20°C storage, however the effects were only significant for P. All varieties except P showed an increase of sucrose after 4°C storage as well, however these results were not significant. B and P was significantly higher than FL and SO when compared regardless of treatment.

3.2.6 Pigments

For total carotenoids, almost all varieties showed an increase after both storage treatments (Figure 5, Table 5). This increase was highest after 20°C storage and have a tendency to be significant. Highest increase was 47% for SO after 20°C storage. FL (1.3 ± 0.2 µg/g DW) and B (1.3 ± 0.2 µg/g DW) had highest amounts of total carotenoids and SE (1.0 ± 0.2 µg/g DW) was lowest. Figure 5 clearly show that total carotenoids increase during 20°C storage. Total carotenoids also showed significant differences between varieties.

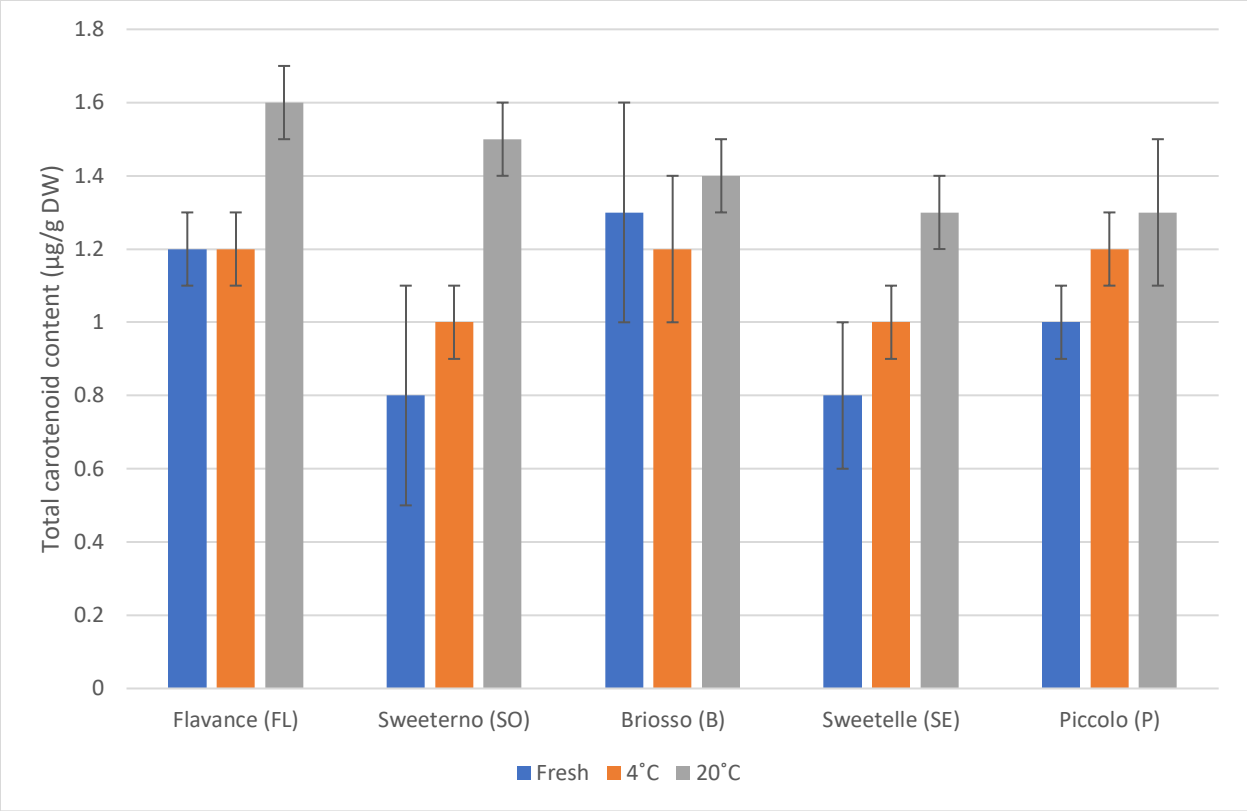

Figure 5. Total carotenoid content (µg/g DW) for different tomato varieties for fresh harvested fruit, 4°C storage and 20°C storage. Mean values ± SD are shown. n=5.

Sum of Chl*a* and Chl*b* was calculated as total chlorophyll. Chlorophyll content was low in general. Only SE showed significant increase after both storage conditions. Total chlorophyll also showed significant differences between varieties.

3.3 Volatiles

In order to evaluate how post-harvest storage conditions affect composition of VOCs, volatile profiles of the tomatoes were obtained using HS-SPME/GC-MS analysis (chapter 2.5). In total, 76 volatile compounds were identified. Heatmap (appendix, figure A1) and analysis of variance (ANOVA) and Tukey's test results (appendix, table A1) of all identified compounds are shown in appendix. Significant differences were observed for 71 compounds. Out of all flavor related volatiles mentioned in chapter 1.1.4, 14 of these were identified. Relative FC for 14 compounds related to tomato flavor is presented in Table 6.

Table 6. Flavor related volatiles and their fold changes after the two storage conditions 4°C and 20°C compared to fresh fruit. Volatile compounds were identified using compound library (GCMS DB_MoNA-Volatile-KovatsRI) and NIST 2011 compound library, normalized to internal standard and relative fold change (FC) was calculated for each compound. The color scale ranges from 33.77 (green) to 0.00 (red). List of flavor related compounds was retrieved from Baldwin et al. (2000). (n.d.), not detected. n=5.

	Flavance		Sweeterno		Briosso		Sweetelle		Piccolo	
Volatiles	4°C	20°C	4°C	20°C	4°C	20°C	4°C	20°C	4°C	20°C
3-methylbutanal	0.10	0.03	0.50	0.36	0.14	0.22	0.12	0.19	0.52	0.07
2-methylbutanal	0.60	0.21	0.38	0.26	0.27	0.34	0.20	0.15	0.48	0.12
1-penten-3-one	0.19	0.17	0.27	0.26	0.17	0.15	0.32	0.20	0.54	0.11
(E)-2-pentenal	0.26	0.21	0.31	0.29	0.18	0.26	0.30	0.40	0.55	0.58
Hexanal	0.38	0.38	0.50	0.88	0.33	0.62	0.41	1.53	0.63	2.17
(E)-2-hexenal	0.32	0.43	0.37	0.59	0.31	0.40	0.34	0.53	0.60	1.01
1-hexanol	0.06	0.16	2.98	2.77	0.13	0.33	0.36	0.85	0.70	3.80
(E)-2-heptenal	0.36	0.79	0.97	2.64	0.34	0.82	0.53	1.27	0.51	1.21
6-methyl-5-hepten-2-one	1.14	0.58	1.45	1.25	0.36	0.53	0.54	1.98	0.66	1.07
Phenylacetaldehyde	2.80	9.94	0.69	2.88	1.40	2.59	17.19	33.77	0.89	0.64
Linalool	0.30	n.d.	n.d.	n.d.	n.d.	n.d.	6.78	n.d.	1.08	0.08
beta-Cyclocitral	0.71	0.60	0.65	0.83	0.42	0.60	0.83	1.03	0.69	0.71
Neral	1.92	11.22	0.99	20.16	14.15	26.56	0.35	n.d.	1.42	0.02
beta-Damascenone	0.44	0.34	0.17	0.79	9.31	8.44	10.54	0.29	0.67	0.00

There is no clear trend regarding all volatiles (Table 6). For all varieties, hexanal, (E)-2-hexenal, 1-hexanol (except SO), (E)-2-heptenal, phenylacetaldehyde (except P) and neral (except SE and P) was higher after 20°C storage compared to 4°C storage, however not all were increased compared to fresh fruit. Phenylacetaldehyde and neral increased more after 20°C storage by at least 2.59 folds for all varieties except P. 3-methylbutanal, 2-methylbutanal, 1-penten-3-one and (E)-2-pentenal decreased in all varieties after both storage conditions. Table A1 in appendix describe significant differences in detail.

Heatmap for flavor related volatiles (Figure 6) show that SO is clustered together with B4, FL4 and FL20 and generally has low concentration of volatiles. Fresh and two varieties for 20°C storage treatments (FLF, BF, SEF, P20 and SE20) are grouped together at the left side of the heatmap and generally have high intensities for (E)-2-pentenal, hexanal, (E)-2-hexenal, 1-hexanol, (E)-2-heptenal and 6-methyl-5-hepten-2-one, and low intensities for phenylacetaldehyde, linalool, *beta*-Cyclocitral, neral and *beta*-Damascenone. Grouped in the

middle is a mix of treatments (P4, PF, B20 and SE4) with generally the opposite intensities for compounds compared to group to the left.

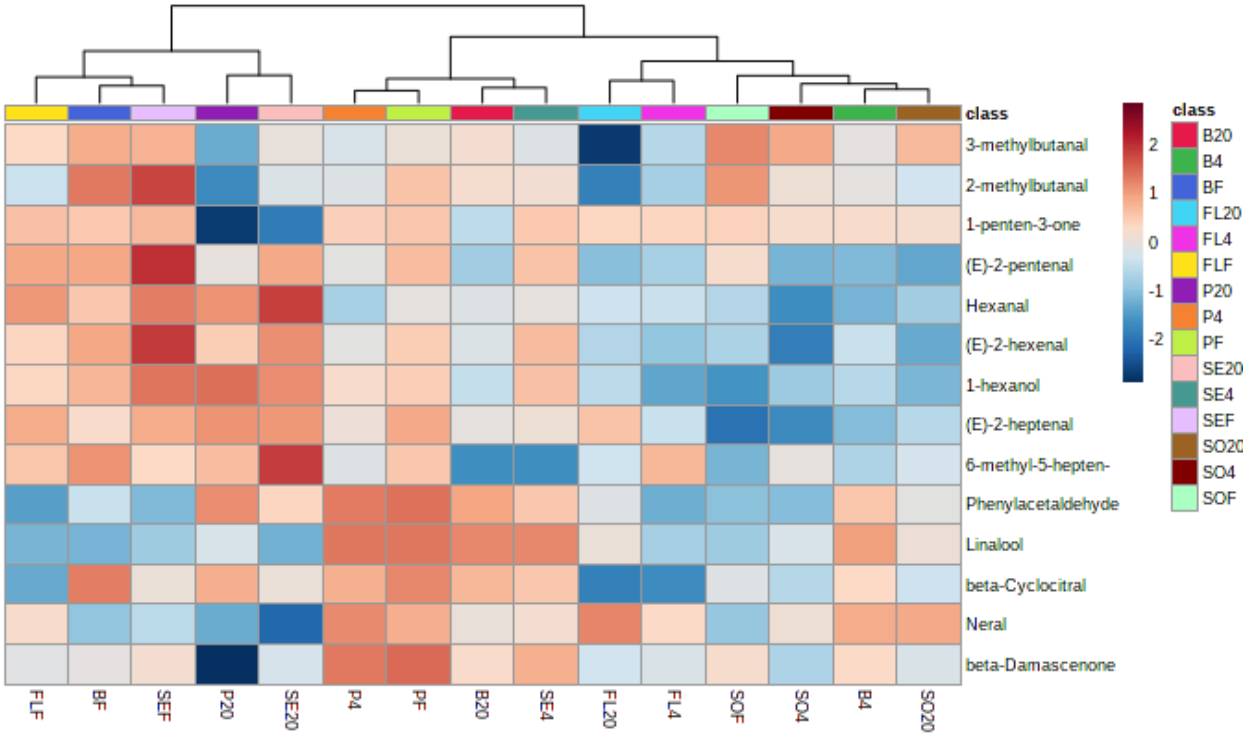

Figure 6. Heatmap for flavor related volatiles. (B), Briosso; (FL), Flavance; (P), Piccolo; (SE), Sweetelle; (SO), Sweeterno; (20), 20˚C storage; (4), 4˚C storage; (F), fresh fruit. List of flavor related compounds was retrieved from Baldwin et al. (2000). n=5.

PCA analysis (Figure 7) show that there is no clear clustering between all treatments but there are some clustering among varieties (SE and B). Both F and 4°C storage treatment for P, SO and FL are relatively close together. SE and B does not show this trend as clearly. For all varieties, 20°C storage treatment has shifted upward. However, the plot show that there is a large variation within 20°C storage treatment for most varieties. Groups of 20°C storage treatment is more separated from each other based on PC2, while within each group for this treatment there is more differences based on PC1. The PCA plot gives the indication that 20°C storage treatment affected composition of flavor related compounds in a grater scale than 4°C storage treatment.

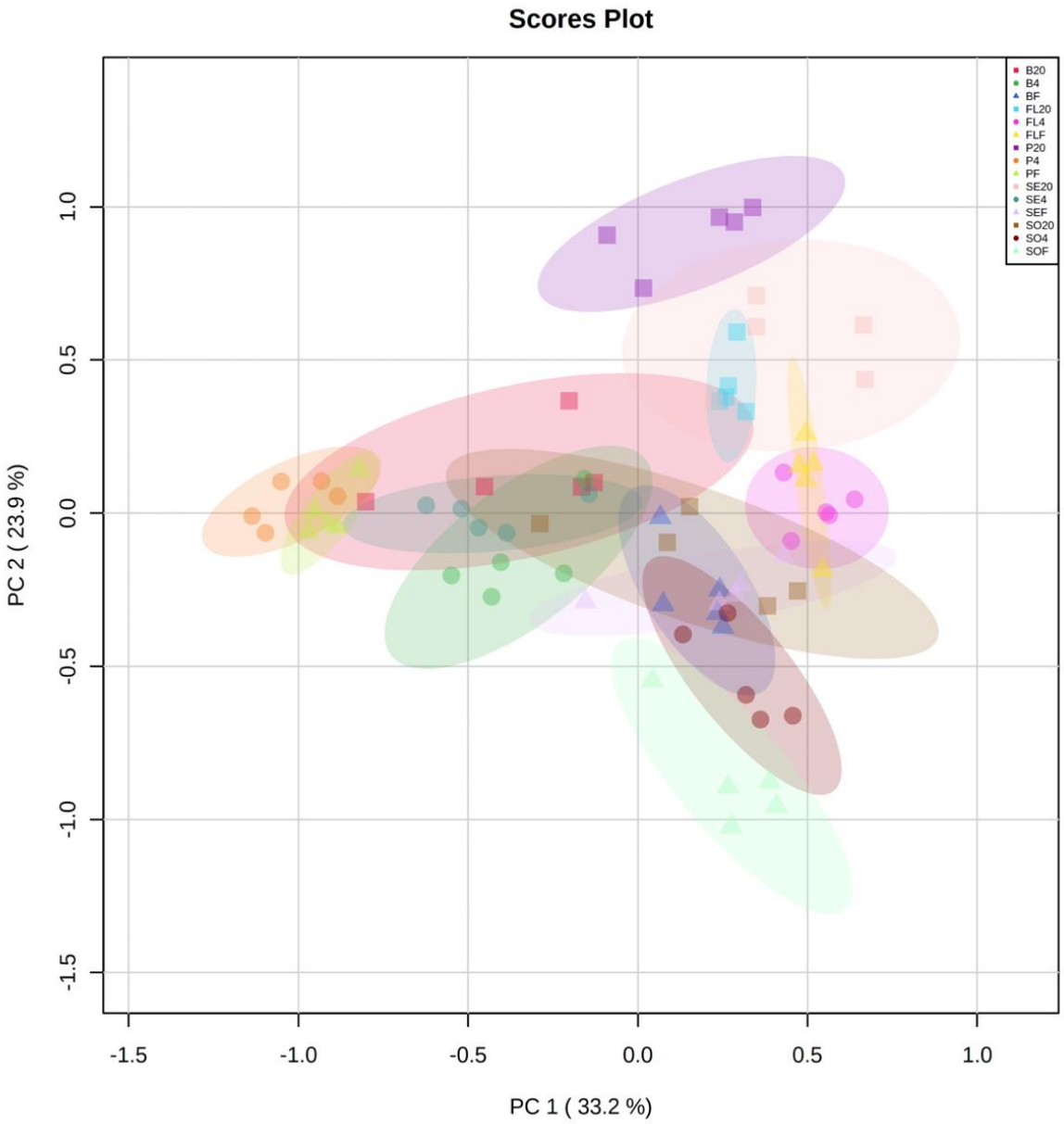

Figure 7. Principal component analysis (PCA) scores plot for flavor related volatiles. Data is normalized to sum of peak heights of identified metabolites and transformed to cube root, with range scaling. (B), Briosso; (FL), Flavance; (P), Piccolo; (SE), Sweetelle; (SO), Sweeterno; (20), 20°C storage; (4), 4°C storage; (F), fresh fruit. n=5.

PCA biplot (Figure 8) shows that there is a correlation between 1-hexanol and (E)-2-heptenal and these compounds has influences PC2 along with hexanal. There is a negative correlation between mentioned compounds and 3-methylbutanal, 2-methylbutanal, 1-penten-3-one and (E)-2-pentenal which influences PC2 in the other direction. Phenylacetaldehyde, linalool, *beta-*

Damascenone and *beta*-Cyclocitral strongly influence PC1 which is probably variety related. 1-hexanol, (E)-2-heptenal and hexanal most likely contribute to the separation of 20°C storage treatment samples from others.

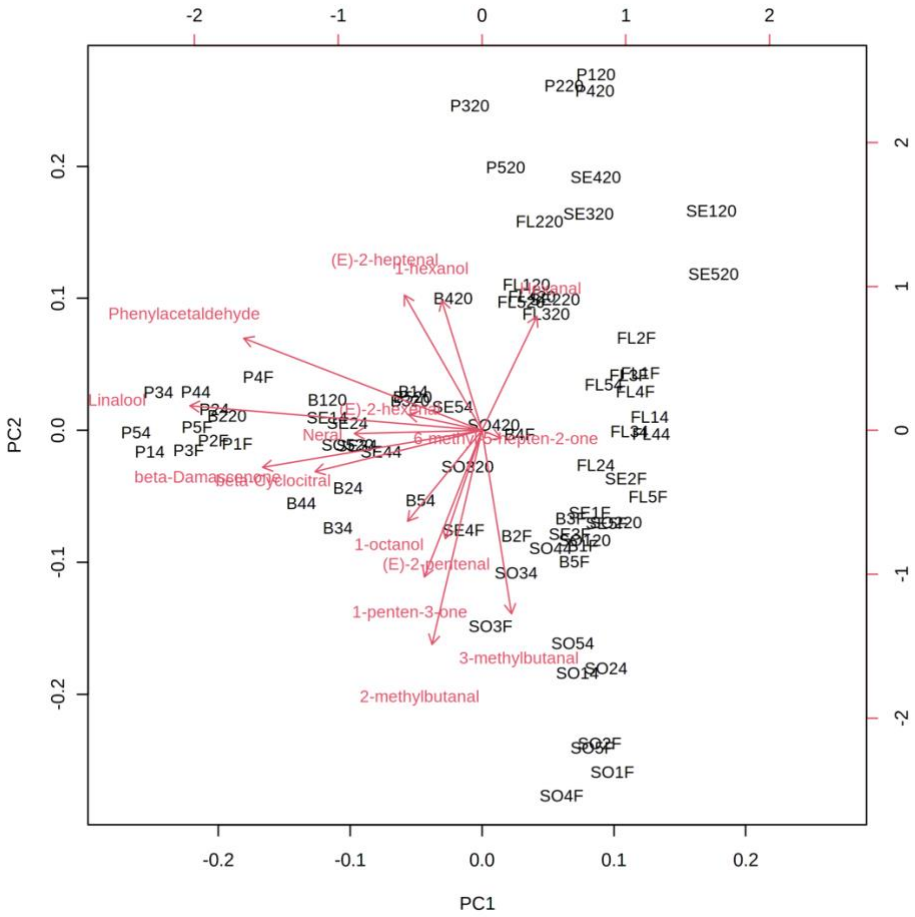

Figure 8. PCA biplot for flavor related volatiles. (B), Briosso; (FL), Flavance; (P), Piccolo; (SE), Sweetelle; (SO), Sweeterno; (20), 20˚C storage; (4), 4˚C storage; (F), fresh fruit. n=5.

3.4 Sensory evaluation

We concluded a sensory evaluation to assess flavor related parameters of different tomato varieties directly after harvest, and after storage at 4°C and 20°C.

Figure 9 show spider webs of sensory evaluation for each variety, and Figure 10 show spider web of sensory evaluation for the varieties regardless of storage conditions. Table A2 (appendix)

show comparison table of sensory evaluation with analysis of variance (ANOVA) and Tukey's Post Hoc test results.

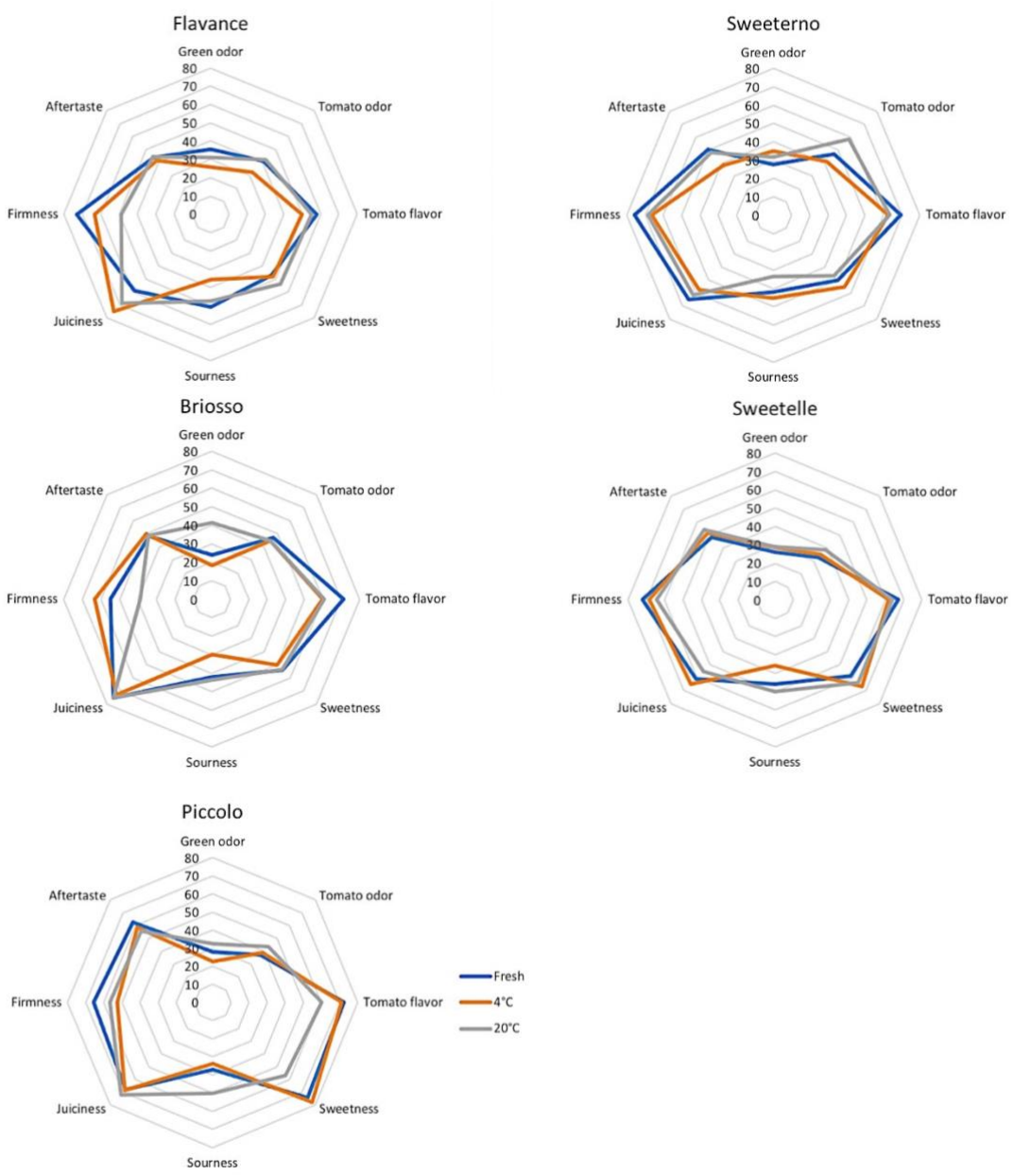

Figure 9. Sensory evaluation of the different varieties. Spider webs show the results for each variety, with the lines representing either fresh fruits, fruits stored at 20°C, or fruits stored at 4°C household storage. Briosso, (B); Flavance, (FL); Sweeterno, (SO); Sweetelle, (SE); Piccolo, (P). (n=6-10).

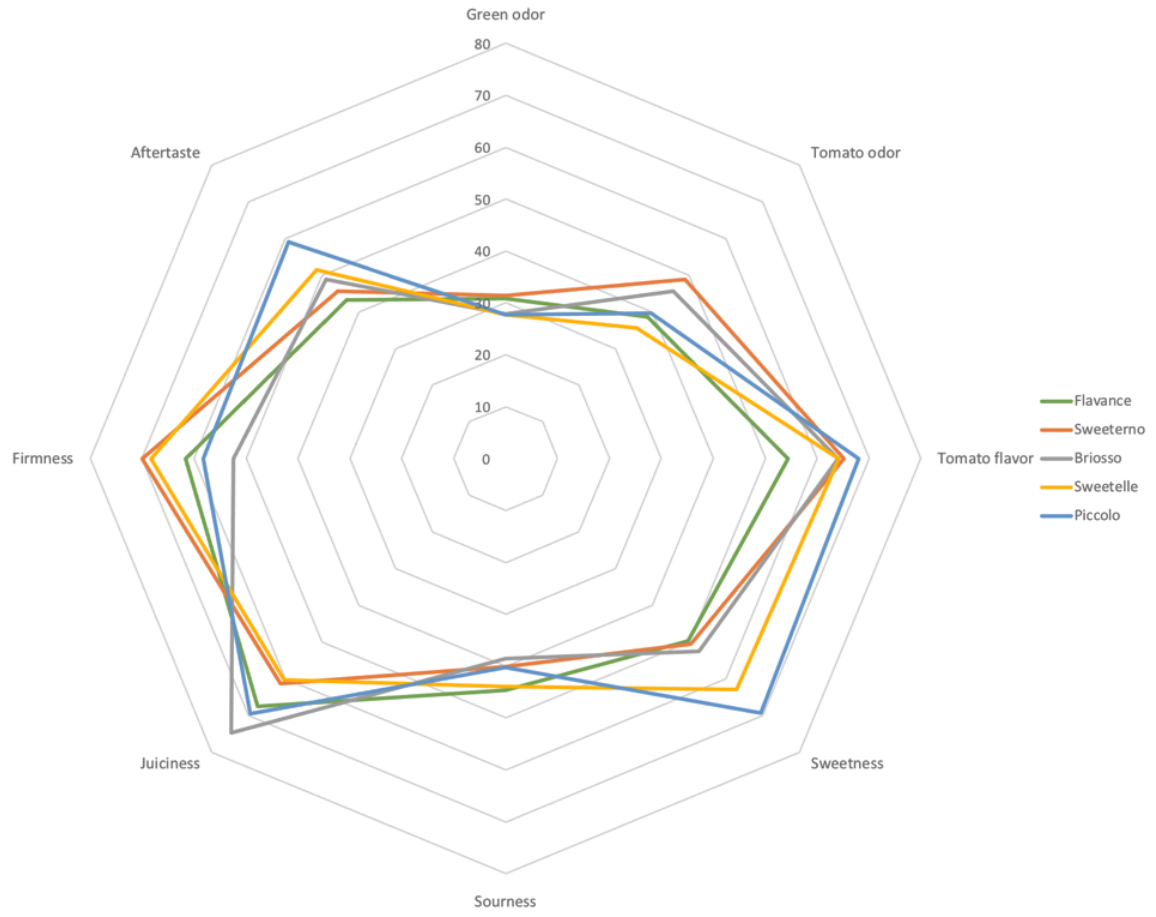

Figure 10. Sensory evaluation of the different varieties regardless of storage. Briosso, (B); Flavance, (FL); Sweeterno, (SO); Sweetelle, (SE); Piccolo, (P). (n=6-10).

The sensory evaluation showed no significant differences for green/grassy odor, tomato-typical odor, sourness and aftertaste. Sweetness, juiciness, tomato-typical flavor and firmness showed significant differences between varieties regardless of treatment. P showed significantly higher score for tomato-typical flavor than FL. P showed significantly higher score for sweetness than FL, SO and B. B showed significantly higher score for juiciness than SO and SE, and significantly lower firmness of the fruit peel than SO and SE (table A2). Average grade for the tomato-typical flavor was decreases after both 20°C and 4°C storage for all varieties, however the decrease was only significant for B after 4°C storage (table A2). According to sensory evaluation, firmness of

the fruit peel was decreased after 20°C storage for all varieties and after 4°C storage for all varieties except B. The score was only significantly reduced after 20°C storage for FL. B showed significant difference between 20°C and 4°C storage (table A2). Figure 10 show negative correlation between firmness and juiciness.

Tomato-typical flavor decreased in all varieties after both storage conditions, and it generally decreased more after 4°C storage, with highest decrease of 12% for B. Firmness also generally decreased after both storage conditions. Regardless of treatment, P and SE showed highest score for tomato-flavor and sweetness (Figure 10). Juiciness and sweetness show no clear storage effect. Sourness decreased for all varieties (except SO) after 4°C storage.

In summary, fresh and 20°C storage generally have higher scores for tomato-typical flavor while 4°C storage have lower scores for sourness. Tomato-typical odor have generally higher scores after 20°C storage as well. Combining tomato-typical flavor and odor, the trend is that 20°C storage gives more overall tomato taste than 4°C storage based on sensory results.

Discussion

The objectives of this study was to establish an analytical procedure to analyze VOCs from tomato fruit, analyze how post-harvest storage conditions affect important tomato fruit quality parameters, and to evaluate how the post-harvest chain affect VOC composition in tomatoes.

4.1 Establishing methodology for analysis of VOCs from tomato fruit

In order to find a suitable analytical method and fiber to analyze VOCs from tomato fruit, literature review was done (Table 3). It was found that HS-SPME/GC-MS was frequently used with 50/30 μm DVB/CAR/PDMS fiber for VOC profiling. SPME was used as opposed to HS alone to increase sensitivity.

Rambla, Alfaro, et al. (2015) pointed out that different sample processing methods produces characteristic volatile patterns, thus it is difficult to compare results received using different fiber coating materials.

Column program as used by Cortina et al. (2017) was tested and showed good separation of peaks. However, it was necessary to see if post-run would enhance separation as oven needed more cooling time between samples. The results showed that 10 min post-run was beneficial. This gave increased throughput and allowed for sample extraction while previous sample was analyzed. The method for sample preparation and analysis of VOCs was based on the method by Cortina et al. (2017), where extraction time was 35 min. However, they used 1 cm fiber whereas in this study used 2 cm fiber was used. Optimization for extraction time was done to find appropriate time in regards to sensitivity of fiber and efficiency. Extraction times of 15 min and 35 min were tested and 15 min extraction time showed to be sufficient. This may be due to the fiber reaching its maximum capacity already at 15 minutes.

It was also necessary to find appropriate sample preparation method. As viewed in literature review (Table 3), addition of salt (NaCl or $CaCl_2$) and/or EDTA was often used. Optimization was done to find a method with good repeatability and reproducibility. The results showed that the addition of 1 mL EDTA saturated with $CaCl_2$ gave low variation of IS and relatively low CV (Table 4). Compared to the other methods in optimization, it also gave relatively high number of peaks. While, the addition of 1 mL EDTA with solid $CaCl_2$ gave high total peak height and number of peaks, whereas the variation of IS was bigger. Based on this, addition of 1 mL EDTA saturated with $CaCl_2$ was the chosen method.

Identification of VOCs was done using integrated compound library (GCMS DB_MoNA-Volatile-KovatsRI). The compound library contains information of fragmentation pattern, retention index, retention time and quantitative mass for approx. 200 VOCs. To maximize number of identified compounds, the library was expanded by spectral identification of peaks from tomato fruit samples using NIST MS search 2.0 (DB v. 2011). Information about approx. 200 more VOCs were added to the library. Both spectrum and retention index similarity was used in identification.

Out of eight tomato varieties obtained from local greenhouses, five varieties with the most significant differences in size were chosen for this: two red salad cultivars (FL and SO), one red cocktail tomato (B), one red grape tomato (SE) and one red cherry tomato (P). All these varieties are consumer oriented, meaning they have above average taste quality compared to the

varieties typically grown in Norway. Due to competition from imported tomatoes, only high value varieties are grown in winter in Jæren, Norway.

The impact of the post-harvest chain on tomatoes was investigated for two storage conditions (20°C with light and 4°C in darkness). The post-harvest chain in Jæren, Norway was taken into account including packaging and transport, retail and consumer storage. The time frame for the post-harvest chain varies, but for this study it was assumed that 1 day is spent for harvesting, 3 days for packaging and transport, 2 days in retail and 4 days consumer storage in either refrigerator (4°C) or on the kitchen counter (20°C).

4.2 Influence of post-harvest storage conditions on important fruit quality parameters and sensory evaluation

Sugars, titratable acidity, dry matter content, firmness and pigments was analyzed in order to evaluate how post-harvest storage conditions affect important tomato fruit quality parameters.

Fruit quality parameters show that there is a large variation between the different varieties. Smaller tomato varieties SE and P had generally higher values for quality parameters (except for carotenoids and firmness) meaning they were more tasty, as expected.

TA and firmness decreased after both storage conditions. TA and firmness decreased more after 20°C storage. Decrease in firmness may indicate water evaporation. Measurement of average tomato weight before storage could be helpful in identification of evaporation. Carotenoids increased after both storage treatments, and was highest after 20°C storage. Results on carotenoid content were similar with results from Kanski et al. (2020) and Farneti et al. (2015). Main carotenoids in tomato include lycopene and *beta*-carotene and they are responsible for the distinctive red color (Vogel et al., 2010).

TSI was not affected by storage, which is consistent with results from Kanski et al. (2020). Zhang et al. (2016) also found that sugar content was not affected by cold temperatures, however they found that acids were not affected by this either. This study found significant reduction in TA after both storage conditions for two varieties only (FL and SE). Considering TSI, there is no

difference between varieties. TSS show large difference between varieties, and this parameter is proportional to the content of soluble carbohydrates. However, taking into account TSI, we do not see this trend. TSS was measured on fresh weight basis, while individual sugars were measured using dry material. This indicates that there was a dilution effect between varieties, they may have the same sugar content but different concentrations due to fruit size. As Dzakovich et al. (2015) found, variation in sugar concentrations does not necessarily change perceived sweetness.

Sensory evaluation was conducted to assess flavor related parameters of different tomato varieties, and the results show that subjective tomato-typical flavor and firmness generally decreased during storage (Table A3 in appendix). Juiciness and sweetness show no clear storage effects. Sourness was also generally decreased. These results correlate with laboratory analysis of fruit quality parameters. Combining tomato-typical flavor and odor, the trend is that 20°C storage gives more overall tomato taste than 4°C storage based on sensory evaluation, however it still decreased compared to fresh fruit. These results are in contrast to Kanski et al. (2020) which found no differences between treatments. However, Kanski et al. (2020) performed sensory evaluation with trained panel to be able to distinguish between different tastes and odors. In our study, the panel was not trained and there were few panelists (6-10) which probably explains high standard deviation and low significance of our sensory evaluation results.

Based on the results of sensory evaluation (firmness, juiciness, sweetness, tomato-typical flavor and aftertaste) it is possible to distinguish different tomato varieties (Table A3 in appendix). Similarly to laboratory results, P and SE showed highest score for tomato-flavor and sweetness.

Tomato-typical flavor decreased in all varieties after both storage conditions, also the amount of volatiles excluding carotenoid derived volatiles decreased after storage as well.

4.3 Influence of post-harvest storage conditions on volatiles and important precursors

In order to evaluate how post-harvest storage conditions affect VOC composition of tomatoes, VOC profiles were analyzed using HS-SPME/GC-MS. As described in chapter 1.1.4, around 30

volatile compound has been thought to contribute to tomato flavor. In our study, we were able to identify 14 of these flavor related volatiles. This may be due to chosen method and fiber.

The results indicate that 20°C storage treatment is more different from fresh harvested fruit than 4°C storage treatment in their VOC composition. 1-hexanol, (E)-2-heptenal and hexanal are generally perceived as fresh green aroma and contribute to the separation of 20°C storage treatment samples from others (Figure 8). Concentrations of these compounds were higher after 20°C storage compared to 4°C storage for all varieties, however not all was increased compared to fresh fruit. Hexanal decreased after 4°C storage in all varieties, and after 20°C storage for all varieties except P and SE. Renard et al. (2013) also found a decrease of hexanal after cold storage of tomato fruits, in contrast to Kanski et al. (2020). Hexanal is one of the most abundant volatile in tomato fruit, and is generally described as green or grassy. 1-hexanol, (E)-2-heptenal and hexanal are products of fatty acids of *alpha*- and *beta*-oxidation and lipoxygenase pathway (Buettner, 2017).

3-methylbutanal, 2-methylbutanal, 1-penten-3-one and (E)-2-pentenal also drives the separation of 20°C storage treatment samples from others (Figure 8), however these compounds are lower for both storage treatments when compared to fresh fruit. Generally, in this study concentration of these compounds decreased during storage. They are amino acid derivatives and are often associated with floral scents and fruit and vegetable aromas (Buettner, 2017).

Phenylacetaldehyde, linalool, *beta*-Damascenone and *beta*-Cyclocitral drives the separation between varieties (Figure 8). Phenylacetaldehyde increased after 20°C storage by at least 2.88 folds for all varieties except P. It is a phenylpropanoid/benzoid (amino acid) derivative. Klee & Tieman (2018) found that phenylacetaldehyde positively correlated with consumer preference of tomato. Linalool, *beta*-Damascenone and *beta*-Cyclocitral belong to the group terpenoids, and often contribute to specific fruit and vegetable aroma due to low-odor threshold (Buettner, 2017). *beta*-Damascenone is a carotenoid derived volatile. 6-methyl-5-hepten-2-one is also an intermediate in the synthesis of terpenoids and this was increased after 20°C storage for both SE and P, however it showed no effect on the separation between different treatments. Klee & Tieman (2018) found that 6-methyl-5-hepten-2-one also positively correlated with consumer

preference of tomato. Even though it did not separate between different treatments according to the PCA biplot in this study, it has been found by Hu et al. (2020) that the compound is active at very low concentrations and a slight increase can lead to significant flavor enhancement. This is consistent with sensory and laboratory results showing that SE and P is generally more tasty. Vogel et al. (2010) found that a reduction in carotenoid derived volatiles had negative impact on flavor and sweetness acceptability. Some important carotenoid derived volatiles in tomato such as *beta*-ionone and geranylacetone (Vogel et al., 2010) were not identified probably due to chosen extraction and chromatographic separation methodology or they were not present.

In summary, flavor related VOCs generally decrease during storage, with a few exceptions (Table 6). As seen on the PCA plot and biplot (Figure 7, Figure 8), fatty acid derivatives separated samples stored at 20°C from fresh fruit ones and from the ones stored at 4°C. Amino acid derivatives decrease after storage in general, except phenylacetaldehyde. Amino acid and fatty acid content was not measured, therefore any correlations are not known. Total carotenoid content generally increased during storage, and increased more after 20°C storage. However, carotenoid derived volatiles or intermediates did not show any clear trends.

This experiment investigated for relatively short transport and retail period, while the post-harvest chain could be much longer. As Maul et al. (2000) found there was increasing effects of storage temperature as exposure time increased, meaning that longer storage time may give more pronounced differences. To improve results, storage period could be increased. Also, another sampling method could be considered. Weighing of tomatoes before and after storage, including fatty acid analysis and training sensory panel could also be done to improve results.

Conclusion

This study was conducted to establish an analytical procedure to analyze VOCs from tomato fruits, analyze how post-harvest storage conditions affect important tomato fruit quality parameters, and to evaluate how the post-harvest chain affect VOC composition in tomatoes.

Comprehensive analysis of fruit quality parameters, combined with sensor evaluation and VOC composition analysis using HS-SPME/GC-MS showed no severe effects of cold storage on fruit quality and volatile profile compared to other studies. The profile for flavor related volatiles was slightly different after 20°C storage compared to fresh fruit and 4°C storage, mainly due to the fatty acid derived volatiles 1-hexanol, (E)-2-heptenal and hexanal. These compounds were generally higher after 20°C storage compared to 4°C storage. Perceived overall tomato taste generally decreased after storage, especially after 4°C storage in darkness compared to 20°C storage with light. The larger differences were between varieties, and there was a trend showing that the difference was largest between the smaller and bigger varieties. In summary, tomato flavor and quality is more dependent on variety then storage conditions when tomato fruits are harvested ripe.

Future perspectives

In this experiment it was investigated a relatively short transport and retail time period, whilst the post-harvest chain could potentially be much longer. Also it is important to note that the fruits were harvested ripe. Further studies could be on longer post-harvest chain period, or if different varieties needs different storage conditions in order to preserve tomato flavor. Other methods, sample preparation and selection of fiber may be explored to enhance detection of volatile compounds. Also, analysis of fatty acids and individual carotenoids could be beneficial to investigate correlations with volatile compounds.

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Appendix

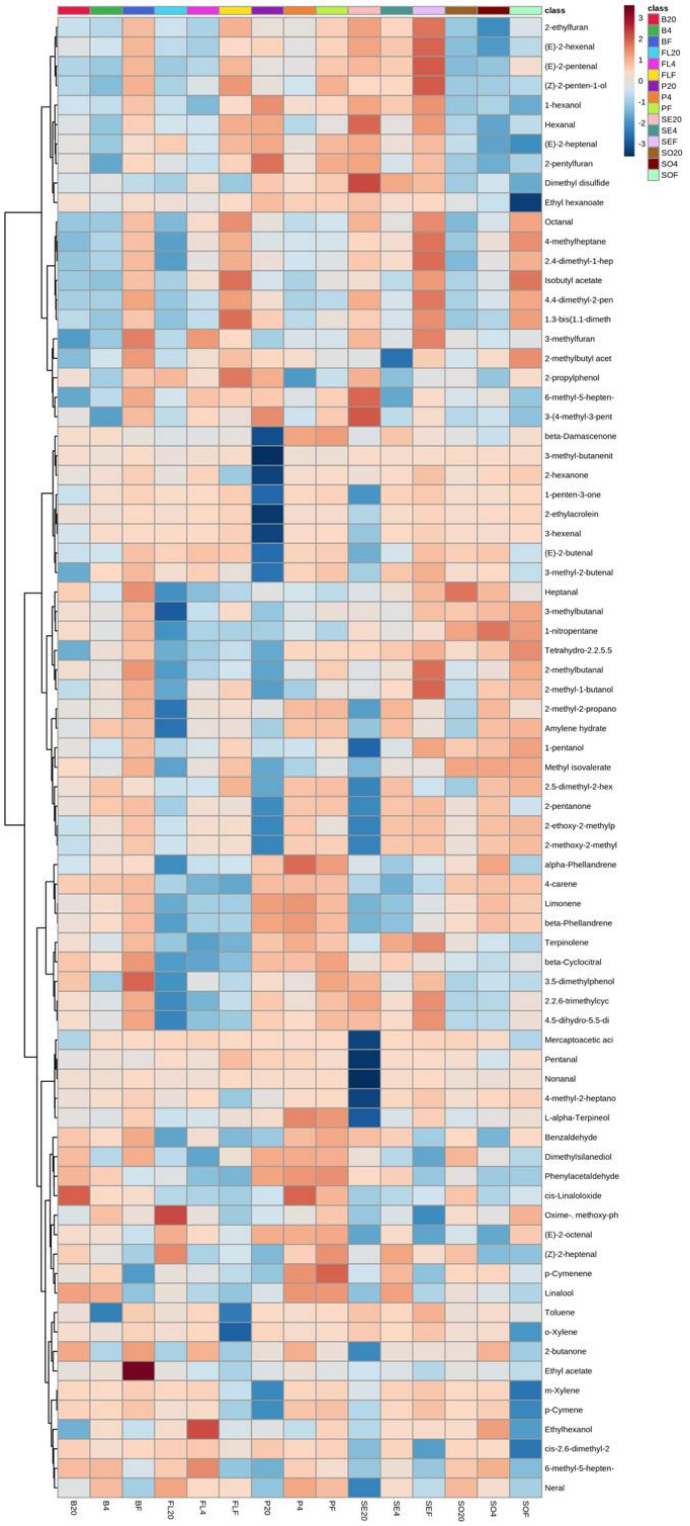

Figure A1. Heatmap for all identified volatiles.

Table A1. ANOVA followed by Tukey-Test ($p \leq 0.05$) for all identified compounds.

Compound	f.value	p.value	-log10(p)	FDR	Tukey's HSD
beta-Damascenone	66.35	4.1857e-31	30.378	3.1812e-29	P4-B20, PF-B20, P4-B4, PF-B4, P4-BF, PF-BF, SE4-BF, P4-FL20, PF-FL20, SE4-FL20, P4-FL4, PF-FL4, SE4-FL4, P4-FLF, PF-FLF, SE4-FLF, P4-P20, PF-P20, SE4-P20, PF-P4, SE20-P4, SE4-P4, SEF-P4, SO20-P4, SO4-P4, SOF-P4, SE20-PF, SE4-PF, SEF-PF, SO20-PF, SO4-PF, SOF-PF, SE4-SE20, SEF-SE4, SO20-SE4, SO4-SE4, SOF-SE4
3-methylbutanal	46.16	8.7513e-27	26.058	3.3255e-25	BF-B20, SEF-B20, SO20-B20, SO4-B20, SOF-B20, BF-B4, SEF-B4, SO20-B4, SO4-B4, SOF-B4, FL20-BF, FL4-BF, FLF-BF, P20-BF, P4-BF, PF-BF, SE20-BF, SE4-BF, SOF-BF, SEF-FL20, SO20-FL20, SO4-FL20, SOF-FL20, SEF-FL4, SO20-FL4, SO4-FL4, SOF-FL4, SEF-FLF, SO4-FLF, SOF-FLF, SEF-P20, SO20-P20, SO4-P20, SOF-P20, SEF-P4, SO20-P4, SO4-P4, SOF-P4, SEF-PF, SO20-PF, SO4-PF, SOF-PF, SEF-SE20, SO20-SE20, SO4-SE20, SOF-SE20, SEF-SE4, SO20-SE4, SO4-SE4, SOF-SE4, SOF-SEF, SOF-SO20, SOF-SO4
1-nitropentane	38.161	1.4034e-24	23.853	3.5553e-23	FL20-B20, SO20-B20, SO4-B20, SOF-B20, BF-B4, SO20-B4, SO4-B4, SOF-B4, FL20-BF, FL4-BF, FLF-BF, P20-BF, P4-BF, PF-BF, SE4-BF, SO4-BF, SE20-FL20, SO20-FL20, SO4-FL20, SOF-FL20, SO20-FL4, SO4-FL4, SOF-FL4, SO20-FLF, SO4-FLF, SOF-FLF, SO20-P20, SO4-P20, SOF-P20, SO20-P4, SO4-P4, SOF-P4, SO20-PF, SO4-PF, SOF-PF, SO20-SE20, SO4-SE20, SOF-SE20, SO20-SE4, SO4-SE4, SOF-SE4, SO20-SEF, SO4-SEF, SOF-SEF, SO4-SO20, SOF-SO4
2-ethylacrolein	36.606	4.1924e-24	23.378	7.3231e-23	BF-B20, FL4-B20, FLF-B20, SEF-B20, SOF-B20, BF-B4, FL4-B4, FLF-B4, SEF-B4, SOF-B4, FL20-BF, FLF-BF, P20-BF, P4-BF, PF-BF, SE20-BF, SE4-BF, SEF-BF, SO20-BF, SOF-BF, FL4-FL20, FLF-FL20, SEF-FL20, SOF-FL20, FLF-FL4, P20-FL4, P4-FL4, PF-FL4, SE20-FL4, SE4-FL4, SEF-FL4, SO20-FL4, P20-FLF, P4-FLF, PF-FLF, SE20-FLF, SE4-FLF, SO20-FLF, SO4-FLF, PF-P20, SE4-P20, SEF-P20, SO20-P20, SO4-P20, SOF-P20, SEF-P4, SOF-P4, SEF-PF, SOF-PF, SEF-SE20, SO4-SE20, SOF-SE20, SEF-SE4, SOF-SE4, SO20-SEF, SO4-SEF, SOF-SO20, SOF-SO4
2-pentylfuran	36.413	4.8179e-24	23.317	7.3231e-23	P20-B20, PF-B20, SE20-B20, SEF-B20, BF-B4, FLF-B4, P20-B4, P4-B4, PF-B4, SE20-B4, SEF-B4, P20-BF, PF-BF, SE20-BF, SO20-BF, SO4-BF, SOF-BF, P20-FL20, PF-FL20, SE20-FL20, SEF-FL20, P20-FL4, PF-FL4, SE20-FL4, SEF-FL4, P20-FLF, SE20-FLF, SO20-FLF, SO4-FLF, SOF-FLF, P4-P20, PF-P20, SE20-P20, SE4-P20, SEF-P20, SO20-P20, SO4-P20, SOF-P20, PF-P4, SE20-P4, SO4-P4, SE4-PF, SO20-PF, SO4-PF, SOF-PF, SE4-SE20, SO20-SE20, SO4-SE20, SOF-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
2-propylphenol	34.253	2.3812e-23	22.623	2.6302e-22	FLF-B20, P20-B20, SE20-B20, BF-B4, FL20-B4, FLF-B4, P20-B4, SE20-B4, FLF-BF, P4-BF, PF-BF, SE4-BF, SO4-BF, FLF-FL20, P4-FL20, PF-FL20, SE4-FL20, SEF-FL20, SO20-FL20, SO4-FL20, FLF-FL4, P20-FL4, SE20-FL4, P20-FLF, P4-FLF, PF-FLF, SE20-FLF, SE4-FLF, SEF-FLF, SO20-FLF, SO4-FLF, SOF-FLF, P4-P20, PF-P20, SE4-P20, SEF-P20, SO20-P20, SO4-P20, SOF-P20, SE20-P4, SE20-PF, SE4-SE20, SEF-SE20, SO20-SE20, SO4-SE20, SOF-SE20
Linalool	34.23	2.4226e-23	22.616	2.6302e-22	P4-B20, PF-B20, P4-B4, PF-B4, P4-BF, PF-BF, P4-FL20, PF-FL20, P4-FL4, PF-FL4, P4-FLF, PF-FLF, P4-P20, PF-P20, SE20-P4, SE4-P4, SEF-P4, SO20-P4, SO4-P4, SOF-P4, SE20-PF, SE4-PF, SEF-PF, SO20-PF, SO4-PF, SOF-PF

6-methyl-5-hepten-2-ol	33.773	3.4366e-23	22.464	3.2647e-22	FL4-B20, FLF-B20, P20-B20, SE20-B20, SEF-B20, SOF-B20, BF-B4, FL4-B4, FLF-B4, P20-B4, PF-B4, SE20-B4, SEF-B4, SOF-B4, FL4-BF, SO4-BF, FL4-FL20, SO4-FL20, FLF-FL4, P20-FL4, P4-FL4, PF-FL4, SE20-FL4, SE4-FL4, SEF-FL4, SO20-FL4, SO4-FL4, SOF-FL4, SO20-FLF, SO4-FLF, SO20-P20, SO4-P20, SO4-P4, SO4-PF, SO20-SE20, SO4-SE20, SO4-SE4, SO20-SEF, SO4-SEF, SOF-SO20, SOF-SO4
(E)-2-hexenal	32.61	8.515e-23	22.07	7.1904e-22	BF-B20, SE20-B20, SEF-B20, BF-B4, SE20-B4, SEF-B4, FL20-BF, FL4-BF, P4-BF, SEF-BF, SO20-BF, SO4-BF, SOF-BF, SE20-FL20, SE4-FL20, SEF-FL20, SE20-FL4, SE4-FL4, SEF-FL4, SE20-FLF, SEF-FLF, SO4-FLF, SE20-P20, SEF-P20, SO20-P20, SO4-P20, SE20-P4, SEF-P4, SE20-PF, SEF-PF, SO20-PF, SO4-PF, SEF-SE20, SO20-SE20, SO4-SE20, SOF-SE20, SEF-SE4, SO20-SE4, SO4-SE4, SOF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
2-methyl-1-butanol	30.118	6.571e-22	21.182	4.9939e-21	BF-B20, SEF-B20, SOF-B20, BF-B4, SEF-B4, FL20-BF, FL4-BF, P20-BF, P4-BF, PF-BF, SE20-BF, SEF-BF, SO20-BF, SEF-FL20, SOF-FL20, SEF-FL4, SEF-FLF, SEF-P20, SOF-P20, SEF-P4, SOF-P4, SEF-PF, SOF-PF, SEF-SE20, SOF-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SEF, SOF-SO20
(E)-2-pentenal	28.378	2.9834e-21	20.525	2.0613e-20	BF-B20, FLF-B20, SE20-B20, SEF-B20, BF-B4, FLF-B4, PF-B4, SE20-B4, SE4-B4, SEF-B4, FL20-BF, FL4-BF, P4-BF, SEF-BF, SO20-BF, SO4-BF, FLF-FL20, PF-FL20, SE20-FL20, SEF-FL20, FLF-FL4, SE20-FL4, SEF-FL4, SEF-FLF, SO20-FLF, SO4-FLF, SEF-P20, SE20-P4, SEF-P4, SEF-PF, SO20-PF, SO4-PF, SEF-SE20, SO20-SE20, SO4-SE20, SEF-SE4, SO20-SE4, SO4-SE4, SO20-SEF, SO4-SEF, SOF-SEF
2-methylbutanal	27.731	5.3406e-21	20.272	3.3824e-20	BF-B20, SEF-B20, SOF-B20, BF-B4, SEF-B4, SOF-B4, FL20-BF, FL4-BF, FLF-BF, P20-BF, P4-BF, PF-BF, SE20-BF, SE4-BF, SEF-BF, SO20-BF, SO4-BF, PF-FL20, SEF-FL20, SOF-FL20, SEF-FL4, SOF-FL4, SEF-FLF, SOF-FLF, PF-P20, SEF-P20, SOF-P20, SEF-P4, SOF-P4, SEF-PF, SEF-SE20, SOF-SE20, SEF-SE4, SOF-SE4, SO20-SEF, SO4-SEF, SOF-SEF, SOF-SO20, SOF-SO4
2-ethylfuran	25.938	2.8545e-20	19.544	1.6688e-19	BF-B20, FLF-B20, SE20-B20, SEF-B20, BF-B4, FLF-B4, SE20-B4, SEF-B4, FL20-BF, FL4-BF, SEF-BF, SO20-BF, SO4-BF, SOF-BF, FLF-FL20, SE20-FL20, SEF-FL20, FLF-FL4, SE20-FL4, SEF-FL4, P20-FLF, P4-FLF, SEF-FLF, SO20-FLF, SO4-FLF, SOF-FLF, SE20-P20, SEF-P20, SE20-P4, SEF-P4, SE20-PF, SEF-PF, SE4-SE20, SEF-SE20, SO20-SE20, SO4-SE20, SOF-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
1-penten-3-one	25.141	6.2007e-20	19.208	3.3661e-19	BF-B20, FLF-B20, PF-B20, SE4-B20, SEF-B20, FLF-B4, PF-B4, SE4-B4, SEF-B4, FLF-BF, P20-BF, SEF-BF, SO20-BF, SO4-BF, FLF-FL20, SEF-FL20, FLF-FL4, SEF-FL4, P20-FLF, P4-FLF, SE20-FLF, SE4-FLF, SEF-FLF, SO20-FLF, SO4-FLF, SOF-FLF, PF-P20, SE4-P20, SEF-P20, SEF-P4, SEF-PF, SO20-PF, SO4-PF, SEF-SE20, SEF-SE4, SO20-SE4, SO4-SE4, SO20-SEF, SO4-SEF, SOF-SEF
(Z)-2-penten-1-ol	22.741	7.2877e-19	18.137	3.6924e-18	BF-B20, FLF-B20, PF-B20, SEF-B20, BF-B4, FLF-B4, PF-B4, SEF-B4, FL20-BF, FL4-BF, P4-BF, SEF-BF, SO20-BF, SO4-BF, SOF-BF, FLF-FL20, PF-FL20, SEF-FL20, FLF-FL4, PF-FL4, SEF-FL4, P20-FLF, P4-FLF, SE20-FLF, SE4-FLF, SEF-FLF, SO20-FLF, SO4-FLF, SOF-FLF, SEF-P20, PF-P4, SEF-P4, SEF-PF, SO20-PF, SO4-PF, SOF-PF, SEF-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
2.2.6-trimethylcyclo-hexanone	21.725	2.205e-18	17.657	9.8904e-18	BF-B20, SE20-B20, SEF-B20, BF-B4, SE20-B4, SEF-B4, FL20-BF, FL4-BF, FLF-BF, P4-BF, SE4-BF, SO20-BF, SO4-BF, SOF-BF, P20-FL20, PF-FL20, SE20-FL20, SEF-FL20, P20-

					FL4, PF-FL4, SE20-FL4, SEF-FL4, P20-FLF, PF-FLF, SE20-FLF, SEF-FLF, SE20-P20, SEF-P20, SO20-P20, SO4-P20, SE20-P4, SEF-P4, SEF-PF, SO20-PF, SO4-PF, SE4-SE20, SO20-SE20, SO4-SE20, SOF-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
1-pentanol	21.722	2.2123e-18	17.655	9.8904e-18	BF-B20, SEF-B20, SO4-B20, SOF-B20, BF-B4, SEF-B4, SO20-B4, SO4-B4, SOF-B4, FL20-BF, FL4-BF, P20-BF, P4-BF, SE4-BF, SEF-BF, SOF-BF, SEF-FL20, SO20-FL20, SO4-FL20, SOF-FL20, SEF-FL4, SO4-FL4, SOF-FL4, SEF-FLF, SOF-FLF, SEF-P20, SO20-P20, SO4-P20, SOF-P20, SEF-P4, SO20-P4, SO4-P4, SOF-P4, SEF-PF, SOF-PF, SEF-SE20, SOF-SE20, SEF-SE4, SO4-SE4, SOF-SE4, SO20-SEF, SO4-SEF, SOF-SO20, SOF-SO4
(E)-2-heptenal	19.75	2.1431e-17	16.669	9.0488e-17	FLF-B20, P20-B20, PF-B20, SE20-B20, SEF-B20, FL20-B4, FLF-B4, P20-B4, PF-B4, SE20-B4, SEF-B4, P20-BF, SE20-BF, SO4-BF, SOF-BF, FL4-FL20, SE20-FL20, SO20-FL20, SO4-FL20, SOF-FL20, FLF-FL4, P20-FL4, PF-FL4, SE20-FL4, SEF-FL4, P4-FLF, SE4-FLF, SO20-FLF, SO4-FLF, SOF-FLF, P4-P20, SE4-P20, SO20-P20, SO4-P20, SOF-P20, PF-P4, SE20-P4, SEF-P4, SE4-PF, SO20-PF, SO4-PF, SOF-PF, SE4-SE20, SO20-SE20, SO4-SE20, SOF-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
Hexanal	19.526	2.8053e-17	16.552	1.1221e-16	FLF-B20, P20-B20, SE20-B20, SEF-B20, FLF-B4, P20-B4, SE20-B4, SEF-B4, SE20-BF, SO4-BF, FLF-FL20, P20-FL20, SE20-FL20, SEF-FL20, FLF-FL4, P20-FL4, SE20-FL4, SEF-FL4, P4-FLF, PF-FLF, SE20-FLF, SE4-FLF, SO20-FLF, SO4-FLF, SOF-FLF, P4-P20, PF-P20, SE20-P20, SE4-P20, SO20-P20, SO4-P20, SOF-P20, SE20-P4, SEF-P4, SE20-PF, SEF-PF, SE4-SE20, SEF-SE20, SO20-SE20, SO4-SE20, SOF-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
p-Cymenene	19.111	4.6518e-17	16.332	1.7677e-16	P4-B20, PF-B20, P4-B4, PF-B4, P4-BF, PF-BF, SE4-BF, P4-FL20, PF-FL20, P4-FL4, PF-FL4, P4-FLF, PF-FLF, P4-P20, PF-P20, PF-P4, SE20-P4, SEF-P4, SO20-P4, SO4-P4, SOF-P4, SE20-PF, SE4-PF, SEF-PF, SO20-PF, SO4-PF, SOF-PF, SEF-SE4
o-Xylene	18.994	5.3675e-17	16.27	1.9425e-16	SE20-B20, SEF-B20, BF-B4, SE20-B4, SEF-B4, FLF-BF, SE20-BF, SEF-BF, SO20-BF, SOF-BF, SE20-FL20, SEF-FL20, FLF-FL4, SE20-FL4, SEF-FL4, SOF-FL4, P20-FLF, SE20-FLF, SE4-FLF, SEF-FLF, SE20-P20, SEF-P20, SOF-P20, SE20-P4, SEF-P4, SE20-PF, SEF-PF, SE4-SE20, SO20-SE20, SO4-SE20, SOF-SE20, SEF-SE4, SOF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
3-methyl-2-butenal	17.99	1.9001e-16	15.721	6.5639e-16	BF-B20, SEF-B20, BF-B4, SEF-B4, FL20-BF, FL4-BF, FLF-BF, P20-BF, P4-BF, SE20-BF, SO20-BF, SO4-BF, SOF-BF, SEF-FL20, P20-FL4, SE20-FL4, SEF-FL4, SEF-FLF, PF-P20, SE4-P20, SEF-P20, SEF-P4, SE20-PF, SEF-PF, SOF-PF, SE4-SE20, SEF-SE20, SEF-SE4, SOF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
Phenyl-acetaldehyde	17.789	2.4643e-16	15.608	8.143e-16	FL4-B20, FLF-B20, P4-B20, PF-B20, SEF-B20, SO4-B20, SOF-B20, P20-B4, P4-B4, PF-B4, P20-BF, P4-BF, PF-BF, P20-FL20, P4-FL20, PF-FL20, P20-FL4, P4-FL4, PF-FL4, SE20-FL4, P20-FLF, P4-FLF, PF-FLF, SE20-FLF, SE4-P20, SEF-P20, SO20-P20, SO4-P20, SOF-P20, SE20-P4, SE4-P4, SEF-P4, SO20-P4, SO4-P4, SOF-P4, SE20-PF, SE4-PF, SEF-PF, SO20-PF, SO4-PF, SOF-PF, SEF-SE20, SO4-SE20, SOF-SE20
Ethyl hexanoate	16.786	9.3056e-16	15.031	2.9468e-15	P20-B20, SE20-B20, P20-B4, P4-B4, PF-B4, SE20-B4, P20-BF, SE20-BF, P20-FL20, SE20-FL20, P20-FL4, P4-FL4, PF-FL4, SE20-FL4, P20-FLF, SE20-FLF, P4-P20, PF-P20, SE4-P20, SEF-P20, SO20-P20, SO4-P20, SOF-P20, SE20-P4,

					SO20-P4, SO4-P4, SOF-P4, SE20-PF, SO20-PF, SO4-PF, SOF-PF, SE4-SE20, SEF-SE20, SO20-SE20, SO4-SE20, SOF-SE20
4.5-dihydro-5.5-dimethyl-4-isopropylidene-1H-pyrazole	16.175	2.1509e-15	14.667	6.5388e-15	BF-B20, FL20-B20, SEF-B20, BF-B4, SE20-B4, SEF-B4, FL20-BF, FL4-BF, FLF-BF, P4-BF, SE4-BF, SO20-BF, SO4-BF, SOF-BF, P20-FL20, P4-FL20, PF-FL20, SE20-FL20, SE4-FL20, SEF-FL20, P20-FL4, PF-FL4, SE20-FL4, SEF-FL4, P20-FLF, PF-FLF, SE20-FLF, SEF-FLF, SEF-P20, SEF-P4, SO20-PF, SO4-PF, SO20-SE20, SO4-SE20, SOF-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
Toluene	15.57	5.055e-15	14.296	1.4776e-14	BF-B20, SE20-B20, SEF-B20, BF-B4, SE20-B4, SEF-B4, FLF-BF, SEF-BF, SO20-BF, SOF-BF, SE20-FL20, SEF-FL20, SEF-FL4, SE20-FLF, SEF-FLF, SE20-P20, SEF-P20, SE20-P4, SEF-P4, SE20-PF, SEF-PF, SE4-SE20, SEF-SE20, SO20-SE20, SO4-SE20, SOF-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
1.3-bis(1.1-dimethylethyl)-benzene	13.675	8.6507e-14	13.063	2.435e-13	FLF-B20, SEF-B20, SOF-B20, FLF-B4, SEF-B4, SOF-B4, FLF-BF, FLF-FL20, SEF-FL20, SOF-FL20, FLF-FL4, SEF-FL4, SOF-FL4, P20-FLF, P4-FLF, PF-FLF, SE20-FLF, SE4-FLF, SO20-FLF, SO4-FLF, SEF-P4, SOF-P4, SEF-PF, SOF-PF, SEF-SE4, SOF-SE4, SO20-SEF, SO4-SEF, SOF-SO20, SOF-SO4
beta-Cyclocitral	13.634	9.2252e-14	13.035	2.504e-13	BF-B20, FL20-B20, FL4-B20, FLF-B20, SO4-B20, BF-B4, PF-B4, FL20-BF, FL4-BF, FLF-BF, SE4-BF, SO20-BF, SO4-BF, SOF-BF, P20-FL20, P4-FL20, PF-FL20, SE20-FL20, SE4-FL20, SEF-FL20, P20-FL4, P4-FL4, PF-FL4, SE20-FL4, SE4-FL4, SEF-FL4, P20-FLF, P4-FLF, PF-FLF, SE20-FLF, SE4-FLF, SEF-FLF, SO20-P20, SO4-P20, SO4-P4, SE4-PF, SO20-PF, SO4-PF, SOF-PF, SO4-SE20, SO4-SEF
3-methyl-butanenitrile	13.242	1.7214e-13	12.764	4.5112e-13	P20-B20, SE20-B20, BF-B4, SE20-B4, SEF-B4, SO20-B4, SO4-B4, SOF-B4, FL20-BF, FL4-BF, P20-BF, P4-BF, PF-BF, FLF-FL20, SE20-FL20, SEF-FL20, SO20-FL20, SO4-FL20, SOF-FL20, SE20-FL4, SO20-FL4, SO4-FL4, P20-FLF, P4-FLF, SE20-P20, SEF-P20, SO20-P20, SO4-P20, SOF-P20, SE20-P4, SEF-P4, SO20-P4, SO4-P4, SOF-P4, SE20-PF, SEF-PF, SO20-PF, SO4-PF, SOF-PF, SE4-SE20, SO20-SE4, SO4-SE4
Methyl isovalerate	13.201	1.8379e-13	12.736	4.6559e-13	SO20-B20, SO4-B20, SO20-B4, SO4-B4, SOF-B4, SO20-BF, SO4-BF, SO20-FL20, SO4-FL20, SOF-FL20, SO20-FL4, SO4-FL4, SOF-FL4, SO20-FLF, SO4-FLF, SOF-FLF, SO20-P20, SO4-P20, SOF-P20, SO20-P4, SO4-P4, SOF-P4, SO20-PF, SO4-PF, SOF-PF, SO20-SE20, SO4-SE20, SOF-SE20, SO20-SE4, SO4-SE4, SOF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
Tetrahydro-2.2.5.5-tetramethylfuran	12.952	2.7488e-13	12.561	6.7389e-13	FLF-B20, SOF-B20, FLF-B4, SOF-B4, FLF-BF, SOF-BF, FLF-FL20, SOF-FL20, FLF-FL4, SOF-FL4, P20-FLF, P4-FLF, PF-FLF, SE20-FLF, SE4-FLF, SEF-FLF, SO20-FLF, SO4-FLF, SOF-P20, SOF-P4, SOF-PF, SOF-SE20, SOF-SE4, SOF-SEF, SOF-SO20, SOF-SO4
Benzaldehyde	12.883	3.0776e-13	12.512	7.3093e-13	BF-B20, PF-B20, BF-B4, PF-B4, FL20-BF, FL4-BF, FLF-BF, P20-BF, SE20-BF, SE4-BF, SO20-BF, SO4-BF, SOF-BF, P4-FL20, PF-FL20, SEF-FL20, P4-FL4, PF-FL4, SEF-FL4, PF-FLF, PF-P20, PF-P4, SO4-P4, SE20-PF, SE4-PF, SEF-PF, SO20-PF, SO4-PF, SOF-PF, SO4-SEF
2-pentanone	12.243	8.9376e-13	12.049	2.0584e-12	BF-B20, FL4-B20, SEF-B20, BF-B4, FL4-B4, SEF-B4, FL20-BF, FLF-BF, P20-BF, P4-BF, SE20-BF, SO20-BF, SOF-BF, FL4-FL20, SEF-FL20, FLF-FL4, P20-FL4, SE20-FL4, SO20-FL4, SOF-FL4, SEF-FLF, SEF-P20, SO4-P20, SEF-P4, SEF-PF, SE4-SE20, SEF-SE20, SO4-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SEF

3.5-dimethylphenol	11.88	1.6624e-12	11.779	3.7159e-12	B4-B20, BF-B20, FL20-B20, BF-B4, PF-B4, SE20-B4, SEF-B4, FL20-BF, FL4-BF, FLF-BF, P20-BF, P4-BF, SE20-BF, SE4-BF, SEF-BF, SO20-BF, SO4-BF, SOF-BF, P20-FL20, PF-FL20, SE20-FL20, SEF-FL20, PF-FL4, PF-FLF, SE20-FLF, SEF-FLF, SO20-PF, SO4-PF, SOF-PF, SO20-SE20, SO4-SE20, SOF-SE20, SO20-SEF
(E)-2-octenal	11.333	4.3402e-12	11.362	9.4243e-12	FL20-B20, PF-B20, FL20-B4, P20-B4, P4-B4, PF-B4, FL20-BF, P20-BF, P4-BF, PF-BF, SE20-FL20, SEF-FL20, SO4-FL20, SOF-FL20, PF-FL4, SE20-FL4, SEF-FL4, SO4-FL4, PF-FLF, SE20-P20, SEF-P20, SO4-P20, SOF-P20, SE20-P4, SEF-P4, SO4-P4, SOF-P4, SE20-PF, SE4-PF, SEF-PF, SO20-PF, SO4-PF, SOF-PF
4-methyl-2-heptanone	11.186	5.6486e-12	11.248	1.1925e-11	BF-B20, FL4-B20, FLF-B20, SEF-B20, SOF-B20, BF-B4, SEF-B4, FL20-BF, P20-BF, SE20-BF, SO20-BF, FL4-FL20, FLF-FL20, SEF-FL20, SOF-FL20, P20-FL4, SE20-FL4, SEF-FL4, P20-FLF, SE20-FLF, SO20-FLF, SEF-P20, SOF-P20, SEF-P4, SEF-PF, SE4-SE20, SEF-SE20, SO4-SE20, SOF-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SO20
4-methylheptane	10.514	1.9366e-11	10.713	3.978e-11	FLF-B20, SEF-B20, SOF-B20, FLF-B4, SEF-B4, SOF-B4, FLF-BF, SEF-BF, FLF-FL20, SEF-FL20, SOF-FL20, FLF-FL4, SEF-FL4, P20-FLF, P4-FLF, PF-FLF, SE20-FLF, SE4-FLF, SO20-FLF, SO4-FLF, SEF-P20, SOF-P20, SEF-P4, SOF-P4, SEF-PF, SOF-PF, SEF-SE20, SEF-SE4, SOF-SE4, SO20-SEF, SO4-SEF, SOF-SO20, SOF-SO4
2.4-dimethyl-1-heptene	10.349	2.6409e-11	10.578	5.2819e-11	FLF-B20, SEF-B20, SOF-B20, FLF-B4, SEF-B4, SOF-B4, FL20-BF, SEF-BF, SO20-BF, FLF-FL20, SEF-FL20, SOF-FL20, FLF-FL4, SEF-FL4, P20-FLF, P4-FLF, PF-FLF, SE4-FLF, SO20-FLF, SO4-FLF, SEF-P20, SEF-P4, SEF-PF, SEF-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SO20
m-Xylene	10.205	3.4667e-11	10.46	6.7557e-11	SEF-B20, SEF-B4, FLF-BF, SEF-BF, SOF-BF, SEF-FL20, SEF-FL4, SE20-FLF, SEF-FLF, SEF-P20, SEF-P4, SEF-PF, SEF-SE20, SOF-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
2-hexanone	9.8595	6.744e-11	10.171	1.2814e-10	BF-B20, FLF-B20, SEF-B20, SOF-B20, SEF-B4, FL20-BF, P20-BF, SEF-BF, FLF-FL20, SEF-FL20, SOF-FL20, P20-FL4, SEF-FL4, P20-FLF, SE20-FLF, SEF-FLF, SEF-P20, SOF-P20, SEF-P4, SEF-PF, SEF-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
1-hexanol	9.8143	7.3648e-11	10.133	1.3652e-10	P20-B20, SE20-B20, SEF-B20, P20-B4, SE20-B4, SEF-B4, P20-BF, P20-FL20, SE20-FL20, SEF-FL20, P20-FL4, SE20-FL4, SEF-FL4, P20-FLF, SEF-FLF, P4-P20, PF-P20, SE4-P20, SO20-P20, SO4-P20, SOF-P20, SE20-P4, SEF-P4, SE20-PF, SEF-PF, SO20-SE20, SO4-SE20, SOF-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
Octanal	9.4827	1.416e-10	9.8489	2.5622e-10	FLF-B20, SE20-B20, SEF-B20, SOF-B20, FLF-B4, SE20-B4, SEF-B4, SOF-B4, FL20-BF, SO20-BF, FLF-FL20, SE20-FL20, SEF-FL20, SOF-FL20, P20-FLF, P4-FLF, PF-FLF, SE4-FLF, SO20-FLF, SO4-FLF, SEF-P20, SE20-P4, SEF-P4, SOF-P4, SEF-PF, SOF-PF, SO20-SE20, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SO20, SOF-SO4
2-methoxy-2-methylbutane	9.302	2.0334e-10	9.6918	3.5939e-10	FLF-B20, SOF-B20, FLF-B4, SOF-B4, FLF-BF, SOF-BF, FLF-FL20, SOF-FL20, P20-FL4, SE20-FL4, P20-FLF, P4-FLF, PF-FLF, SE20-FLF, SE4-FLF, SO20-FLF, SO4-FLF, SEF-P20, SOF-P20, SOF-P4, SOF-PF, SEF-SE20, SOF-SE20, SOF-SE4, SOF-SO20, SOF-SO4
Isobutyl acetate	8.6052	8.544e-10	9.0683	1.4758e-09	FLF-B20, SEF-B20, SOF-B20, FLF-B4, SEF-B4, SOF-B4, FLF-BF, FLF-FL20, SEF-FL20, SOF-FL20, FLF-FL4, SOF-FL4, P20-FLF, P4-FLF, PF-FLF, SE20-FLF, SE4-FLF, SO20-FLF, SO4-

					FLF, SOF-P20, SEF-P4, SOF-P4, SOF-PF, SOF-SE20, SEF-SE4, SOF-SE4, SO20-SEF, SOF-SO20, SOF-SO4
(E)-2-butenal	8.2803	1.7073e-09	8.7677	2.8834e-09	BF-B20, FL4-B20, SEF-B20, BF-B4, FL4-B4, SEF-B4, FL20-BF, P20-BF, P4-BF, PF-BF, SE20-BF, SO20-BF, SOF-BF, FL4-FL20, SEF-FL20, P20-FL4, P4-FL4, PF-FL4, SE20-FL4, SE4-FL4, SO20-FL4, SOF-FL4, SEF-FLF, SEF-P20, SEF-P4, SEF-PF, SEF-SE20, SEF-SE4, SO20-SEF, SOF-SEF
Dimethyl disulfide	8.0854	2.6041e-09	8.5843	4.3025e-09	SE20-B20, SE20-B4, SE20-BF, SE20-FL20, SEF-FL20, SE20-FL4, SE20-FLF, SE20-P20, SE20-P4, SE20-PF, SE4-SE20, SEF-SE20, SO20-SE20, SO4-SE20, SOF-SE20, SO20-SEF, SOF-SEF
6-methyl-5-hepten-2-one	7.7318	5.6845e-09	8.2453	9.1919e-09	SE20-B20, BF-B4, SE20-B4, SE20-BF, SOF-BF, SE20-FL20, SE20-FL4, SE20-FLF, SE20-P20, SE20-P4, SE20-PF, SE4-SE20, SEF-SE20, SO20-SE20, SO4-SE20, SOF-SE20
3-(4-methyl-3-pentenyl)furan	7.7164	5.8839e-09	8.2303	9.3162e-09	SE20-B20, BF-B4, P20-B4, PF-B4, SE20-B4, SE20-BF, P20-FL20, SE20-FL20, SE20-FL4, SE20-FLF, P4-P20, SE4-P20, SO20-P20, SO4-P20, SOF-P20, SE20-P4, SE20-PF, SE4-SE20, SEF-SE20, SO20-SE20, SO4-SE20, SOF-SE20
Neral	7.4338	1.1141e-08	7.9531	1.728e-08	BF-B20, FL4-B20, FLF-B20, P20-B20, SE20-B20, SE4-B20, SEF-B20, SO4-B20, SOF-B20, FL20-BF, P4-BF, SO20-BF, FL4-FL20, FLF-FL20, P20-FL20, SE20-FL20, SE4-FL20, SEF-FL20, SO4-FL20, SOF-FL20, SO20-FL4, P4-FLF, SO20-FLF, P4-P20, SO20-P20, SE20-P4, SE4-P4, SEF-P4, SO4-P4, SOF-P4, SO20-SE20, SO20-SE4, SO20-SEF, SO4-SO20, SOF-SO20
3-methylfuran	7.3143	1.4647e-08	7.8343	2.2263e-08	BF-B20, FL4-B20, SE20-B20, SEF-B20, BF-B4, FL4-B4, SEF-B4, FL20-BF, P20-BF, P4-BF, PF-BF, SE4-BF, SO20-BF, SO4-BF, SOF-BF, FL4-FL20, SEF-FL20, P20-FL4, SEF-P20, SEF-P4, SEF-PF, SEF-SE4, SO20-SEF, SO4-SEF, SOF-SEF
2-ethoxy-2-methylpropane	6.9137	3.7286e-08	7.4284	5.5564e-08	FLF-B20, SOF-B20, FLF-B4, SOF-B4, FLF-BF, SOF-BF, FLF-FL20, SOF-FL20, SE20-FL4, P20-FLF, P4-FLF, PF-FLF, SE20-FLF, SE4-FLF, SEF-FLF, SO20-FLF, SO4-FLF, SOF-P20, SOF-P4, SOF-PF, SOF-SE20, SOF-SE4, SOF-SO20, SOF-SO4
cis-Linaloloxide	6.8796	4.0417e-08	7.3934	5.9071e-08	FLF-B20, SE20-B20, SEF-B20, FLF-B4, SE20-B4, SE20-BF, SE20-FL20, P4-FL4, P4-FLF, PF-FLF, P4-P20, SE20-P4, SEF-P4, SOF-P4, SE20-PF, SEF-PF, SOF-PF, SE4-SE20, SO20-SE20
Heptanal	6.6301	7.3406e-08	7.1343	1.0526e-07	SO4-B20, SO4-B4, FL20-BF, SO20-FL20, SO4-FL20, SOF-FL20, SO20-FL4, SO4-FL4, SO20-FLF, SO4-FLF, SO4-P20, SO20-P4, SO4-P4, SO20-PF, SO4-PF, SO4-SE20, SO4-SE4, SO4-SEF
Amylene hydrate	6.357	1.4284e-07	6.8452	2.0103e-07	FLF-B20, SOF-B20, FLF-B4, SOF-B4, FLF-BF, FLF-FL20, SOF-FL20, FLF-FL4, P20-FLF, P4-FLF, PF-FLF, SE20-FLF, SE4-FLF, SEF-FLF, SO20-FLF, SO4-FLF, SOF-P20, SOF-P4, SOF-PF, SOF-SE20, SOF-SE4, SOF-SO20, SOF-SO4
Nonanal	6.2469	1.875e-07	6.727	2.5909e-07	SE20-B20, SE20-B4, SE20-BF, SE20-FL20, SE20-FL4, SE20-FLF, SE20-P20, SE20-P4, SE20-PF, SE4-SE20, SEF-SE20, SO20-SE20, SOF-SE20
2.5-dimethyl-2-hexanol	5.7463	6.6387e-07	6.1779	9.0096e-07	FLF-B20, FLF-B4, SOF-B4, FLF-BF, FLF-FL20, SOF-FL20, FLF-FL4, SOF-FL4, P20-FLF, P4-FLF, PF-FLF, SE20-FLF, SE4-FLF, SEF-FLF, SO20-FLF, SO4-FLF, SOF-P20, SOF-P4, SOF-PF, SOF-SE20, SOF-SE4, SOF-SO20, SOF-SO4
2-methyl-2-propanol	5.5625	1.0686e-06	5.9712	1.4248e-06	FLF-B20, SOF-B20, FLF-B4, SOF-B4, FLF-BF, FLF-FL20, SOF-FL20, FLF-FL4, P20-FLF, P4-FLF, PF-FLF, SE20-FLF, SE4-FLF, SEF-FLF, SO20-FLF, SO4-FLF, SOF-P20, SOF-P4, SOF-SE20, SOF-SO20

Ethyl acetate	4.9177	5.9671e-06	5.2242	7.819e-06	FLF-B20, SEF-B20, FLF-B4, SE20-B4, SEF-B4, FLF-BF, SEF-BF, FLF-FL20, SEF-FL20, P20-FLF, P4-FLF, PF-FLF, SE4-FLF, SO20-FLF, SO4-FLF, SEF-P4, SEF-PF, SO20-SEF, SO4-SEF
cis-2.6-dimethyl-2.6-octadiene	4.377	2.6845e-05	4.5711	3.458e-05	SE20-B20, SE20-B4, SE20-BF, SOF-FL4, SE20-FLF, SE20-P4, SE20-PF, SE4-SE20, SEF-SE20, SO20-SE20, SO4-SE20, SOF-SE20
3-hexenal	4.0405	7.0401e-05	4.1524	8.9175e-05	FLF-B4, SEF-B4, P20-FLF, P4-FLF, SO20-FLF, SO4-FLF, SE20-P20, SEF-P20, SEF-P4, SO20-SEF, SO4-SEF
4.4-dimethyl-2-pentanone	3.7911	0.00014576	3.8364	0.0001816	SEF-B20, SEF-B4, SEF-FL20, SEF-FL4, SEF-P20, SEF-P4, SEF-PF, SEF-SE4, SO20-SEF, SO4-SEF
Pentanal	3.6878	0.00019769	3.704	0.00024079	FLF-B20, FLF-B4, FLF-BF, FLF-FL20, FLF-FL4, P4-FLF, SE20-FLF, SE4-FLF, SO20-FLF, SO4-FLF, SOF-FLF
(Z)-2-heptenal	3.6845	0.0001996	3.6998	0.00024079	FL20-BF, PF-BF, FL4-FL20, P20-FL20, SO4-FL20, SOF-FL20, PF-FL4, PF-P20, SO4-PF, SOF-PF
Limonene	3.5713	0.00027933	3.5539	0.00033171	P20-FL20, P20-FL4, P20-FLF, SE20-P20, SE4-P20
beta-Phellandrene	3.5261	0.00031962	3.4954	0.00037371	P20-FL20, P20-FL4, P20-FLF, SE20-P20, SE4-P20
2-methylbutyl acetate	3.3615	0.00052327	3.2813	0.00060256	SOF-B20, SOF-B4, SOF-FL20, SOF-FL4, SOF-P4, SOF-PF, SOF-SE20, SOF-SE4, SOF-SO20, SOF-SO4
L-alpha-Terpineol	3.2198	0.00080246	3.0956	0.00091025	P4-B20, P4-B4, PF-B4, P4-FL20, PF-FL20, P4-FL4, PF-FL4, P4-FLF, P4-P20, SE20-P4, SE4-P4, SEF-P4, SO20-P4, SO4-P4, SOF-P4, SE4-PF, SO20-PF
4-carene	3.1774	0.00091245	3.0398	0.0010198	P20-FLF
Terpinolene	3.1005	0.0011523	2.9385	0.0012692	P4-FL20, P4-FL4, SEF-FL4, P4-FLF, SOF-P4
p-Cymene	2.9007	0.0021191	2.6739	0.0023007	PF-FL20
alpha-Phellandrene	2.2527	0.015408	1.8123	0.016493	P20-FL20, SE4-P20, SOF-P20

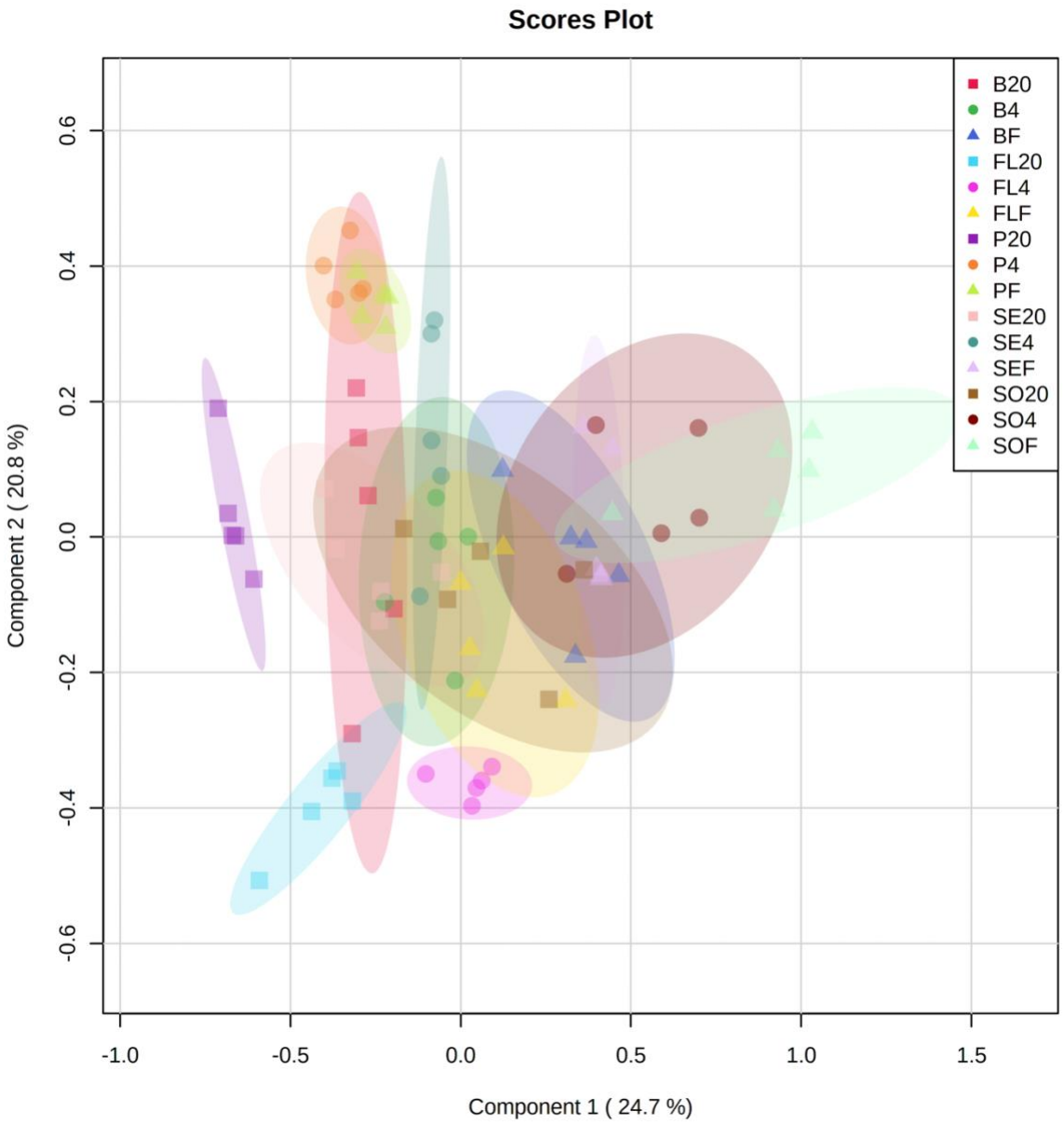

Figure A2. Partial Least Squares Discriminant Analysis (PLS-DA) for flavor related volatiles.

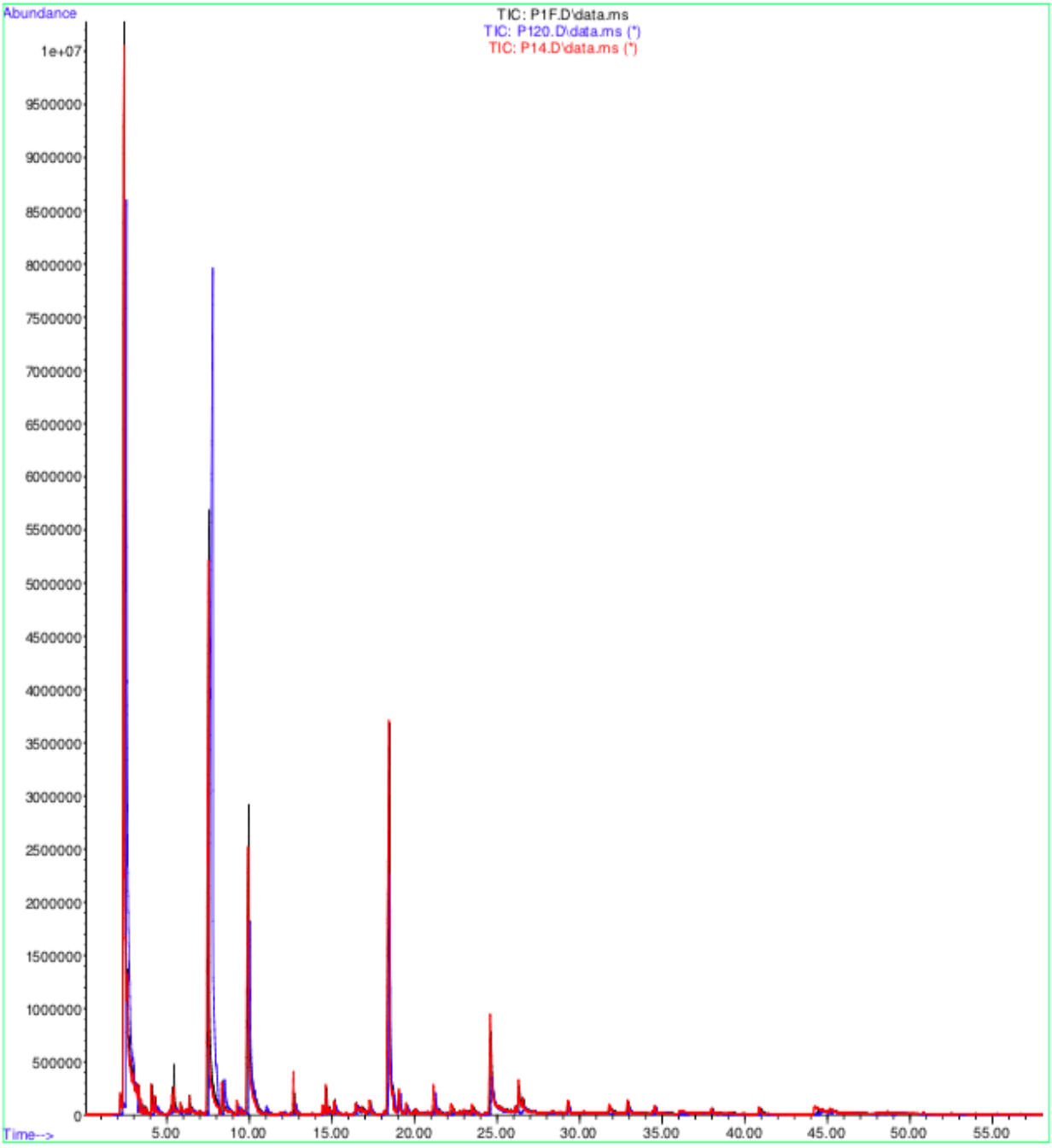

Figure A3. Overlaying chromatograms for fresh fruit (F) sample, and fruit stored at 20°C and 4°C for Piccolo (P).

Table A2. List of all identified compounds with identifiers. (RT), Retention time; (RI), Retention index.

Metabolite name	Average RT(min)	Average RI	Quantitative mass
2-methyl-2-propanol	2.64	673.66	59.10
2-butanone	2.84	678.29	72.06
(E)-2-butenal	2.84	678.40	70.06
Ethyl acetate	3.09	684.06	43.07
3-methylfuran	3.11	684.57	82.06
Oxime-. methoxy-phenyl-	3.11	684.71	133.00
2-ethoxy-2-methylpropane	3.22	687.21	59.10
Amylene hydrate	3.49	693.52	59.09
3-methylbutanal	3.63	696.63	41.10
2-methylbutanal	3.76	699.72	57.10
2-ethylacrolein	3.81	700.97	55.07
2-methoxy-2-methylbutane	3.94	703.90	73.08
1-penten-3-one	4.09	707.37	55.00
2-pentanone	4.12	708.04	43.08
Pentanal	4.33	713.03	44.04
2-ethylfuran	4.36	713.60	81.02
3-methyl-butanenitrile	5.16	732.25	43.09
1-pentanol	5.36	736.93	55.10
2-methyl-1-butanol	5.45	738.91	57.10
Dimethyl disulfide	5.47	739.51	94.00
(E)-2-pentenal	5.88	748.90	55.09
Tetrahydro-2.2.5.5-tetramethylfuran	6.08	753.49	43.06
Toluene	6.19	756.03	91.05
4-methylheptane	6.24	757.28	43.10
Dimethylsilanediol	6.37	760.36	77.00
Isobutyl acetate	6.54	764.20	43.05
Methyl isovalerate	6.58	765.14	74.02
(Z)-2-penten-1-ol	6.58	765.25	57.05
4.4-dimethyl-2-pentanone	6.70	767.91	43.04
3-methyl-2-butenal	6.95	773.82	84.06
2-hexanone	7.07	776.45	43.03
3-hexenal	7.50	786.58	69.10
Hexanal	7.57	788.22	56.10
2.4-dimethyl-1-heptene	9.28	822.91	43.10
(E)-2-hexenal	9.94	835.31	41.10
m-Xylene	10.64	848.73	91.06
1-hexanol	10.99	855.44	56.09

2-methylbutyl acetate	11.59	866.84	43.05
o-Xylene	11.91	872.94	91.05
2.5-dimethyl-2-hexanol	12.41	882.32	59.05
1-nitropentane	12.75	888.79	43.10
Heptanal	12.75	888.86	70.10
4-methyl-2-heptanone	14.70	920.43	43.08
3.5-dimethylphenol	14.88	923.16	122.08
(Z)-2-heptenal	16.48	947.32	83.09
(E)-2-heptenal	16.53	948.00	41.08
Benzaldehyde	16.75	951.40	106.00
6-methyl-5-hepten-2-one	18.48	977.36	43.10
2-pentylfuran	18.71	980.78	81.03
4-carene	19.02	985.52	121.10
6-methyl-5-hepten-2-ol	19.11	986.79	95.10
Ethyl hexanoate	19.37	990.69	88.10
alpha-Phellandrene	19.50	992.70	93.08
Octanal	19.56	993.55	41.09
p-Cymene	20.83	1011.12	119.10
Limonene	21.19	1015.95	91.07
beta-Phellandrene	21.21	1016.16	93.10
2.2.6-trimethylcyclohexanone	21.50	1019.98	82.10
Ethylhexanol	21.66	1022.14	57.08
2-propylphenol	21.86	1024.77	107.02
Phenylacetaldehyde	22.36	1031.30	91.05
cis-2.6-dimethyl-2.6-octadiene	23.08	1040.75	69.10
4.5-dihydro-5.5-dimethyl-4-isopropylidene-1H-pyrazole	23.30	1043.65	82.04
(E)-2-octenal	23.50	1046.38	55.07
cis-Linaloloxide	24.41	1058.33	59.00
1-octanol	24.65	1061.50	56.10
p-Cymenene	25.49	1072.53	117.04
3-(4-methyl-3-pentenyl)furan	26.18	1081.72	69.10
Linalool	26.38	1084.28	71.08
Nonanal	26.61	1087.35	57.09
Terpinolene	26.77	1089.46	93.09
L-alpha-Terpineol	31.70	1173.47	59.05
beta-Cyclocitral	32.96	1195.83	137.10
Neral	34.60	1230.89	69.10
1.3-bis(1.1-dimethylethyl)benzene	34.61	1231.21	175.13
beta-Damascenone	40.91	1365.75	69.03

Mercaptoacetic acid. bis(trimethylsilyl)-	45.16	1474.39	73.06

*Table A3. Sensory evaluation (mean ± standard deviation) of the different varieties for fresh harvested fruits (fresh), after 20°C and 4°C storage and sensory results (mean ± standard deviation) of the different varieties regardless of the treatment, n =. Different letters indicate significant differences between fresh harvested fruits, after 20°C and 4°C storage for each variety (Tukey-Test p ≤ 0.05) and indicate differences between the varieties lines regardless of treatment (Tukey-Test p ≤ 0.05). A two-way ANOVA was performed as well. ns = not significant, * = p<0.05, ** = p<0.01, *** = p<0.001*

Variety	Treatment	Green/grassy odor n=6-10	Tomato-typical odor n=6-10	Tomato-typical flavor n=6-10	Sweetness n=6-10	Sourness n=6-10	Juiciness n=6-10	Firmness of the fruit peel n=6-10	Aftertaste n=5-10
Flavance	Fresh	35.5 ± 27.3a	41.0 ± 27.0a	58.0 ± 19.3a	47.0 ± 17.0a	50.5 ± 15.4a	59.0 ± 17.9a	73.0 ± 14.9a	44.0 ± 25.9a
	4°C	25.8 ± 20.6a	32.5 ± 23.2a	50.0 ± 11.0a	48.3 ± 24.0a	35.8 ± 30.7a	75.0 ± 12.3a	63.3 ± 17.5ab	41.7 ± 9.8a
	20°C	31.3 ± 20.3a	42.5 ± 25.5a	55.0 ± 18.5a	53.8 ± 13.0a	47.5 ± 13.9a	68.8 ± 14.6a	48.8 ± 20.3b	44.3 ± 16.2a
Sweeterno	Fresh	27.5 ± 22.0a	47.0 ± 31.0a	69.5 ± 18.6a	50.0 ± 18.3a	42.0 ± 9.2a	65.0 ± 19.6a	75.5 ± 13.4a	50.5 ± 25.9a
	4°C	35.0 ± 21.4a	41.3 ± 22.3a	62.5 ± 16.7a	55.0 ± 12.0a	45.0 ± 20.7a	57.5 ± 21.9a	66.3 ± 20.7a	38.6 ± 19.5a
	20°C	31.7 ± 25.6a	58.3 ± 14.7a	63.3 ± 20.7a	46.7 ± 20.7a	33.3 ± 20.7a	61.7 ± 16.0a	68.3 ± 19.4a	48.0 ± 23.9a
Briosso	Fresh	24.0 ± 18.4a	47.0 ± 27.5a	76.1 ± 12.2a	54.0 ± 13.5a	42.0 ± 12.3a	75.5 ± 11.7a	55.0 ± 15.8ab	48.0 ± 26.6a
	4°C	18.3 ± 21.4a	45.0 ± 15.2a	60.0 ± 12.7b	50.0 ± 15.5a	30.0 ± 19.0a	73.3 ± 18.6a	63.3 ± 18.6a	50.0 ± 17.3a
	20°C	41.3 ± 25.9a	45.0 ± 12.0a	60.6 ± 11.5ab	53.8 ± 18.5a	43.8 ± 14.1a	75.6 ± 12.4a	38.8 ± 14.6b	48.6 ± 20.4a
Sweetelle	Fresh	26.0 ± 22.7a	33.0 ± 26.3a	67.0 ± 20.0a	58.5 ± 19.2a	46.0 ± 18.4a	61.0 ± 17.9a	72.0 ± 18.7a	48.5 ± 24.5a
	4°C	28.3 ± 17.2a	35.0 ± 16.4a	61.7 ± 19.4a	66.7 ± 12.1a	35.8 ± 27.6a	65.0 ± 18.7a	68.3 ± 9.8a	51.7 ± 20.4a
	20°C	28.8 ± 28.0a	38.8 ± 18.9a	63.8 ± 10.6a	63.8 ± 10.6a	50.0 ± 16.9a	55.0 ± 10.7a	64.3 ± 12.4a	54.3 ± 16.2a
Piccolo	Fresh	28.0 ± 22.5a	37.0 ± 27.2a	72.5 ± 14.0a	74.0 ± 17.0a	37.0 ± 20.6a	68.0 ± 16.9a	65.5 ± 9.0a	62.5 ± 25.1a
	4°C	22.5 ± 13.9a	38.8 ± 25.3a	71.3 ± 15.5a	77.5 ± 20.5a	33.8 ± 17.7a	68.8 ± 10.1a	52.5 ± 17.5a	58.6 ± 17.7a
	20°C	32.5 ± 23.6a	43.3 ± 20.7a	60.0 ± 12.7a	56.7 ± 23.4a	50.0 ± 25.3a	71.7 ± 14.7a	56.7 ± 15.1a	56.0 ± 19.5a
Variety		ns	ns	ns	***	ns	*	**	ns
Treatment		ns	ns	*	ns	ns	ns	**	ns
Variety x Treatment		ns	ns	ns	ns	ns	ns	ns	ns
Flavance		31.7 ± 22.9a	39.4 ± 24.8a	55.0 ± 16.9b	49.6 ± 17.3b	45.8 ± 19.8a	66.3 ± 16.4ab	62.5 ± 19.8ab	43.5 ± 19.2a
Sweeterno		31.0 ± 22.0a	47.9 ± 24.9a	65.6 ± 18.0ab	50.8 ± 16.7b	40.8 ± 16.7a	61.7 ± 19.0b	70.6 ± 17.3a	46.1 ± 23.1a
Briosso		28.3 ± 23.0a	45.8 ± 19.8a	66.5 ± 13.9ab	52.9 ± 15.2b	39.6 ± 15.2a	75.0 ± 13.3a	51.7 ± 18.3b	48.6 ± 21.9a
Sweetelle		24.8 ± 18.6a	35.4 ± 21.1a	64.6 ± 16.7ab	62.3 ± 14.9ab	44.8 ± 20.4a	60.0 ± 15.9b	68.5 ± 14.7a	51.1 ± 20.5a
Piccolo		27.3 ± 19.8a	39.2 ± 24.2a	69.0 ± 14.6a	70.8 ± 20.8a	39.2 ± 21.0a	69.2 ± 16.1ab	59.0 ± 14.4ab	59.8 ± 21.0a