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AGRICULTURAL, FOOD AND ENVIRONMENTAL SCIENCE

XXXIII CYCLE

Studies on qualitative profile of greenhouse tomatoes in relation to technical factors

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Abstract

Tomato (Solanum lycopersicum L.) is one of the crops with the highest economic value on a global scale and a fundamental food for human nutrition. The progressive spread of cultivars with high production capacity, the consequent high fruit load per plant and, with reference to certain types of tomatoes, the practice of harvesting before complete ripening (to prolong their shelf life), had negative effects on certain organoleptic (flavour) and nutraceutical characteristics of the product (e.g. carotenoids content). From an agronomic and technological point of view, this suggests the opportunity to carry out investigations on how the needs of the crop (in the pre-harvest phase) and of the product (in the post-harvest phase) can be better satisfied, in order to match the growing needs expressed by stakeholders and consumers. Indeed, these overall require a product that is pleasant in appearance and taste, rich in substances with health effects and durable over time. This doctoral thesis has the objective of investigating whether and how the quality of tomatoes for fresh consumption produced in cold greenhouses can be improved through technical guidelines such as the choice of the genotype, the use of herbaceous grafting, the application of plant biostimulants or the maintenance of different storage temperatures of the product in the postharvest phase. From the results obtained from this PhD research project, it emerges that the quantitative and qualitative profile of different tomato cultivars for fresh consumption can be managed setting up most proper technical aspects, such as grafting combination, the application of biostimulants or the implementation of specific post-harvest storage conditions.

Sommario

Il pomodoro (Solanum lycopersicum L.) è una delle colture a più alto valore economico su scala globale e un alimento fondamentale per la nutrizione umana. La progressiva diffusione di cultivar con elevate capacità produttive e contemporaneità di maturazione, il conseguente elevato carico di frutti per pianta e, in riferimento a talune tipologie di pomodoro, la prassi di raccogliere le bacche prima della completa invaiatura (per prolungarne la shelf life), hanno avuto anche effetti negativi su alcune caratteristiche organolettiche (sapore) e nutraceutiche del prodotto (contenuto in carotenoidi in primis). Sotto il profilo agronomico e tecnologico, ciò suggerisce l'opportunità di realizzare indagini su come le esigenze della coltura (nella fase di pre-raccolta) e del prodotto in modo da corrispondere al meglio le esigenze espresse dagli stakeholder di filiera e dei consumatori, che complessivamente esigono un prodotto gradevole nell'aspetto e nel gusto, ricco di sostanze con effetti salutistici e durevole nel tempo.

La presente tesi di dottorato si è posta l'obiettivo di indagare se e come la qualità del pomodoro da mensa coltivato in serra possa essere influenzata attraverso indirizzi tecnici quali la scelta del genotipo, l'impiego dell'innesto erbaceo, l'applicazione di prodotti biostimolanti o il mantenimento di differenti temperature di conservazione del prodotto nella fase di post-raccolta. Dai risultati ottenuti dal presente progetto di ricerca di dottorato emerge che il profilo quantitativo e qualitativo dei pomodori destinati al consumo fresco può essere migliorato mediante pratiche agronomiche, quali l'innesto erbaceo, l'applicazione di sostanze

biostimolanti o la conservazione in specifiche condizioni in post raccolta.

1.Quality characteristics of horticultural products

1.1 What is Quality and How do Consumers Perceive it?

The term "horticultural product" is referred to all products, raw or processed, that arise from the horticultural sector. Usually, products from horticultural industry go to market still respiring, as a fresh produce. The quality of fruits and vegetables, or globally horticultural products, represents a dynamic concept which derives from the different interests along the production chain up to the last ring, the consumers (Watada, 1980). Over the years, several researchers have tried to answer this question. According to Kramer and Twigg (1983) "Quality is the composite of those characteristics that differentiate individual units of a product, and have significance in determining the degree of acceptability of that unit by the buyer", or to Steenkamp (1990) "Perceived quality is an idiosyncratic value judgement with respect to the fitness for consumption which is based upon the conscious and/or unconscious processing of quality cues in relation to relevant quality attributes within the context of significant personal and situational variables". Following Abbott (1999) "The term quality implies the degree of excellence of a product or its suitability for a particular use". A more recent definition of quality could be the following "… a set of characteristics that the product must have to satisfy the consumer needs, and which determines its value" (Peri et al., 2004).

When horticultural products and quality are involved, the concept becomes more complex. Considerable efforts have been made to give a complete and universal definition of horticultural quality that could be valid for different products,

distinguishing between intrinsic characteristics inherent to the nature of the product and extrinsic characteristics inherent to different cultures, food practices and marketing factors which reflect on the product acceptability by consumers (Schreiner et al., 2013).

Some objective qualitative evaluation criteria for fresh horticultural products have been established in Europe and in Central and North America, where some of the world's main commercial hubs reside. These standards establish qualitative characteristics that strongly influence the market value of the products, setting as a reference some intrinsic qualitative characteristics such as size, shape and color, freshness and absence of defects or deterioration (Schnitzler and Gruda, 2002). However, the concentration of pesticides in plant tissues are not taken into consideration as well other important qualitative aspects, such as texture, flavour and compounds with a healthy action are not considered, although these can promote consumer satisfaction and the products saleability.

Researches on quality of horticultural products can be targeted considering two different perspectives, *market/product-oriented* and *consumer-oriented*. The first approach (**Figure 1**) prioritizes the needs of key intermediaries in the process of production, marketing and quality standardization: growers and traders-distributors, which promote preferentially quantifiable traits, relating mostly to product appearance and shelf-life. In recent years, the increased attention regarding nutritional characteristics, hedonistic aspects, environmental and socio-economic impact has moved the consumer, with all his requests, to the center of the chain of interest. The consumer has become the subject more involved in the perception of quality and in the product acceptability (Kyriacou and Rouphael, 2018).

Furthermore, in recent years consumer interest toward the quality of horticultural products has increased, especially for their beneficial effects on human health. Thus, the system has changed from *market/product-oriented* to *consumer-oriented*, considering not only the common characteristics desirable from all the intermediaries in the supply chain (such as appearance or integrity of the product) but also quality aspects related to sensory stimuli (taste, touch, smell) and to expectations (real or imaginary) relating to healthiness and health–related compounds that promote a state of good health (Leonardi et al., 2017). As already pointed out by several authors, including Huyskens-Keil and Schreiner (2003) and Gruda (2005), both perspectives are necessary for an adequate definition of quality, and for both fields there is considerable difficulty in assessing the impact of quality on consumer preferences and choice. An interaction between the consumer and the producer is therefore essential.

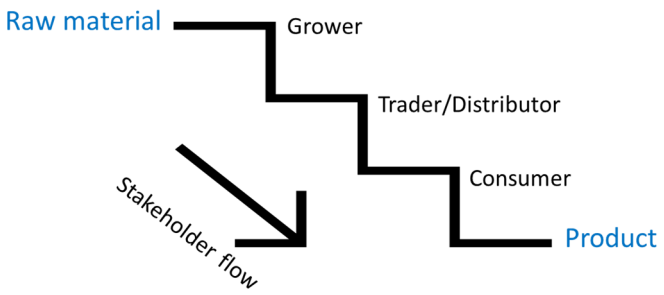

Figure 1. Scheme of the *market/product oriented* system of horticulture quality. Passing from the seed to the final product the consumer is the last actor while grower, trader and

distributor, the chain intermediates, have a higher priority in driving/setting the quality aspects.

1.2 Quality standards and regulations

The need to regulate and establish official and objective criteria for horticultural products has resulted in the creation of various protocols, laws, regulations pertaining to different categories and organizations. If the quality can be defined as the combination of attributes or characteristics of the products having significance in determining the degree of acceptability of the product to a user/consumer, then these attributes can be measured. Generally, consumers quality parameters are more linked to the satisfaction derived by the use of a product and concern abstract and less quantifiable concepts. On the other hand, the quality attributes of an object evaluation have to be pertinent to visual or aesthetic measurable attributes and belong from the market/product oriented perspective (Shewfelt, 2000). In this view food quality is still defined in terms of clearly measurable characteristics rather than in terms of consumer acceptability. These official quality standards and grades influence market value of vegetables and refer strictly to intermediates of the stakeholder chain. The quality standards of fresh and processed fruit or vegetable products vary with their intended use, but as a common approach they focus the evaluation on few parameters quickly determinable. Most of them are external quality attributes, such as simple morphometric traits (shape, size, or colour), integrity or degree of visible defects and decay (Shewfelt, 2000). Some examples are the United Nations UNECE Standards for Strawberry (UNECE, 2018) and the European Regulation (EC No 843/2002 May 21 2002): although the strawberry is clearly recognized for its characteristics of freshness, aromaticity and sweetness, the

characteristics relating to taste, aroma, texture or sugar content are not considered in these official regulations. References to the organoleptic composition are rarely found in the standards protocols of fruit and vegetables, and when present, they limit themselves to defining minimum thresholds in terms of total soluble solids content (SSC) or acidity. These regulations are more closely linked to the needs of the global logistics supply chain, which needs homogeneous, standardized and "easy to move" products, setting minimum thresholds of acceptability that can be evaluated with fast, low-cost and non-invasive means than to the needs of the consumer, who claims quality and product excellence. This is the case of the United Nations UNECE standards for table grapes (UNECE, 2017), demanding a refractometric index of at least 16 °Brix as guaranty of sufficient ripeness level or the U.S. Standards for Grades of Table Grapes (USDA,1999), which set a specific maturity table based on different grape varieties.

As above reported, the raising consumers' expectations toward organoleptic or nutraceutical traits of horticultural products are almost neglected by the current regulation (Schreiner et al., 2013). A partial attempted to fill this gap is represented by the establishment of *voluntary standards* by the companies that with their corporate standards (generally reflecting the characteristics desired by the consumer), become a symbol of the quality of a product. Furthermore, the complex network of actors along the supply chain, from commercial quality assurance to the standardization system, favours collaboration between the various stakeholders, allowing the presence on the market of high quality products in a globalized way, crossing seasonality and production (Kader, 2008).

Obviously, an unchanging consumer with consistent preferences does not exist, due to the personal physiological reactions which are a result of past experience, training, individual preference and power of perception. On the other hand, the horticultural supply chain addresses the needs of a very diverse types of consumers with different requests and desires (Schreiner, 2009). Moreover, consumer preferences are also strongly oriented not only to the satisfaction of organoleptic expectations but are even more attracted by nutritional and functional aspects of horticultural products and the presence of specific phytonutrients (such as carotenoids, phenols or vitamins). Finally, horticultural quality is also linked to the socioeconomic and environmental factor, because of increased health awareness and environmental consciousness (Schreiner et al., 2013).

To design, develop and product plant-based healthy foods, looking at the different points of the production chain from raw materials to consumer is the current dominant trend for fruits and vegetables. In this view, raw material, food processing strategies and development of the final product are tailored to meet different expectations, but the current regulatory context for fruits and vegetables quality have no criteria to evaluate and incorporate these criteria into their own regulation.

1.3 Horticultural quality features

The act of buying fruit and vegetable products is the result of multiple considerations, sometimes even unconscious and preventively to purchase and consumption, through which the consumer evaluates the product using both intrinsic and extrinsic quality characteristics (Rezvani and Salehi, 2012; Tijskens et al., 2001).

The attributes that allow qualifying a horticultural product are related to its appearance, texture, organoleptic compounds, health-promoting compounds, and the presence of contaminants.

1.3.1 Appearance

Along the production chain, fruits/vegetables appearance is used as primary tool to set the quality of individual units of product (Kays, 1999). For consumers, the appearance of a product is crucial, especially considering the current trend that requires products free from visible defects. The appearance of the product involves numerous traits, including size, shape, colour, exocarp characteristics and absence of defects. Fruit size and uniformity are important parameters because are among the few characteristics reported in the official regulations and influences the product destination (minimum size threshold for sale, division in classes on the basis of weight/diameter or other), this represents one of the first characteristics considered by consumers (Leonardi et al., 2017).

Fruit shape, often described through the ratio among diameters (e.g., longitudinal and transversal), depends by the considered product. This trait is often used as a tool for cultivar description, plant variety or cultivar patents and evaluation of consumer decision performance (Costa et al., 2011).

Another key component often associated to food quality is colour, strictly linked to consumer perception and associated with quality traits as freshness, maturity, desirability, and food safety (McCaig, 2002). Being one of the first grading factors, colour is often a primary consideration of consumers when making purchasing decisions. Its evaluation and perception depend by the object properties, by the consumers

ability/sensibility, by illumination environment and condition, and by the angles of illumination and viewing (McCaig, 2002). Many industries adopt a quantitative measurement through reproducible colour values in accordance with standards developed by the Commission Internationale de l'Éclairage (CIE) (Schanda, 2007). The *L*a*b** values are calculated from the visible spectral data, are accredited as CIELAB system and are widely used in food and agricultural industries (Arias et al., 2000; Wulf and Wise, 1999; Ortolá et al., 1998).

During ripening and senescence, many horticultural products undergo changes in peel and pulp colour, and for some vegetables and fruits it is an index related to the eating quality and shelf-life, as in tomato.

Exocarp characteristics (such as thickness) and absence of defects (such as cracking, shrivelling, burns, blossom-end-rot, discoloured area, pulp vitrescence, etc…) can depend on multiple factors in which pre- and post-harvest management are involved. Normally the presence of defects or undesirable characteristics causes that the product is distinguished into different product classes on which the final price for the consumer depends.

1.3.2 Texture

"Texture is the sensory and functional manifestation of the structural, mechanical and surface properties of foods, detected through the senses of vision, hearing, touch and kinaesthetic" (Szczesniak, 2002). Texture is normally experienced after the purchase of the product and is an important aspect for shelf life, acceptability, and transportability. Among the physiological factors influencing fruit texture have to be considered the tissutal concentration of calcium, water relations, transpiration, wax layers, cell-to-

cell adhesion, cell-wall architecture and solubilization, and cell-wall protein status (Huxham et al., 1999; Saladie et al., 2007). Texture is a multifactorial trait, whose description falls into two categories, i.e. mechanical (firmness, hardness, stiffness, and elasticity) and acoustic (crispness and crunchiness) (Szczesniak, 2002; Costa et al., 2012). Firmness is one of the attributes that is used to describe and measure the consistency of the agricultural products (Leonardi et al., 2017). Many traits related to texture such as juiciness, turgidity, and crispness, flesh firmness, mealiness, meltiness influence human perception of flavour and taste of fruits and vegetables.

1.3.3 Flavour compounds

The flavour of agricultural products is a function of taste (the equilibrium among the perceived sweetness and sourness) and aroma (presence and concentrations of a pool of odour active volatile compounds). Although taste and aroma are well integrated in their contribution to the overall organoleptic properties of food, it seems that aroma is sometimes able to overcome the role of taste (Kader, 2008; Voilley and Etiévant, 2006).

1.3.3a Sugar content and acidity

Among the main non-volatile components contributing to the taste of the agricultural products there are sugars, organic acids, free amino acids, and salts.

Sweetness is determined by the concentrations of the predominant sugars, which are ranked relative to sucrose in the following order of sweetness: fructose (1.2) > sucrose (1.0) > glucose (0.64). On the other hand, sourness/acidity is determined by the concentrations of the predominant organic acids, which are ranked relative to citric acid in the following

order of sourness: citric (1.0) > malic (0.9) > tartaric (0.8) (Kader, 2008).
The presence of some amino acids, such as aspartic and glutamic, or minerals, such as calcium, phosphorus, and potassium might also drive the sourness altering the buffering capacity of the matrix and, consequently, the taste. As for amino acids, their combination is determinant for the taste of food; indeed, glycine and alanine present a sweet taste, while valine and leucine a bitter one, and aspartic acid and glutamate have sour and umami tastes, respectively (Lemieux and Simard, 1992). In food, the organoleptic sensation of astringency is elicited primarily by flavanol polymers (proanthocyanidins or condensed tannins), and variations in proanthocyanidin composition, including polymer size, extent of galloylation, and formation of derivatives affect astringency perception (Lesschaeve and Noble, 2005).
A great benefit for plant breeders and technicians is represented by soluble solids index in fruits and vegetables, which can be quickly measured by refractometers and include a plenty of compounds such as sugars, organic acids, soluble pectins, anthocyanins and other phenolic compounds, and ascorbic acid and is linked to consumer acceptance (Kader, 2008). The perceived quality of a food is the result of the quantity of sugars, acids and flavouring substances present in the product. Factors like genotype, the maturity stage and time of delivery to the consumers are important factors influencing flavour quality of fruits and vegetables.

1.3.3b Aroma volatiles

Volatile organic compounds (defined as volatiles or VOCs) of plants are generated from both primary and secondary metabolism and are generally low-molecular-weight compounds. More than 7000 aroma volatiles have been

identified and catalogued from different food matrices (Goff and Klee, 2006). Aroma profile is a complex mixture of different volatiles, whose composition is species-specific and often cultivar-specific (Sanz et al., 1996). Moreover, different volatiles are produced in plant tissues at specific developmental stages, e.g. during flowering, ripening, or fruit ripening.

Although many fruits and vegetables share similar aromatic compounds, each food has a distinctive aroma, whose perception is also a function of the consumers' characteristics (Krumbein et al., 2004; Tucker, 1993). Food flavour is usually described as a combination of taste and smell, but other extrinsic features such as appearance, texture, temperature, mouth feel, and past experience also play a pivotal role.

Volatile compounds are mostly represented by esters, alcohols, aldehydes, and ketones and their contribution to overall flavour can be evaluated by threshold concentrations, potency, and interactions with other compounds (Goff and Klee, 2006). Metabolic pathways of main group of volatiles, including those for amino acids, fatty acids, and carotenoids, are partially know but further researches are needed to identify the key substrates and enzymes involved in their biosynthesis in order to target those that can increase desirable aroma compounds.

1.3.4 Health-promoting compounds

In the current diet-health paradigm, many horticultural products have assumed the status of "functional foods", i.e. foods capable of providing additional physiological benefit, such as preventing or delaying chronic diseases, as well as guaranty basic nutritional requirements (Kaur and Kapoor,

2001; Dillard and Bruce German, 2000; Hurtst and Hurst, 2013).

Fruits and vegetables are essential sources of vitamins, minerals, dietary fibre, and antioxidants. The single contribution of each group to human health depends upon its nutritive value, per capita consumption and bioavailability (Kader, 2008). Specifically, the per capita consumption is greatly influenced by consumer preferences and degree of satisfaction from eating the fruit or vegetable (Kader, 2008).

Plants accumulate nutrients and phytochemicals in an organ-specific manner (such as lycopene in tomato fruits). Moreover, biosynthesis, distribution, the accumulation of the health-promoting compounds is influenced by maturity stage, agricultural practices, stresses, temperature and storage conditions (Wang et al., 2007; Cho et al., 2007).

Only recently breeding programs have addressed the concentration of some phytochemical in the horticultural products, with the aim to obtain new cultivars, not only disease-resistant and with a good taste and yield performances, but also appreciated for the health-promoting traits. Horticultural crops such as strawberry, apple, tomato, potato, cabbage, broccoli, lettuce, onion, cranberry and raspberry are currently enrolled in breeding programs in which the phytochemical content is considered a key component (Crosby et al., 2007; Patil et al., 2012).

1.3.4a Carotenoids

Carotenoids are among the most important phytochemical in fruits and vegetables; these are pigments exhibiting red, orange, or yellow colours (**Figure 1.2**). Carotenoids represent by far one of the most studied phytochemical fractions of plants, due to their protective role against age-related degeneration, cancer, and cardiovascular diseases (Martì et

al., 2016; Demmig-Adams and Adams, 2002). Interestingly, some carotenoids (e.g., α-carotene and β-carotene) possess a special role of being provitamin A.

Carotenoids basically consist of a C40-hydrocarbon skeleton chain. More than 750 carotenoids have been characterized in natural matrices, and on the basis on their structures they are classified in two groups: carotenes or pure hydrocarbon carotenoids (lycopene, α-carotene, and β-carotene), and xanthophylls or oxygenated carotenoids (antheraxanthin, lutein, neoxanthin, violaxanthin, and zeaxanthin) (Ngamwonglumlert et al., 2020). Lycopene, β-carotene, lutein, and zeaxanthin are the major carotenoids found in foods. These compounds essential for life cannot be synthesized by animals, so they must be introduced in the diet.

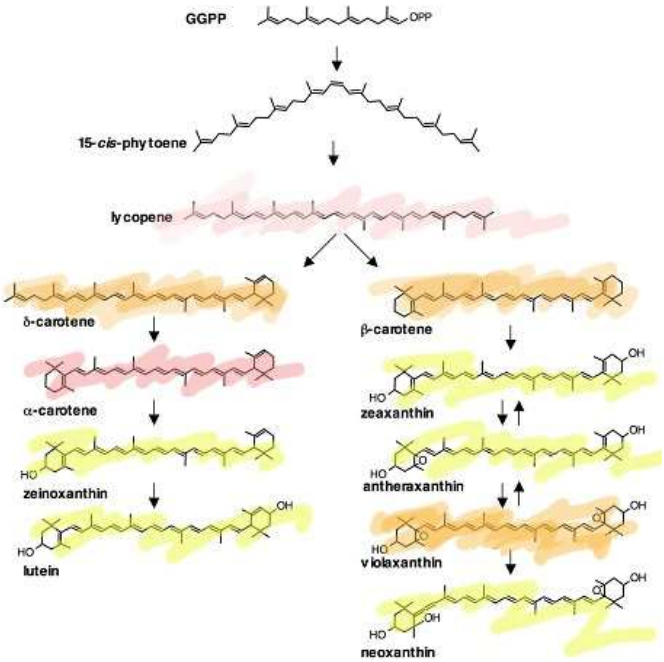

Figure 1.2 Main carotenoid biosynthesis in plants as performed by a pathway of more than ten enzymes (Diretto et al., 2006).

1.3.4b Ascorbic acid

Structurally, vitamin C or L-ascorbic acid (AsA) is one of the simplest vitamins and it is a C6 sugars relative. Vitamin C, including both ascorbic and dehydroascorbic acid, is important in the protection of the vegetable tissues against oxidative damages that might increase with ripening due to enhanced respiration (Slimestad and Verheul, 2005). In both plant and animal systems, ascorbic acid interacts enzymatically and non-enzymatically with damaging oxygen radicals and their derivatives, so-called reactive oxygen species (ROS), and is an important micronutrient and an

essential antioxidant for human diet (Frei et al., 2012; Davey et al., 2000). Moreover, it is involved in cell division and cell wall synthesis and in the interaction of plants with the environment, pathogens and oxidizing agents (Gest et al., 2013).

1.3.4c Phenolic compounds

Fruits and vegetables are a good source of phenolic compounds. Chemically, phenolic compounds consist of a hydroxyl group (-OH) bonded directly to an aromatic hydrocarbon group. Under the term phenolic compounds different molecules with complex structures are grouped, which are distinguished according to the number of carbon atoms that make up the skeleton (Harborne, 1980) (**Table1**). This class of compounds exhibits an antioxidant action and the number of specific compounds depends, as for other compounds, on the species, ripening stage, agricultural practices, stress factors and storage conditions. Plant polyphenols are a wide group of phytochemicals involved in the regulation of plant growth, reproduction and response to the environmental stressors (Sharma et al., 2019).

Table 1. The main classes of phenolic compounds in plants (Harborne 1980)

Number of carbon atoms	Basic skeleton	Class	Examples
6	C6	Simple phenols	Catechol, hydroquinone
		Benzoquinones	2,6-Dimethoxybenzoquinone
7	C6-C1	Phenolic acids	Gallic, salicylic
8	C6-C2	Acetophenones	3-Acetyl-6-methoxybenzaldehyde
		Tyrosine derivatives	Tyrosol
		Phenylacetic acids	p-hydroxyphenylacetic
9	C6-C3	Hydroxycinnamic acids	Caffeic, ferulic
		Phenylpropenes	Myristicin, eugenol
		Coumarins	Umbrelliferone, aesculetin
		Isocoumarins	Bergenon
		Chromones	Eugenin
10	C6-C4	Naphthoquinones	Juglone, plumbagin
13	C6-C1-C6	Xanthones	Mangiferin
14	C6-C2-C6	Stilbenes	Resveratrol
		Anthraquinones	Emodin
15	C6-C3-C6	Flavonoids	Quercetin, cyaniding
		Isoflavonoids	Genistein
18	$(C6-C3)^2$	Lignans	Pinoresinol
		Neolignans	Eusiderin
30	$(C6-C3-C6)^2$	Biflavonoids	Amentoflavone
n	$(C6-C3)n$	Lignins	
	$(C6)n$	Catechol melanins	
	$(C6-C3-C6)n$	Flavolans (Condensed Tannins)	

1.3.4d Minerals and Vitamins

Vegetables and fruits are valuable sources of essential minerals. Their concentration in vegetables depends on a number of factors including the genetic background of the crop, the environmental conditions, soil/substrate characteristics and the ripening stage at harvest (Martínez-Ballesta et al., 2010).

By definition, vitamins are organic compounds needed in small quantities to sustain life. Vitamins can be distinguished in fat-soluble (A, D, E and K.) and water-soluble (B group and C). Vitamins cannot be synthesized by humans but must be introduced through the diet. For example, some beneficial effects of horticultural products is related to vitamin E, which plays an important role as antioxidant toward lipid membranes. These lipid-soluble molecules are, specifically, tocopherols and tocotrienols, collectively known as tocochromanols. On the other hand, Vitamin A, which

derives from α-carotene and β-carotene, is essential to human health systems, such as in embryonic development, immunity, and vision (Demmig-Adams & Adams, 2002; Gong & Rubin, 2013).

1.3.5 Contaminants

In agricultural product safety factors concern the presence of naturally occurring toxicants in certain crops (e.g. glycoalkaloids in potatoes), which vary according to genotype and are routinely monitored to ensure food safety. Other contaminants such as chemical residues and heavy metals are monitored by various chain intermediates to assure compliance with established maximum threshold. Sanitation throughout harvesting and postharvest handling operations is essential to minimize microbial or faecal contamination and procedures that reduce the potential growth and development of mycotoxin-producing fungi are used all long the production cycle (Kader, 1992). Example of naturally occurring toxicants are cyanogenic glucosides in lima beans and cassava, nitrates and nitrites in leafy vegetables, oxalates in rhubarb and spinach, thioglucosides in cruciferous vegetables, and glycoalkaloids (solanine) in potatoes. Contaminants also include chemical residues, heavy metals (mercury cadmium, lead), synthetic residues and pollutants that should never be present (Kader, 1992).

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2. Preharvest and postharvest factors affecting vegetables quality

Over decades, plenty of studies outlined the effects of pre- and post-harvest factors on quality traits of vegetables (Dorais et al., 2001; Ripoll et al., 2014). Preharvest factors proved to have significant effects on physicochemical, organoleptic and functional quality of fruits and vegetables at harvest (Weston and Barth, 1997). The biosynthesis and accumulation of health-promoting compounds depends mainly both on the genetic material and the ripening stage of the produce, but the phytochemical profile is also influenced by agronomic practices and environmental factors (Lei et al., 2007).

2.1 Genetic Background

The choice of the cultivar is a key determinant of quality for fruits and vegetables, as it influences the product appearance, organoleptic and compositional traits, including bioactive compounds with antioxidant activity (Kyriacou et al., 2017; Rouphael et al., 2012; Dorais et al., 2008). Traditionally, horticultural breeding programs were focused on developing cultivars with desirable agronomic characteristics such as high and stable yields, good appearance of the edible product, diseases/pests resistance and long shelf-life (Dorais et al., 2008). Modern hybrid cultivars are usually very responsive to agronomic inputs (e.g. fertilization and water supply), and show plant characteristics that allow easier crop management and market acceptability. Only recently, thanks also to the raised expectation of consumers in terms of quality, healthy and tasty foods, breeding programs shifted their attention to the organoleptic and functional quality traits of several horticultural crops including apple, broccoli, cabbage,

cranberry, lettuce, onion, potato, raspberry and tomato (Khanizadeh et al., 2006a, 2007; Tsao et al., 2006).

Plant breeding programs are now using both traditional and biotechnological approaches to enhance levels of health-promoting compounds in new cultivars, however only limited number of such improved crops have been released on a commercial scale (Lei et al., 2007; Schijlen, et al., 2004). Among the few examples, tomato seed companies are now offering different commercial typology of fruits (cherry, cocktail, grape, plum, round and salad) in different colours (green, red, yellow, orange, pink, purple, brown, black) with higher phytonutrient contents (such as carotenoids o polyphenols) (Chime et al., 2017; Raffo et al., 2002; Gonzali et al., 2009).

Differences in the content of important phytochemicals, such as carotenoids, phenolics and vitamins among different coloured carrots (orange, purple, yellow or white) were reported (Alasalvar et al., 2001). Another example of biotechnological breeding selection is represented by an apple genotype with reduced content of phenolic metabolites implicated in post-cutting oxidation and browning, now suitable for processing into non-browning fresh-cut apple products (Khanizadeh et al., 2007). Also for lettuce some improvement in the concentration of flavonols and phenolic acids was achieved (Pernice et al., 2007). Moreover, some positive results achieved in lettuce concern, also, a reduced tendency to accumulate nitrates (Reinink, 1988).

Genetic improvements has addressed several *Brassicaceae* species such as broccoli, cauliflower and turnip, which are characterized by high concentrations of pungent/bitter-tasting but health-promoting compounds, such as the alkenyl and indole glucosinolates. However, many *Brassicaceae* cultivars have high content of health-relevant metabolites, but are

often characterized by low sugar contents, which is partly incompatible with consumer acceptability and makes the product less desirable, highlighting the need to raise their sugar content for increasing consumer acceptability (Krumbein et al., 2010; Schonhof et al., 2004).

2.2 Climate Conditions

2.2a Solar Radiation

With limited exceptions, usually solar radiation is positively correlated with high qualitative and quantitative performance in several species (Weston and Barth, 1997; Krug, H.,1986). Indeed, under low light, inadequate quantities of photo-assimilates, together with a lower synthesis of sucrose, has been observed in melon, tomato and strawberry (Pardossi et al., 2000; Caruso et al., 2003). A close and direct relationship was observed between light conditions and ascorbic acid content in tomato, lettuce, sweet pepper and strawberry; specifically, lower light intensities were coupled with lower content of ascorbic acid in plant tissues (Shinohara et al., 1987; Lee et al., 2000). The pigments synthesis, which is expressed in the colour of the different fruits and vegetables, is an important quality index for consumers and is influenced by solar radiation (Schreiner et al., 2002). Dorais et al. (2001) reported a reduced synthesis of carotenoids in tomato under low light intensity, resulting in an uneven fruit pigmentation mainly due to a reduced content of lycopene and β-carotene.

Light is the key factor influencing also the nitrate concentration in vegetables. As a general rule, the content of nitrate in leafy vegetables is related to light intensity and growth rate, with higher N concentrations in the edible tissues reported during winter (Santamaria et al., 2006). Fallovo et al. (2009) reported an increased nitrate content in leaves of *Brassica rapa* L. subsp. *nipposinica* var. *chinoleifera* and

Brassica juncea L. when grown at a low level of daily photosynthetic active radiation (5.0 mol m^{-2}) compared to a medium level (6.8 mol m^{-2}). Similarly, a reduction in light level has been accompanied by an increase in nitrate accumulation in lettuce and spinach, the latter being associated to a reduced activity of the nitrate reductase enzyme (Chadjaa et al., 1995; Gaudreau et al., 1995).
Low light intensity promotes, also, the content of some antinutritional factors, such as the calcium oxalate. This health-hazardous compound can lead to nephrolithiasis, or calcium oxalate renal calculi, and it can impair the uptake of iron and calcium from food in the gut (Han et al., 2015; Lönnerdal, 2010). Leafy green vegetables belonging to *Chenopodiaceae* family (e.g. swiss chard and spinach) are quite rich in oxalates. According to Proietti et al. (2004) spinach leaves grown under low photon flux density (200 μmol m^{-2} s^{-1}) showed higher amount of oxalates and nitrates in comparison with plants grown under more proper conditions (800 μmol m^{-2} s^{-1}).
Severe light intensity has detrimental effect too on yield and quality of fruits and vegetables. Extreme light can generate the green shoulder in tomato or can cause sunscald in a large number of crops, such as tomato, bell pepper, eggplant or pepino (Geissler et al., 1985; Kays, 1999).

2.2b Temperature

Suboptimal temperatures during plant growth can greatly affect yield and product quality in vegetable crops. Ottosen et al. (2003) reported reduced fruits size and quality, as well as delayed harvests in bell peppers grown at temperatures below 17°C, while tomato fruits grown in cold greenhouse during off-season are often described as less juicy and flavourful (Gruda, 2005). Suboptimal temperature has a well-known

effect also on fruits and vegetable colour (Dorais, et al.,2001; Geissler, 1985). One of most studied plant pigments, due to its health-promoting role, is lycopene. Similarly to other carotenoids, its synthesis is actively influenced by the environmental factors, with an optimum temperature between 22 and 25 °C (Dumas et al., 2003), while air temperature below 10 °C seems to inhibit its accumulation (Dorais, et al., 2001; Geissler, 1985). On the other hand, it is known that slightly unfavourable temperatures can sometimes positively affect the quality in crops and enhance the content of some bioactive compounds such as glucosinolates in cabbages. Charron and Sams (2004) reported a higher concentration of total glucosinolates in leaves of *Brassica oleracea* grown at 12 °C than at 22 °C, because of a higher activity of the enzyme myrosinase.

Exposure to high temperature can alter many morphological, physiological and metabolic traits of the crops, leading to a decay of nutritional and organoleptic quality of the products (Moretti et al., 2010; Neugart et al., 2012). Extreme warm temperatures often result in quality losses, such as alterations in shape, uneven colour distribution and altered texture of tomato, pepper, cucumber and eggplant fruits (Geissler, 1985; Gross, 1991). Direct negative effects include damage to cellular membranes, proteins, and nucleic acids (Kays, 1999). Quality deterioration also affects the synthesis of pigments and the degradation of the existing ones, including symptoms of suns cars or sunburns (Kays, 1999). In greenhouse bell pepper fruits, when temperatures exceed 35 °C, burn spots are shown on the top of the fruits (Geissler, 1985).

2.3 Agronomic Practices

2.3a Soilless Culture and Fertilization

Soilless cultures offer the possibility to actively modify the product quality in vegetable systems, through the rapid setting of electrical conductivity (EC) of the nutrient solution, chemical forms of the elements, nutrients concentration, temperature of the nutrient solution or pH (Gruda, 2009). An effective tool to improve the vegetable quality in soilless system is represented by the management of ion concentration and composition of the nutrient solution. Several experimental reports showed as with a moderate salinity stress, realized by adding NaCl, is possible to enhance the acidity and soluble solids content in tomato fruits (De Pascale et al., 2001; Martorana et al., 2004; Wang et al., 2008). Similar results were reported by for dry matter and total carbohydrates in zucchini squash when the salinity of the nutrient solution was raised from 2.0 to 4.1 dS m^{-1}. The concentration of health-promoting compounds such as vitamin C, lycopene, β-carotene and phenols were enhanced by properly increased EC in sweet pepper, cucumber, eggplant, celery or watermelon (Dumas et al., 2003; Rouphael et al., 2006; Sonneveld and Van der Burg, 1991; Trajkova et al., 2006; Savvas and Lenz, 1994; Pardossi et al., 1999; Colla et al., 2006b).

The chemical composition of the nutrient solution can influence the quality of vegetables too (Wang et al., 2008). Fanasca et al. (2006) studied tomato fruits quality of the high pigment hybrid cultivar "Lunarossa" as affected by the macrocation proportions (K/Ca/Mg) in a soilless culture. It was reported an increased fruit dry matter, total soluble solids and lycopene content associated to a higher proportion of K in the nutrient solution, whereas a higher proportion of Mg resulted in an improved total antioxidant activity. Finally, in soilless systems the presence of undesirable compounds such as nitrates can be limited by applying proper strategies, such

as by eliminating or reducing the N supply before the harvesting of lettuce, celery or endive (*Chicorium endivia* L. var. *crispum* Hegi) (Martignon et al., 1993; Gonnella et al., 2004). Interestingly, a targeted biofortification of vegetables can be achieved by adding desired elements such as selenium and iodine into the nutrient solution. The opportunity to increase the presence of the above-mentioned elements, as well as calcium, copper, iron, magnesium, and zinc, is becoming a convenient agronomic strategy to achieve an efficient increase of their concentration in the edible parts of several vegetables (Tomasi et al., 2015).

2.3b Vegetable Grafting

The choice of appropriate rootstocks for specific scions is crucial for reaching optimal yields and fruit quality standards. Strictly, grafting is the technique to join two or more pieces of living plants that grows as a single plant. In vegetable crops, grafting has become a worldwide common practice for *Solanaceae* and *Cucurbitaceae*, in order to manage many soil borne diseases, to improve abiotic stress tolerance, and recently to manage fruit quality too (Rouphael et al., 2010; Schwarz et al., 2010). It has been reported that grafting is able to influence many quality traits in vegetables, such as fruit shape, epicarp colour, skin or rind smoothness, flesh texture and colour and soluble solids concentration in many crops, such as watermelons, cucumbers, eggplants, tomatoes and melons (Kyriacou et al., 2017). It was, also, reported that sugars, flavour, colour, carotene content and texture can be affected by grafting, mainly as a function of the genotypic scion/rootstock combination (Davis et al., 2008).

In *Cucurbitaceae* such as cushaw pumpkin (*Cucurbita argyrosperma*) and squash (*C. pepo*), C. *maxima* × *C. moschata* hybrids and *L. siceraria* are usually used as

rootstocks. It has been reported that watermelon fruits obtained from grafted plants onto *L. siceraria* were firmer than control (ungrafted plants) (Yetisir et al., 2003). Similarly, watermelon fruits obtained using the hybrid rootstock *C. maxima* × *C. moschata* showed a higher firmness than control fruits (from ungrafted plants), irrespective of the growth conditions (greenhouse vs. open field) (Yetisir et al., 2003; Huitrón-Ramírez et al., 2009).

The rootstock-scion combination seems to affect the fruit soluble solid content (SSC) too, even if the results are sometimes contrasting, maybe as a consequence of different growth conditions (Colla et al., 2006a; Crinò et al., 2007). Indeed, Huitrón-Ramírez et al. (2009) reported no SSC differences among grafted and ungrafted watermelons, while Salam et al. (2002) reported an increased SSC in grafted watermelons onto bottle gourd (*L. siceraria* Standl). Furthermore, Di Gioia et al. (2010) found no significant differences in SSC in tomato "Oxheart" grafted onto two interspecific *S. lycopersicum* × *S. habrochiates* rootstocks, and a decrease by 14-20 % in vitamin C content when tomato plants were grafted onto Beaufort F_1 and Maxifort F_1. In general, for *Cucurbitaceae*, the use of pumpkin rootstocks seems to be negatively linked to the sweetness of watermelons, pointing out the need to carefully evaluate the rootstock effects on SSC before introducing it on a large scale.

Several phytochemicals also seem to be influenced by the rootstock genotype. Lycopene, total vitamin C and dehydroascorbate contents in mini-watermelon plants grafted onto a hybrid rootstock (*C. maxima* × *C. moschata*), were found to be higher by 40%, 7% and 13%, respectively, than control plants (Proietti et al., 2008). Similar results were obtained by Huang et al. (2009) from cucumber plants grafted

onto *C. ficifolia* and *L. siceraria* for vitamin C. In tomato, fruits from 'Fanny' plants grafted onto the hybrid rootstock 'AR-9704' showed a doubled content of lycopene than control plants grown under the same conditions (Fernández-García et al., 2004). On the other hand, the effects deriving from vegetable grafting are often dependent on the combination between scion and rootstock, giving a genotype-dependent response. Fruits from grafted eggplants onto *S. torvum* and *S. sisymbrifolium* as rootstocks were negatively affected in terms of vitamin C content, firmness and sensory attributes (Arvanitoyannis et al., 2005).

The mineral content of vegetables is influenced by grafting too. Rouphael et al. (2008) reported an improved concentrations of K and Mg in fruits of watermelon plants grafted onto the hybrid rootstock 'PS 1313' (*C. maxima* × *C. moschata*), whereas no differences were reported for P and Ca concentrations. On the other hand, higher Ca contents for fruits obtained from tomato plants grafted onto 'He-Man' (tomato interspecific hybrids), in comparison to control plants, have been reported by Khan et al. (2006) when plants were grown in a greenhouse.

2.3c Biostimulants Application

In recent years, modern agriculture aims to reduce the external inputs without reducing the yield and quality of vegetables. Among the agricultural practices, the use of biostimulants is gaining growing interest (Du Jardin, 2015).

Different attempts were made to set a universal and complete definition of biostimulants and the first one was reported in web journal dedicated to turf maintenance professionals, called Ground Maintenance, by Zhang and Schmidt in 1997 who defined biostimulants as "materials that, in minute quantities, promote plant growth". Followed other

definitions, such as “biostimulants are materials, other than fertilisers, that promote plant growth when applied in low quantities” (Kauffman et al., 2007); or “plant biostimulants include several substances and microorganisms that enhance plant growth” (Calvo et al., 2014). Agricultural industries and companies played a crucial role in the definition and promotion of the concept of biostimulants, also, by creating sector associations as the “European Biostimulants Industry Council” (EBIC) in Europe and the “Biostimulant Coalition” in the USA, to have a direct dialogue with other stakeholders, regulators, and scientists (Du Jardin, 2015). Recently, European Union established the official definition of biostimulant as “any substances, mixtures and micro-organisms that stimulate plants’ natural nutrition processes… such products aim solely at improving the plants’ nutrient use efficiency, tolerance to abiotic stress, quality traits or increasing the availability of confined nutrients in the soil or rhizosphere, they are by nature more similar to fertilising products than to most categories of plant protection products. They act in addition to fertilisers, with the aim of optimising the efficiency of those fertilisers and reducing the nutrient application rates” (Reg EU 1009/2019).

From all definitions, it emerges that the concept of biostimulant is not based on their materials and ingredients, but on its effect on plants. Moreover, by using the words “minute quantities” there was an initial endeavour to distinguish biostimulants from nutrients and soil amendments, which are usually applied in higher quantity (Du Jardin, 2015). The purpose of a biostimulant is to stimulate the nutrition processes of plants regardless of the nutrient content of the formulation, by promoting nutrient use efficiency, tolerance to abiotic stressors, crop quality traits or availability of confined nutrients in the soil and rhizosphere.

In general, biostimulants, including plant-based extracts, contain a wide range of bioactive compounds, including mineral elements, humic substances, vitamins, amino acids, chitin, chitosan, and polysaccharides or oligosaccharides (Berlyn & Russo 1990; Hamza & Suggars 2001; Du Jardin, 2015). It is reported that biostimulant mostly enhance seed and seedling vigour, stimulate vegetative growth, improve nutrient acquisition and partitioning within the plant, increase antioxidants content in plant tissues, contribute to stress tolerance, and rise plant yield and fruit quality (Parađiković et al., 2019; Bulgari et al., 2015).

Spray applications of red grape skin extract and alfalfa hydrolysed protein extract on hot pepper plants (*Capsicum chinense* L.) resulted in higher fresh weight and higher content of total phenols, ascorbic acid and antioxidant activity compared to non-treated plants (Ertani et al., 2015).

Baby spinach plants (*Spinacia oleracea* L.) treated with different plant and seaweed-based extracts showed a higher fresh yield, leaf dry biomass, as well an improved total polyphenols and total ascorbic acid content compared to untreated plants (Rouphael et al., 2018).

Biostimulant applications are reported to positively affect the growth and development of the root system, due to the presence of bioactive compounds. Root growth-promoting activities were observed in lettuce (*Lactuca sativa* L.), and an improved uptake of nitrogen and sulfate in winter rapeseed (*Brassica napus* L.) under limited nutrients availability, as a consequence of root applications of seaweed-derived biostimulants (Vernieri et al., 2006; Jannin et al., 2013).

During transplanting of many vegetable and ornamental crops stress may occur, as reported by Parađiković et al. (2017) for wax begonia (*Begonia semperflorens*) in which a biostimulant treatment (Radifarm®, Valagro) enhanced the

level of N, K^+, Ca^{2+} and Mg^{2+}, as well fresh and dry weight of roots, improved the root/shoot ratios, the number of leaves and flowers in treated plants.
Among the biostimulants, those plant-derived seem promising. Caruso et al. (2019) tested the response of "Piennolo del Vesuvio" D.O.P. tomato plants treated with a tropical plant extract and a legume-derived protein hydrolysate biostimulant. Biostimulants proved to be effective in increase total phenols and ascorbic acid as well as lycopene content, and lipophilic antioxidant activity compared to the non-treated plants.
Unfortunately, different species and different cultivar display various and sometimes contrasting effects, depending on genetic and environmental factors, and on the dose and time of application of the biostimulant (Kunicki et al. 2010).

2.3d Irrigation Management and Salinity
Many sites devoted to fruits and vegetables production around the Mediterranean Basin are located in zones where water quality and/or availability are often inadequate to meet the crops' demands, with subsequent relevant effects on vegetables quality (Rouphael et al., 2012). Several water-saving irrigation strategies (e.g., deficit irrigation-DI, regulated deficit irrigation-RDI or partial root-zone drying-PRD) have been proposed as potential tool to optimize water use efficiency, by subjecting crops to mild or controlled water stress with no/marginal effects on yield, while often acting as eustressors when product quality is concerned (Costa et al., 2007).
The application of DI strategy seems very useful for tree crops, apples, olives and grapevines (Costa et al., 2007). A strategy to control the vegetative growth and improve the fruit quality is represented by the regulated deficit irrigation

(RDI). This consists in removing or reducing the irrigation inputs during specific periods of the crop cycle (Karam et al., 2011). An increased concentrations of soluble sugars and higher colour intensity were reported for "Virosa" tomato fruits under RDI (the foliar water potential (Ψ) of –1.0 to –1.2 MPa was set as intervention thresholds to maintain a moderate level of water stress, –0.5 MPa was the control value) regimes by Pulupol et al. (1996), even if a yield loss was registered. Dorji et al. (2005) showed as hot pepper grown in greenhouse and subjected to RDI resulted in lower fruit load and in an increased soluble solids content (by about 20%), due to favoured carbon partitioning into fruits. Partial root-zone drying (PRD) consist in temporary keeping part of the root-zone in a dry soil and the rest of the root-zone well-watered (Sepaskhah and Ahmadi, 2012). It seems to be a valid strategy for several crops to save water and optimize or stabilize yield and improve fruit quality of vegetables (Santos et al., 2015; Dry and Loveys, 1999). The assumption behind this strategy is that a localized water stress in the root system should stimulate the plant, via the xylem, to increase water use efficiency (Dodd et al., 1996). As a result, a better fruit quality in terms of higher content of anthocyanins, phenols and glycosylglucose in grapevine fruits and a reduction in canopy density have been reported (Dry, 1996). However, vegetable crops are characterized by shallow root systems and tend to suffer even mild water stress conditions. This often leads to significant yield and quality losses, although some positive effects have been reported in tomato, where the reduced yield is compensated by an increase in quality and a lower water consumption (Costa et al., 2007; Zegde et al., 2006).

The irrigation water quality has profound effects on vegetables. Often the use of saline water has been associated

to reduced yields of vegetable crops, but also to improved fruit quality (Francois and Maas, 1994). The rise in rhizosphere salinity have been reported to improve fruit quality, by increasing the dry matter and total soluble solids contents in crops such as melon, watermelon, and zucchini squash (Colla et al., 2006a,b; Rouphael et al., 2006). Furthermore, a mild salt stress seems to activate antioxidant signals and boost the accumulation of health-promoting compounds, like carotenoids in tomato fruits (Gomez et al., 1999). The extent of the functional improvement depends on cultivar, growth conditions as well as on the specific phytonutrients considered. For instance, De Pascale et al. (2001) reported that salinity of the irrigation water between 4.0-4.4 mS cm^{-1} was associated to an increased total carotenoids and lycopene concentrations in tomato fruits, while an increased level up to 15.7 mS cm^{-1} led to an enhanced content of ascorbic acid. Water salinity seems to alter the mineral uptake by the plants, as reported for tomato plants, for which the increased NaCl concentrations were associated to reduced fruit accumulation in terms of P, K, Mg and Zn (Giuffrida et al., 2009; De Pascale et al., 2001). Finally, too high salinity threshold can result in a reduced Ca uptake by the plants, leading to higher incidence of physiological disorders (e.g. blossom end rot of fruits in tomato and pepper, or tip burn in leaves of lettuce) increasing the unmarketable fraction.

2.4 Postharvest Factors

The quality of fruits and vegetables is an evolving condition, starting from the harvest and ending up to final consumption. Our knowledge regarding the main physical, chemical and physiological parameters of quality has progressed considerably, also, due to advances in postharvest physiology

and technology (Kader, 2002). The preservation of quality in fresh horticultural products after the harvest is a result of proper harvesting, packaging and handling procedures along the supply chain, with the aim to prolong the shelf-life and reduce the product losses (Kader, 2008).

2.4a Storage Temperature

Storage temperature is one of most important determinants of fresh produce deterioration rate during postharvest. Indeed, it can extend the shelf-life and reduce the product loss, but it might also have repercussions on many qualitative aspects (Tsaniklidis et al., 2014). Low temperatures slow down the product metabolism and the activity of microorganisms responsible for quality deterioration (Ahmad and Siddiqui, 2015). Many fruits and vegetables, such as apples, pears, potatoes, oranges, tomatoes and chillies are stored at low temperature. However, their tolerance to low temperature during storage is cultivar specific and varies from one cultivar to another, so that improper cold storage (in terms of temperature threshold and storage duration) can lead to severe chilling injury (Ahmad and Siddiqui, 2015). For instance, apple cv. "McIntosh" is highly susceptible to chilling injury at 0 °C and requires storage at 2 - 4 °C (depending storage duration), while "Granny Smith" can be stored at 0°C (Little and Holmes 2000 ; Watkins 2002).

For climacteric and macrothermal crops, such as tomato, the management of storage temperature is directly related to respiration and metabolic activities (Arah et al., 2015). Specifically, chilling injury symptoms (premature softening, irregular colour development, surface pitting, browning of seeds, water-soaked lesions, off-flavour development, and increased postharvest decay) can occur in tomato fruits stored

at temperatures below 10-12°C (Raison and Lyons; 1986; Luengwilai et al., 2012).
The maintenance of a constant optimal temperature throughout the postharvest handling chain (from the field to the consumer) is one of the most difficult tasks and often a determinant factor of product quality. Nunes et al. (2001) simulated two different temperature regimes (semi-constant and fluctuating) for storing 'Opus' snap beans. Pods from the semi-constant temperature regime lost less weight, had better aspect in terms of colour, firmness and shriveling, and lower incidence of browning and bruising than those stored in fluctuating temperatures.

2.4b Postharvest Chemicals Application
Several chemical treatments (such as 1-MCP, polyamine, oxalic acid treatment, spermidine, calcium chloride, salicylic acid, methyl jasmonate and methyl salicylate, lecithin) can influence the postharvest metabolism of fruits, so their use to improve the postharvest shelf life have been studied (Arah et al., 2015; Valero et al., 2002; Opara et al., 2015).
For instance, it is well known the pivotal role of ethylene, a naturally occurring plant hormone, with manifold effects on fruit ripening, postharvest quality and life (Saltveit, 1999). The post-harvest reduction or suppression of ethylene action is readily applicable through scrubbing technologies and the use of ethylene inhibitors. For instance, 1-methylcyclo-propene allows to enhance, preserve and prolong quality and shelf-life inhibiting the ethylene production; on the other hand tailored applications of ethylene allow the control of postharvest ripening process in various vegetables (Blankenship and Dole, 2003; Watkins, 2006).
Polyamines are natural compounds involved in many growth and developmental processes and may play an important role

in postharvest storage and enhancing keeping quality of fruits. Polyamines have been described as anti-senescence agents, and in postharvest storage proved to increase firmness in strawberry (Ponappa, Scheerens, & Miller, 1993), tomato (Law, Davies, & Mutschler, 1991), and lemon (Martìnez-Romero et al., 1999).

Salicylic acid and methyl salicylate are compounds exhibiting a high potential in controlling postharvest losses of horticultural products (Asghari and Aghdam, 2010). These compounds could delay the ripening of fruits through inhibition of ethylene biosynthesis and could enhance resistance to pathogens in a large number of products (Asghari and Aghdam, 2010). Horticultural products are classified either as climacteric or non-climacteric on the basis of respiration and ethylene production rates after harvest, and on this depends the applicable strategy for increasing shelf life with different chemical compounds.

2.4c Modified Atmosphere Packaging (MAP) and Controlled Atmosphere (CA)

Controlled atmosphere (CA) and modified atmosphere packaging (MAP) are quite established means able to decrease the respiratory activity and increase the shelf-life in many fruits and vegetables. Optimal CA and MAP for fresh products vary according to the species, its ripening stage, the storage temperature, and the storage duration (Brecht et al., 2003). Both CA and MAP work by changing the normal composition of the storage atmosphere, thus altering product respiration, transpiration, ethylene production and sensitivity, relative humidity, diffusion of O_2 and CO_2 (Zagory and Kader, 1988). Specifically, CA is a system in which product shelf life is extended by altering the gaseous environment of storage area or package. MAP it can be distinguished in

active and passive one. Active modified atmosphere packaging is defined by FDA as "the displacement of gases in the package, which is then replaced by a desired mixture of gases" whereas passive modified atmosphere packaging as "when the product is packaged using a selected film type, and the desired atmosphere" (FDA, 2001).

MAP is referred to a gas composition that is initially modified, while CA is usually referred to a continuously controlled gas atmosphere (Zagory and Kader, 1988; Kader et al., 1989; FDA, 2001). Nunes et al. (1995) tested the strawberry cv. 'Chandler' in 5% O_2+/15% CO_2 and 10% O_2+/20% CO_2, stored for up to 2 weeks at 4 or 10 °C. Beyond the temperature affects, fruits stored in CA with 5% O_2+/15% CO_2 maintained a higher fresh weight, firmness, colour parameters (higher lightness, hue, and chroma), acidity and soluble solids content than those stored in 10% O_2+/20% CO_2. However, these techniques have not always shown adequate results in order to preserve the flavour profile, appearance and nutraceutical properties of vegetables, because of the stress induced by the altered atmospheric composition on metabolites biosynthetic pathways (Stern et al., 1994; Auerswald et al., 1999). As reported by Majidi et al. (2012) green-mature tomatoes stored under CA or MAP conditions (with an initial pressure of 5 kPa O_2 and 3kPa CO_2, respectively) resulted in an extended shelf-life compared to conventional cold storage used as control treatment. Controlled atmosphere proved to be the best solution in maintaining firmness and colour, whereas the maximum value of total soluble solids was observed in control fruits after 20 days of storage. Recently, cabbage stored under CA of 2% O_2 and 5% CO_2 proved to have a shelf-life up to three months, three time longer than in at room conditions. Nevertheless, isothiocyanate concentration decreased during

storage period, but more slowly under CA conditions, despite in a genotype-dependent manner (Osher et al., 2018).

Using active MAP technology to preserve product quality during postharvest storage has often shown good results. For instance, Bailén et al. (2007) kept tomato fruits cv. "Beef" for up to 28 days (8 °C, 90% RH) into selectively permeable plastic bags alone (control), or containing granular-activated carbon (GAC), either alone or in combination with palladium as ethylene absorber (GAC-Pd), to reduce the ethylene accumulation inside the packages. The addition of ethylene absorbers efficiently reduced the ethylene accumulation inside the MAP packages, so allowing a more convenient equilibrium between O_2 and CO_2 concentration, once the steady-state package atmosphere composition was reached. Consequently, some parameters related to fruit ripening such as changes in colour, softening, and weight loss evolved more slowly in tomatoes packaged with GAC or GAC-Pd, with a resulting lower product spoilage at the end of the storage period.

It must be pointed out that all postharvest handling techniques manage the "potential quality" of the product, this last depending on genotypic and agro-environmental factors during preharvest, and affect only the pace at which ripening, senescence and loss of quality occur (Crisosto and Mitchell, 2002; Weston and Barth, 1997). In the case of climacteric fruits, such as tomato, postharvest practices delay or inhibit the climacteric peak in respiratory activity, slowing down ripening and senescence, but potentially leading to altered appearance or flavour profile (Kader, 2008). In non-climateric products, such as apple, maximum quality is obtained at harvest, and postharvest technique are mainly aimed to manage the respiration and transpiration rates. For both physiological class of fruits, maturity at harvest

represents a crucial factor for postharvest quality. To define the optimal stage for harvest imposes a critical management decision because several quality traits of fruits and vegetables (e.g. flavour quality, sugars content, phytochemical composition, and so on) are often optimized by harvesting ripe products, whereas postharvest shelf-life ameliorates by harvesting not completely ripe products (Toivonen and Beveridge, 2005; Reid et al., 2002). Harvest maturity indices have to be crop- and cultivar-specific and should aspire to a balancement between preharvest ripening stage and postharvest performance (Reid et al.,2002).

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3. Quality characteristics of tomato for fresh consumption

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3.1 Introduction

Tomato (*Solanum lycopersicum* L.) is the second most economically important vegetable crops in the world after potato, being cultivated on a surface area of 4.8 Mha, generating an export value of 14.1 billion dollars, considering both raw and processed product (FAO, 2020). The crop is mainly grown in Asia (2.6 Mha), followed by Africa (1.3 Mha), Europe (0.4 Mha), Americas (0.4 Mha) and Oceania (0.01 Mha). The wide diffusion of the species flows from its adaptability to different growth conditions (both in open field and greenhouse), as well as from the versatility of the product, which is exploitable both for fresh consumption and industrial processing. Its climacteric, fleshy berries are a rich source of minerals (mainly K, P and Mg), vitamins (ascorbic acid, niacin, tocopherols and tocochromanols), carotenoids (lycopene, β-carotene and lutein) and polyphenols (chlorogenic acid, quercetin, naringenin), making tomato an ideal component of the modern diets (Wang and Seymour, 2017; Chaudhary et al., 2018). Given its economic and nutritional importance, since the 1980s the interest toward this crop has grown rapidly, so that tomato has become a model plant from a physiological and biotechnological viewpoint (Bertin and Génard., 2018). Over time, this has led breeders to permanently insert genes from wild *Solanum* species into domesticated tomatoes, in the perspective to improve crop yield, adaptability and disease resistances (Bay et al., 2007). Many qualitative traits of tomato berries such as colour, shape, firmness and shelf life have also been improved through the evolution of breeding techniques, with the aim to enhance the product attractiveness and consumer

satisfaction. Nonetheless, over the last decades consumers have started complaining about the poor flavour of modern tomatoes, so breeders, agronomists and food technologists have begun to pay more attention to this trait (Klee, 2010). The interest toward this topic is evidenced by the rapid increase in the number of Scopus® papers dealing with VOCs over the last 30 years, especially from the early 2000s. However, designing tomato quality with improved flavour is a difficult task, as it involves multiple traits mainly related to fruit taste and aroma (Baldwin et al., 2000). Fruit taste is related to sugars (glucose, fructose and sucrose) and organic acids (mainly citric and malic) concentration, while its aromatic profile involves numerous volatile organic compounds (VOCs), whose interaction with taste and texture results in flavour perception (i.e. and integrated perception of taste and retronasal olfaction) (Baldwin et al., 2008; Kyriacou et al., 2018). These intrinsic properties represent an intricate weave involving many polygenic systems (Carli et al., 2011), generating a physiological complexity making difficult to response to the emerging consumer demands. In the case of aroma, more than 400 volatile constituents have been identified in tomato, even if a relatively limited number (less than 10%) is actually reputed of major organoleptic importance (Petró-Turza, 1986; Klee and Tieman, 2013). This polygenic nature implies that, beyond the genetic background, external factors such as environmental conditions, crop management, ripening stage, postharvest handling, storage and processing can generate significant effects on tomato flavour (Causse et al., 2003; Farneti et al., 2015). Recently Tieman et al. (2017) have identified major alleles influencing some key volatiles contributing to consumer appreciation, so paving the way toward a new era in breeding for tomato quality traits. However, the

understanding about the determinism linking the influence of environmental and technical factors on tomato volatiles is still in its infancy, making still impossible a volatile-based product quality design.
For this reason, the purpose of this chapter is to examine the current knowledge on features which affect tomato fruits quality, focusing on the crucial role of aroma volatiles composition of tomato and health-promoting compounds, such as carotenoids and tocochromanols. An introduction on VOCs will be provided with reference to their chemical nature, biosynthetic pathways and their rising role in determining tomato quality for fresh consumption. Then, will follow a section on the importance of carotenoids and tocochromanols, with some references to their chemical nature, biosynthetic pathways, and their rising role in human health.

3.2 Tomato quality: evolution and emerging aspects

Since the second post-war period, tomato has undergone an intense phase of breeding, leading to a strong cultivar specialization, so actually a broad range of genotypes is available on the market, widely differing in fruit size, shape, pigmentation and average composition. The increase in yield, the improvement of fruit appearance and shelf life have been key-breeding steps in enhancing the crop profitability and product consumption on a global scale. Breeding activities oriented to an adaptation to processing industry have led to the development of specific varieties with high level of dry mater content, adapted to field cropping and mechanical harvesting (William and Stanley, 1992). Moreover, breeding for tolerance/resistance to abiotic and biotic stressors had a pivotal role in improving the sustainability of the crop and the

toxicological profile of the product. Nonetheless, such intense breeding activity nowadays is reputed to have contributed to consumers' dissatisfaction toward the lack of flavour in modern tomato cultivars (Krumbein et al., 2004). Improved traits such as higher yield potential and the concentration of fruit set over shorter periods have led to an increased fruit load, generating in turn a dilution effect of many chemicals responsible for flavour perception (Lahoz et al., 2016). On the other hand, the improvement of tomato shelf life, the practice of harvesting at earlier ripening stages and postharvest refrigeration, all necessary traits to bridge the spatial and temporal gap among production and consumption, nowadays are considered among the main responsible of making tomato fruits less flavourful (Maul et al., 2000; Mauro et al., 2020). The improvement of fruit appearance has played a role in worsening the aromatic profile of tomato too. Improved cultivars for uniform fruit ripening, lacking the trait "green shoulder" (u-mutants fruits), which are deemed more appealing by consumers, have brought with them fewer chloroplasts and, consequently, a lower level of carotenoids and soluble solids, so negatively contributing to the overall flavour (Powell et al., 2012). To the industry side of view, quality aspect was mainly focused on the ability of fruits to produce textured purees, as flavour and nutraceutical content were a less pregnant demand in a context where ingredients, correcting off- or low flavour, were used without consumer reproaches (William and Stanley, 1992).

On the other hand, the awareness of consumers on the importance of potential benefits of many fruits and vegetables are, more and more, driving the interest of research institutes and food industries to focus the knowledge on the quality of raw materials for fresh consumption and to design food products enriched with nutraceutical substances (Schreiner et

al., 2013). Improving flavour and nutraceutical content is currently one of most important challenges for prompting further tomato consumption on a global scale, going far beyond a merely hedonistic task. Indeed, more flavoursome and health-promoting vegetables are expected to influence in the future the consumers' eating habits, shifting away from less healthy snack food alternatives and reduce additives in processed foods, so having positive reflexes on the incidence of chronic, non-communicable diseases and public health expenditure (Klee, 2010).

3.3 VOCs in tomato: their role, classification and biosynthetic pathways

3.3.1 Contribution of VOCs to tomato flavour

Volatile organic compounds are non-nutritional constituents produced by fruits, which spread up in the air and affect the overall aroma and flavour of tomatoes (Kegge, et al., 2013). Decades of breeding and technical evolution of the crop have largely neglected these constituents, also because of objective difficulties in their quantification and in establishing their role in contributing to tomato eating quality (Klee and Tieman, 2013). Indeed, tomato volatiles are often present at picomolar or nanomolar concentrations, so that complex gas chromatography-mass spectrometry (GC-MS) equipment and procedures are needed for their quantification (Tieman et al., 2017; Wang et al., 2016). Among the over 400 volatile compounds found in tomato, differences of many orders of magnitude exist between their abundances, with ~30 of them showing an appreciable concentration. Thus, the most abundant compounds, such as (Z)-3-hexenal or hexanal, can reach several $\mu g\ g^{-1}$ of fresh weight (FW) while others, such as β-damascenone or β-ionone, are present in the order of ng g^{-1} FW or less (Buttery and Ling, 1993).

To overcome the difficulties in pyramiding the contribution of volatiles to tomato flavour, a widely accepted approach is based on the use of the odour thresholds and odour units (Buttery et al., 1987). The first variable refers, for a given compound, to the minimum concentration perceptible by the human nose, through its orthonasal olfaction (Guadagni et al., 1963). The second variable derives from the ratio among log of concentration of a compound and its corresponding odour threshold (Guadagni et al., 1966). Positive log odour units designate a significant contribution to tomato aroma (Wang et al., 2016). At least 16 tomato volatiles have positive odour units, including cis-3-hexenal, hexanal, 3-methylbutanal, trans-2-hexenal, trans-2-heptenal, 2-phenylacetaldehyde, β-ionone, 1-penten-3-one, β-damascenone, 6-methyl-5-hepten-2-one, cis-3-hexenol, 2-phenylethanol, 3-methylbutanol, 1-nitro-2-phenylethane, 2-isobutylthiazole, and methyl salicylate (Wang et al., 2016). Other volatiles with slightly negative odour units may contribute the background aromatic notes (Wang et al., 2016). However, this approach has some limits. First, it takes into account only the orthonasal perception (aroma) excluding the retronasal one, which is essential for flavour perception (Klee and Tieman, 2013). It has been observed that, for the same compound, different odour thresholds may correspond to these two specific perception channels (Rambla et al., 2014). Secondly, the odour threshold is estimated using a pure standard in water solution, instead of using tomato fruit samples (Bezman et al., 2003). To this end, it has been demonstrated that the volatiles emission is highly influenced by the characteristics of the matrix in which they are dissolved (Bezman et al., 2003). Moreover, the perception of VOCs does not derive from an additive effect, but rather from multiple interactions among different compounds, all contributing to the overall flavour

(Mauro et al., 2020; Rambla et al., 2014). The existence of significant interactions has been demonstrated even between volatile and non-volatile compounds in the fruits, particularly sugars and organic acids, that can alter the perception of a volatile compound (Baldwin et al., 2008; Tandon et al., 2003). On the other hand, is has been demonstrated the role of some aroma volatiles (e.g. the apocarotenoid volatiles) in enhancing the perception of tomato sweetness, regardless of the sugars concentration, so suggesting a different way in enhancing fruits taste perception (Vogel et al., 2010; Tieman et al., 2012).

3.3.2 Chemical classification and biosynthesis of VOCs

Tomato volatiles are mainly included among aldehydes, ketones, alcohols, nitrogen- and oxygen-containing compounds, esters, sulphur- and nitrogen-containing heterocyclic and nitrogen compounds (Wang et al., 2016). Due to this ample chemical heterogeneity, VOCs are commonly grouped also on the basis of their biochemical precursors (**Table 3.1**), as they mainly derive from the degradation of fatty acids, amino acids or carotenoids (**Figure 3.1**) (Causse et al., 2017).

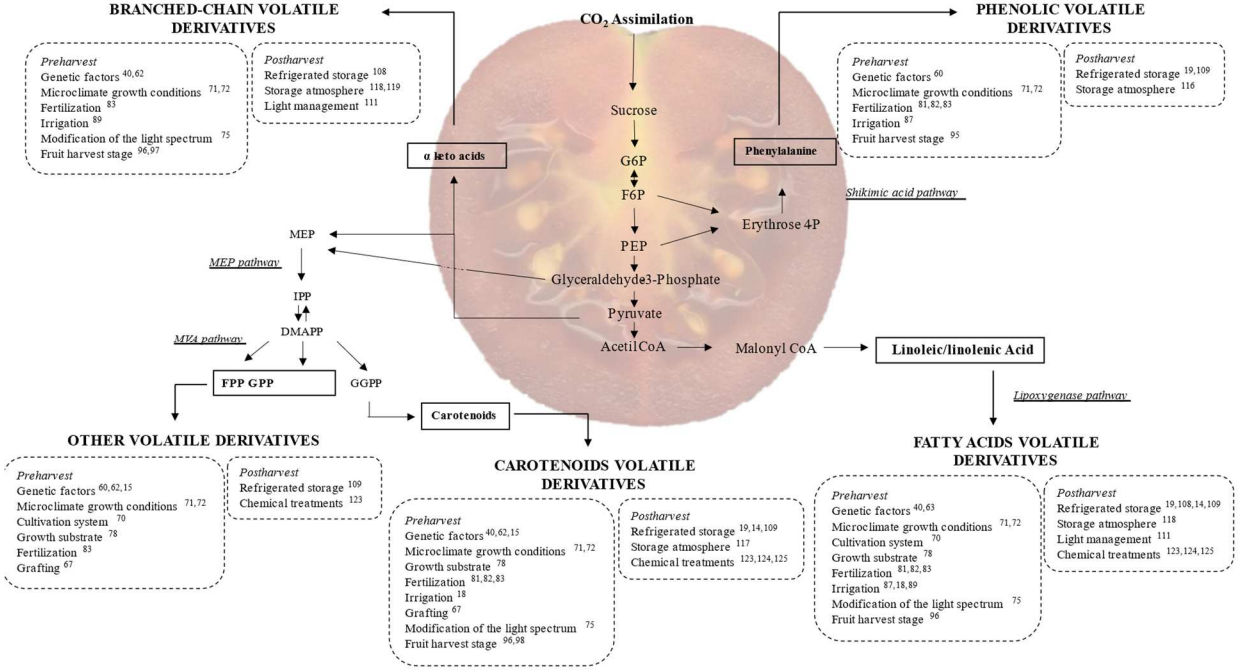


Figure 3.1. Metabolic pathways leading to the biosynthesis of main volatiles in tomato. Pathway names are grey italicized, main classes precursor compounds are in bold and boxed, and volatile classes are in bold and underlined. Abbreviations: Acetyl-CoA, acetyl coenzyme-A; DMAPP,

dimethylallyl diphosphate; Erythrose 4-P, erythrose 4-phosphate; F6P, fructose 6-phosphate; FPP, farnesyl diphosphate; G6P, glucose 6-phosphate; GGPP, geranylgeranyl diphosphate; GPP, geranyl diphosphate; IPP, isopentenyl diphosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; MVA pathway, mevalonate pathway; PEP, phosphoenolpyruvate.

Table 3.1. Main tomato volatiles along with their precursors, concentrations, odour thresholds in water, log odour units, and odour description (modified from Wang et al., 2016).

Volatile compounds	Classification	Average concentration (ng L^{-1})	Odour threshold (ng L^{-1})	Log odour unit	Descriptor
Fatty acids derivatives					
hexanol	Alcohol	7	5000	-1.9	Resin, flower, green
Z-3-hexenal	Aldehyde	12	0.25	3.7	Tomato, green
E-2-heptenal	Aldehyde	60	13	0.7	Green
1-penten-3-ol	Alcohol	110	400	-0.6	Sweet, fruity, grassy
pentanol	Alcohol	120	4000	-1.5	Balsamic
E-2-pentenal	Aldehyde	140	1500	-1	Strawberry, fruity, tomato
Z-3-hexenol	Alcohol	150	70	0.3	Green
E-2-hexenal	Aldehyde	270	17	1.2	Green
1-penten-3-one	Ketone	520	1	2.7	Fruity, floral, green
hexanal	Aldehyde	3100	4.5	2.8	Green, grassy
Caroteoids derivatives					
epoxy-β-ionone	Ketone	1	100	-2	Fruity, sweet, wood
pseudoionone	Ketone	1	800	-1.9	Balsamic
β-damascenone	Ketone	1	0.002	2.7	Fruity
neral	Aldehyde	2	30	-1.2	Lemon
β-cyclocitral	Aldehyde	3	5	-0.2	Mint
β-ionone	Ketone	4	0.007	2.8	Fruity, floral
geranial	Aldehyde	12	32	-0.4	Citrus
geranylacetone	Ketone	57	60	-0.02	Sweet, floral, estery
6-methyl-5-hepten-2-one	Ketone	130	50	0.4	Fruity, floral

Table 3.1 ***Cont.***

Volatile compounds	Classification	Average concentration (ng L^{-1})	Odour threshold (ng L^{-1})	Log odour unit	Descriptor
Amino acids derivatives					
3-methylbutanenitrile	N-compound	13	1000	-1.9	Pungent
2-phenylacetaldehyde	Aldehyde	15	4	0.6	Floral, alcohol
1-nitro-2-phenylethane	N-compound	17	2	0.9	Musty, earthy
3-methylbutanal	Aldehyde	27	0.2	2.1	Musty
2-isobutylthiazole	S- and N-compound	36	3.5	1	Tomato vine, green
methyl salicylate	Ester	48	40	0.008	Wintergreen
3-methylbutanol	Alcohol	380	250	0.2	Earthy, musty
2-phenylethanol	Alcohol	1900	1000	0.3	Nutty, fruity
Others					
linalool	Alcohol	2	6	-0.5	Citrus, fruity, sweet
1-nitro-3-methylbutane	N- and O-compound	59	150	-0.4	-

3.3.2.1 Fatty acids derivatives

The biosynthesis of fatty acid-derived volatiles takes place once the separation between these substrates and some enzyme is lost, due to the disruption of cellular tissues. The C18 linoleic (18:2) and linolenic (18:3) acid, through lipid degradation by the lipoxygenase (LOX) and hydroperoxide lyase (HPL) enzymes, produces the corresponding short chain C6 aldehydes such as hexanal, cis-3-hexenal, 1-hexanol and the C5 volatile 1-penten-3-one (Rambla et al., 2014).

Specifically, cis-3-hexenal might be isomerized to trans-2-hexenal either by enzymatic or non-enzymatic reactions. The aldehydes, such as hexanal and cis-3-hexenal, can be reduced into the corresponding alcohols by the enzyme alcohol dehydrogenase (ADH). Then, the alcohols can be metabolized into esters by the alcohol acetyl transferases (AAT) in wild tomato species, such as *Solanum pennellii* Correll, but are normally absent in cultivated tomatoes (Davidovich-Rikanati et al., 2009). The C6 volatiles are the most abundant in tomato fruits and are associated to the odour notes of tomato-like, grassy, and green. Other volatiles derived from fatty acids, such as 1-penten-3-one, 1-penten-3-ol, trans-2-pentenal, pentanal, and pentanol are synthesized through the involvement of isoform of tomato lipoxygenase in their biosynthetic pathway (Shen et al., 2014). These compounds give fruity notes and seem particularly appreciated by the consumers (Shen et al., 2014).

3.3.2.2 Amino acids derivatives

Among the volatiles involved in tomato flavour, many derive from amino acids, and can be classified in two groups: phenolic and branched-chain volatiles (Rambla et al., 2014). The synthesis of phenolic volatiles starts with the shikimic acid pathway, leading to the formation of their precursor phenylalanine. By means of aromatic amino acid decarboxylases (AADCs) phenylalanine is decarboxylated in phenylethylamine, which is then converted to 2-phenylacetonitrile, 1-nitro-2-phenylethane or 2-phenylacetaldehyde through a series of reactions still not fully characterized (Wang et al., 2016). Subsequently, the 2-phenylacetaldehyde is reduced in its respective alcohol, 2-

phenylethanol. This reaction is performed by enzymes belonging to the family of phenylacetaldehyde reductase (PARs), whose synthesis, such as the AADCs enzymes, is not constant during the maturation process (Tieman et al., 2007). 2-phenylethanol and 2-phenylacetaldehyde are important phenolic volatiles, conferring nutty and fruity notes to tomato fruits, while 1-nitro-2-phenethane is perceived as an earthy aroma (Wang et al., 2016).

Branched-chain amino acid derivatives are other important volatiles, but their exact biosynthetic pathway is still not well known. Nowadays it is supposed that their synthesis could begin from either an α-ketoacid and an amino acid (Klee, 2000). Valine, leucine and isoleucine are initially transaminated into their branched-chain α-ketoacids by the action of branched-chain amino acids aminotransferase (BCAT), a family of enzymes located in the chloroplasts, mitochondria and cytoplasm (Gonda et al., 2010). A series of volatiles is thus produced, such as 3-methylbutanal/ol, 2-methylbutanal/ol and 2-isobutyl-thiazole (Klee, 2000; Kochevenko et al., 2012; Rambla et al., 2014). These compounds release earthy, tomato vine, green and musty notes (Wang et al., 2016).

3.3.2.3 Carotenoids derivatives

A key class of tomato volatiles derives from the degradation of carotenoids (apocarotenoid volatiles) (Rambla et al., 2014), so their concentration usually increases during tomato ripening (Mauro et al., 2020). Although they are present in very low concentration, they have a primary role in conferring floral/fruity notes to tomatoes and increasing the product liking (Baldwin et al., 2000; Vogel et al., 2010). In

ripe tomatoes, the oxidative cleavage of multiple linear and cyclic carotenoids operated by the enzymes LeCCD1A and LeCCD1B lead to the biosynthesis of C14 dialdehyde and several C13 volatiles such as pseudoionone, β-ionone, 6-methyl-5-hepten-2-one, β-damascenone and geranylacetone, reported to be generated by oxidative cleavage of phytoene, phytofluene, ζ-carotene, and neurosporene (Simkin et al., 2004; Wang et al., 2016; Tieman et al., 2017). Other significant apocarotenoid volatiles seem to be two aldehyde isomers, geranial and neral ((Z)- and (E)- citral) having notes of citrus and lemon, contributing as background aroma even having negative odour unit value (Tandon et al., 2003). Anyway, several variables including precursors and their levels, enzymatic or non-enzymatic processes, and growing conditions influence the synthesis of carotenoids-derived volatiles (Lewinsohn et al., 2005).

3.3.2.4 Others

There are several chemical families and different biosynthetic pathways, many of which not identified, participating to the biosynthesis of tomato volatiles. Among these, a group of compounds seems to follow the phenylpropanoid biosynthetic pathway and have the (E)-cinnamic acid as a common precursor (Rambla et al., 2014). A not well-established biosynthetic pathway leads to the synthesis of eugenol, catechol and guaiacol, which are thought to confer clove-like and smoky aromas (Koeduka et al., 2006; Mageroy et al., 2012). In addition, methyl salicylate, produced by the methylation of salicylic acid, is one of the few volatiles derived from esters relevant for tomato aroma, following the phenylpropanoids catabolism. Its higher

presence has been found in unripe tomato fruits and, according to its anti-herbivores defence in plant tissues (James et al., 2004; Mauro et al., 2020), seems to be unwelcomed to consumers (Krumbein et al., 1998; Tieman et al., 2010).

Terpenoids generate a heterogeneous set of volatiles, specifically sesquiterpenoids (C15) and monoterpenoids (C10). These compounds are present within the green tissues of tomato plants but poorly present in ripe fruits, where their contribution to aroma seems secondary (Rambla et al., 2014). They derive from the isopentenyl diphosphate (IPP) and from its isomer dimethylallyl diphosphate (DMAPP) and can be synthetized following two alternative pathways. The first precursor pathway is localized in the cytosol, where the mevalonic acid pathway uses acetyl-CoA to produce IPP. The second precursor pathway takes place in plastids, where the methylerythritol phosphate pathway produces DMAPP and IPP from pyruvate and glyceraldehyde-3-phosphate. From these two molecules all the monoterpenoids and sesquiterpenoids are originated through successive synthetic steps (Nagegowda et al., 2010). The main mono- and sesquiterpenoids involved in tomato aroma are limonene, linalool, α-terpinerol and two aldehydes isomer geranial and neral (cis- and trans- citral), having notes of citrus and lemon (Wang et al., 2016). Alternatively, tomato volatiles can be produced by hydrolysis of glycosides. During ripening, volatiles such as phenylacetaldehyde or 3-mehylbutanal could be produced by hydrolysis of glycosides, previously oxidized in the corresponding alcohols by enzymatic way (Williams, 1993).

Another potential important volatile is 4-hydroxy-2,5-dimethyl-3 (2H) -furanone (HDMF), maybe derived from fructose-1,6-diphosphate (Roscher et al., 1998). Although its biosynthetic pathway is still not exactly known and is present at low concentrations, this compound seems to be promising for improving tomato aroma (Baldwin et al., 2000; Klee, 2010). HDMF is also produced during thermal processing, as a by-product of the Maillard reaction. Other compounds like 2-acetylfuran or 2-pentylfuran result from the same reactions, explaining why they are either not present or present as traces in fresh tomatoes (Buttery et al., 1990). Dimethyl sulphide, one of the major VOCs in tomato products, comes also from the heat-driven conversion of the free amino acid S-methionine (Williams et al., 1976).

3.4 Carotenoids in tomato: role, classification and biosynthetic pathways

3.4.1 Chemical structure, classification, and biosynthetic pathways of carotenoids

In plants more than 750 naturally occurring carotenoids have been identified (Britton et al., 1995, 2004), where display their crucial role in plant life, such as photoprotective functions during photosynthesis, providing substrates for biosynthesis of the plant growth regulator abscisic acid, ABA, and perhaps other hormones (Green and Durnford, 1996; Niyogi, 2000; Nambara and Marion-Poll, 2005; Auldridge et al., 2006). Tomato carotenoids content depends on the genetic material (cultivars), ripening stage, and both agronomic practices and environmental conditions during

cultivation (Martínez-Valverde et al., 2002; Dumas et al., 2003). Depending on the plant organ and species, the carotenoid profile shows both quantitative and qualitative differences. For instance, in green organs such as leaves or stems most plants show similar carotenoid profiles and no differences can be reported for zeaxanthin, violaxanthin, antherxanthin and lutein (Goodwin and Britton, 1988). On the contrary, in non-green tissues (e.g. fruits) carotenoids have a distinctive composition that depends on the plant species, with a colour range varying from yellow to orange or red. Tomato fruit accumulates large amounts of lycopene (Distefano et al., 2021); red pepper (*Capsicum annuum* L.) fruit contains mainly capsanthin and capsorubin (Hornero-Méndez et al., 2000); *Bixa orellana* L. is the only plant that accumulates bixin in its seeds, a dicarboxyl monomethyl ester apocarotenoid, also known as annatto, used as a red colour additive (Bouvier et al., 2003); carrots (*Daucus carota* L.) and sweet potatoes (*Ipomoea batatas* L.) accumulate mainly β-carotene and represent an exceptional root level storage site (Desobry et al., 1998; Teow et al., 2007); marigold flower (*Tagetes* spp.) accumulate principally lutein (Bhattacharyya et al., 2010).

In plants, carotenoid biosynthesis starts from a C_5 isoprene unit, the isopentenyl pyrophosphate (IPP), in the plastids, and it is there that the product accumulates (Cunningham and Gantt, 1998) (**Figure 3.2**). Four IPPs units are condensed to form the C_{20} compound geranylgeranyl pyrophosphate (GGPP) and with the condensation of two GGPP molecules by phytoene synthase (PSY) the first C_{40} carotenoid, i.e. phytoene, as a 15-*cis* isomer is synthetized. In tomato, two different types of PSYs (Psy-1 and Psy-2) are present. Psy-1

controls the carotenogenesis in chromoplasts and represents a fruit- and flower-specific isoform. In green tissues, Psy-1 is substituted by its homologous, Psy-2, which is the major contributor to carotenogenesis in chloroplasts (Fraser et al., 1999). Two structurally similar enzymes, phytoene desaturase (PDS) and ζ-carotene desaturase (ZDS) add conjugated double bonds. These desaturations reactions lead to the synthesis of phytofluene, ζ -carotene, neurosporene and lycopene, which differ in the number of conjugated double bonds, i.e. 5, 7, 9 and 11, respectively. The increase in the number of conjugated double bonds shifts light absorption towards longer wavelengths, passing from colourless phytoene and phytofluene, to a pale-yellow ζ -carotene, to orange-yellow neurosporene and to red lycopene. Among these desaturation steps, many reaction intermediates with a *cis*-configuration occur. In tomato the carotenoid isomerase (CRTISO) has been identified and is responsible for the conversion of all-*trans*-lycopene from *cis*- to *trans*-configuration (in the plant kingdom lycopene is naturally found in the *trans* isomeric form, the most thermodynamically stable but less bioavailable form) (Isaacson et al., 2002; Nguyen and Schwartz,1998; Gärtner et al., 1997). Upon exposure to high temperatures, light, catalysts, and/or active surfaces, seven of the double bonds of lycopene can isomerize to the less stable mono- or poly-*cis*-conformations (Shi et al., 2004).

In plants, all-*trans*-lycopene is the favoured substrate for the cyclases activity. The cyclization of lycopene is a crucial step in carotenoid metabolism and lead to carotenoids distinguished by different cyclic end groups: either the addition of beta (β-ring) and/or epsilon (ε-type ring). These

rings are generated by lycopene β-cyclase (LCYB) and lycopene ε-cyclase (LCYE), respectively (Cunningham et al., 1993; Cunningham et al., 1996; Pecker et al., 1996; Ronen et al., 1999). In most plants LCYE adds only one ε- ring to lycopene, therefore carotenoids pathway proceeds leading to carotenoids with one β- and one ε- ring (α-carotene and its derivatives, including its derivate lutein, 3,30-dihydroxy-α-carotene) or two β-rings (β-carotene and its derivatives) (Cunningham and Gantt, 2001; Cunningham et al., 1996; Goodwin, 1980). While β-LCY catalyses cyclization of both ends of lycopene, LCYE typically cyclizes only one end, forming the monocyclic δ-carotene (ε,ψ-carotene), however two ε-rings carotenoids are uncommon in most plants (Goodwin, 1980). α-Carotene and β-carotene are further hydroxylated to produce the oxygenated derivatives, xanthophylls (e.g. lutein and zeaxanthin), which are among the main carotenoid pigments in the photosystems of plants. β-hydroxylase (CHYB) catalyses two hydroxylation reactions, converting β-carotene to zeaxanthin via β-cryptoxanthin. Hydroxylation of the β- and ε-rings is catalysed by β-hydroxylase (CHYB) and ε-hydroxylase (CHYE), respectively.

Zeaxanthin epoxidase (ZEP) hydroxylates β-rings of zeaxanthin in two consecutive steps to form antheraxanthin and then violaxanthin. By neoxanthin synthase (NCED) violaxanthin is converted to neoxanthin, which represents the final step in the core carotenoid biosynthetic pathway (Nisar et al., 2015). Although most of the carotenoid pathway reactions are encoded by single genes, in tomato and *Arabidopsis* multiple carotenoid hydroxylase genes involved in xanthophyll biosynthesis have been identified. These

comprise two ferredoxin-dependent, non-heme β-ring hydroxylases, a P450-type ε-ring hydroxylase (CYP97C1) and a P450-type b-ring hydroxylase (CYP97A3) (Pogson et al., 1996; Sun et al., 1996; Tian and DellaPenna, 2001; Tian et al., 2004). A flower-specific CHYB (CrtR-b2) was identified in tomato (Galpaz et al., 2006). Considering the existence of flower- and fruit-specific PSY, GGPP and β-LCY (tomato expression database, http://ted.bti.cornell.edu/), it seems possible to support the hypothesis that there is a chromoplast-specific carotenoid biosynthesis pathway.

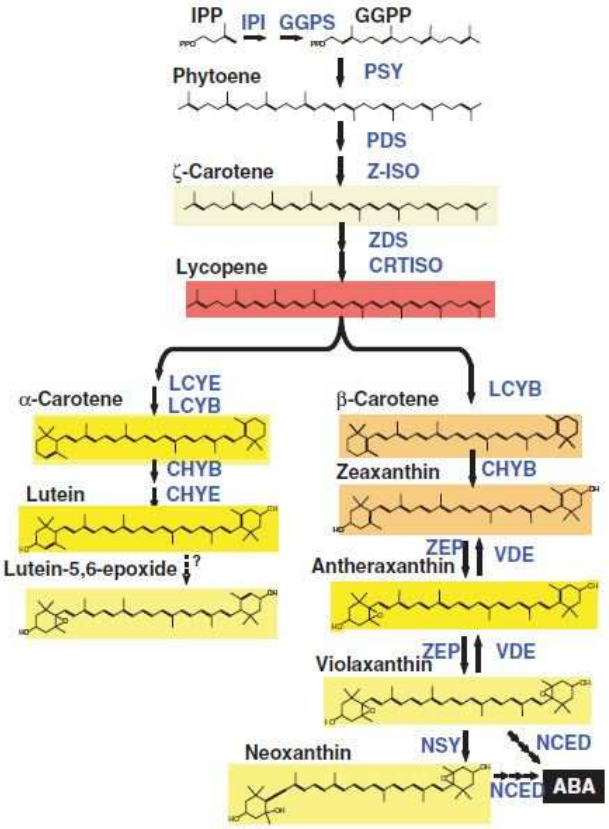


Figure 3.2. Carotenoid biosynthesis pathway in plants (only all-*trans*-configurations are shown). GGDP, geranylgeranyl diphosphate synthase; PSY, phytoene synthase; PDS, phytoene desaturase; ZDS, ζ-carotene desaturase; LCYB, lycopene β-cyclase; LCYE, lycopene ε-cyclase; CHYB, β-ring hydroxylase; CHYE, ε-ring hydroxylase; ZEP, zeaxanthin epoxidase; NSY, neoxanthin synthase; CRTISO, carotenoid isomerase. (Source: Tanaka et al., 2008 with some modifications).

3.4.2 Carotenoids in human nutrition

A large part of the beneficial effects of tomato consumption can be attributed to carotenoids (α-, β-, γ-, ζ- carotene, neurosporene, lutein, phytoene and phytofluene) (**Figure 3.3**). The two main carotenoids of tomatoes are lycopene, which is the most abundant carotenoid compound (representing ~80-90 %), and responsible of the red colour to the fruit, and β-carotene (which is ~7-10% of the total carotenoid content) and is characterized by an orange colour (Distefano et al., 2020). The human body is unable to synthesize lycopene and, therefore, it can only be taken through the diet. Over 80% of the lycopene present in the human body derives from the consumption of tomatoes or its derived products (sauces, gravies, concentrates, other) (Canene-Adams et al., 2005). Lycopene is the most abundant carotenoid in the human body (~0.5 μmol/liter plasma while the tissue levels vary from 1 nmol/g wet weight in adipose tissue to up to 20 nmol/g wet weight in adrenals and testes), followed by β-carotene, lutein and zeaxanthin (Stahl and Sies,1996).

Because of the presence of long-chain conjugated double bonds, carotenoids are well known to have antioxidative activity (Frusciante et al., 2007). Among tomato carotenoids, lycopene, with the presence of 13 conjugated double bonds, is reported to be the most efficient in quenching singlet oxygen *in vitro* (Boileau et AL., 1999) and deactivate an array of free radicals, such as hydrogen peroxide, nitrogen dioxide, thio- and sulphonyl radicals (Böhm and Bitsch,1999; Lu et al., 1995; Mortensen et al., 1997). The conjugated double bonds in lycopene are involved in quenching and scavenging mechanism also *in vivo,* thus lycopene is effective against

lipid peroxy-radicals and the highly destructive hydroxyl radical, responsible of many diseases (Burton and Ingold,1984; Mayne, 1996). Because of these beneficial effects, the consumption of large quantities of carotenoid-containing vegetables, such as tomato, is associated to a reduced the risk of cancer in the upper respiratory and digestive tracks, in lung and stomach (Block et al., 1992; Levy et al., 1995; Sies et al., 1992).

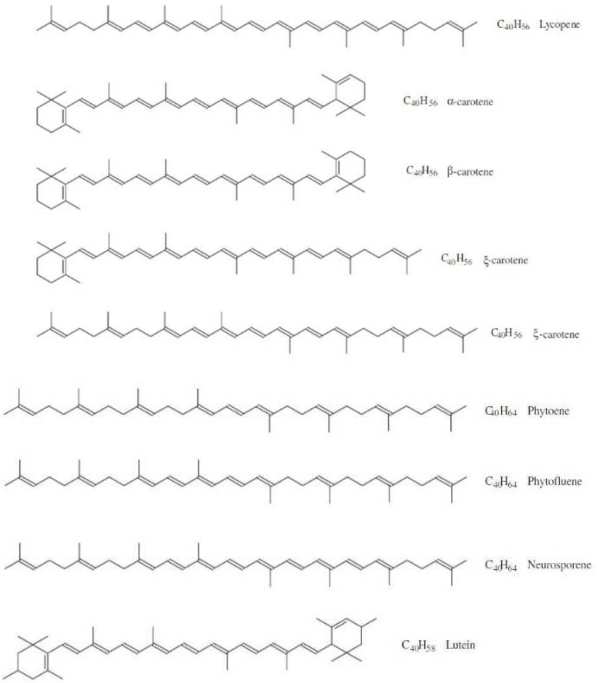


Figure 3.3 Molecular structures of carotenoid species in tomato fruits (Source Shi et al., 2004).

3.5 Tocochromanols in tomato: role, classification and biosynthetic pathways

3.5.1 Chemical structure, classification, and biosynthetic pathways of tocochromanols

Tocochromanols are solely synthesized by photosynthetic organisms, and their content, composition, and presence vary widely in different plant tissues. Both tocochromanols content and composition strongly change under conditions of plant oxidative stress including high light, salinity, drought, and low/high temperatures (Arango and Heise, 1998; Munné-Bosch and Alegre, 2002; Bergmüller et al., 2002; Maeda et al., 2006; Abbasi et al., 2007). Plant growth and development stage affect the levels of tocotrienols content and composition, which changes occurring during senescence, in correspondence of transformation of chloroplast to chromoplast, during fruit ripening or seed development (Arango and Heise, 1998; Falk et al., 2002; Abbasi et al., 2007; Falk and Munné-Bosch, 2010; Arrom and Munné-Bosch, 2010). Green tissues generally contain low levels of tocochromanols (<50 μ/gfw) and high incidence of α-tocopherol; on the contrary seeds contain 10–20 times higher levels of total tocochromanols, but α-tocopherol is most often a minor component (Shintani and DellaPenna, 1998; Grusak, and DellaPenna, 1999; DellaPenna and Last, 2006). From a physiological perspective, tocochromanols combine the polyunsaturated acyl groups and protect membrane lipids (especially polyunsaturated fatty acids) from the oxidative damages, by scavenging lipid peroxy-radicals and quenching or chemically reacting with $^1O_2$ and other reactive oxygen species (ROS) (Schneider, 2005).

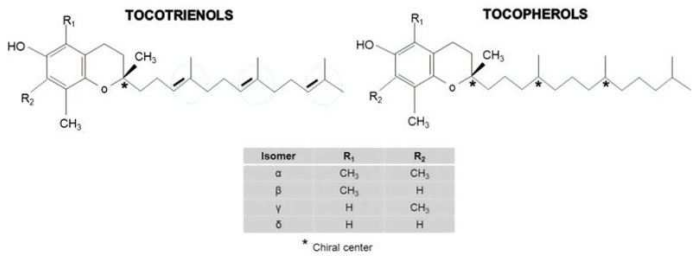
In plants, plastids contain sophisticated biochemical machinery producing a multitude of compounds that perform crucial functions. Plastid isoprenoid synthesis represents a major source of the two major groups of non-enzymatic lipid-soluble antioxidants in photosynthetic tissues, the tocochromanols and carotenoids. As for carotenoids, tocochromanols have isoprenoid precursors at the base of their synthetic pathway. The isopentenyl pyrophosphate (IPP) production is operated by two different pathways: cytosolic mevalonic acid pathway (MVA) and methylerythritol 4-phosphate (MEP) pathway. The latter, combining glyceraldehyde-3-phosphate and pyruvate lead to deoxy-D-xylulose 5-phosphate, and through subsequent reaction form IPP and then geranylgeranyl diphosphate (GGDP) (Lichtenthaler, 1999). Tocochromanols are amphipathic molecules, and the polar head group is derived from aromatic amino-acid metabolism, whereas the saturated tail is derived from phytyl-diphosphate (phytyl-DP) or (GGDP) for tocopherols and tocotrienols, respectively.

It is reported that a considerable proportion of tocopherols is synthesized from free phytol, suggesting that excess amounts of phytol released from chlorophyll breakdown during stress conditions or senescence might be use as substrate to form tocopherols in chloroplasts (Rise et al., 1989). Indeed, during stress events, such as high light, drought, salt or heat treatment, and during senescence, chlorophyll degradation and tocopherols accumulation are inversely correlated due to higher the activity of chlorophyllases and the porphyrin ring system activities (Collakova and DellaPenna 2003; Hörtensteiner 2006). This hypothesis was also corroborated by results obtained after isolation of the tocopherol-deficient

VTE5 mutant of *Arabidopsis*, which encodes an enzyme with phytol kinase activity (Valentin et al. 2006)

The α-, β-, γ- and δ forms differ for the number and position of methyl substituents on the aromatic ring (**Figure 3.4**). To have the headgroup synthesis, p-hydroxyphenylpyruvate (HPP) is converted into homogentisate (HGA) by the enzyme HPP dioxygenase HPPD). Successively, phytyl-PP or GGDP are condensed with HGA by homogentisate phytyl transferases (VTE2) to produce 2-methyl-6-phytylquinol (MPBQ) and 2-methyl-6-geranylgeranylbenzooquinol (MGGBQ), intermediates in tocopherol and tocotrienol synthesis, respectively.

The vitamin E biosynthesis pathway from the reduction of hydroxyphenylpyruvate to homogentisate is known as the "vitamin E core pathway". α-, β-, δ-, and γ- tocopherols are products of the reactions catalysed by 2-methyl-6-phytyl-1,4-benzoquinol methyltransferase (VTE3), tocopherol cyclase (VTE1) and γ-tocopherol methyltransferase (VTE4) that converts δ- and γ-tocopherols (and tocotrienols) to β- and α-tocopherols (and tocotrienols), respectively (Li et al., 2008; Quadrana et al., 2013; Caspi 2014) (**Figure 3.5a and 3.5b**)



Isomer	R_1	R_2
α	CH_3	CH_3
β	CH_3	H
γ	H	CH_3
δ	H	H

* Chiral center

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Figure 3.4 Tocotrienols and tocopherols and relative substituents that define their isoforms. Tocotrienols have a

characteristic farnesylated tail, which may provide superior anticancer properties as compared to tocopherols (Wong and Radhakrishnan, 2012).

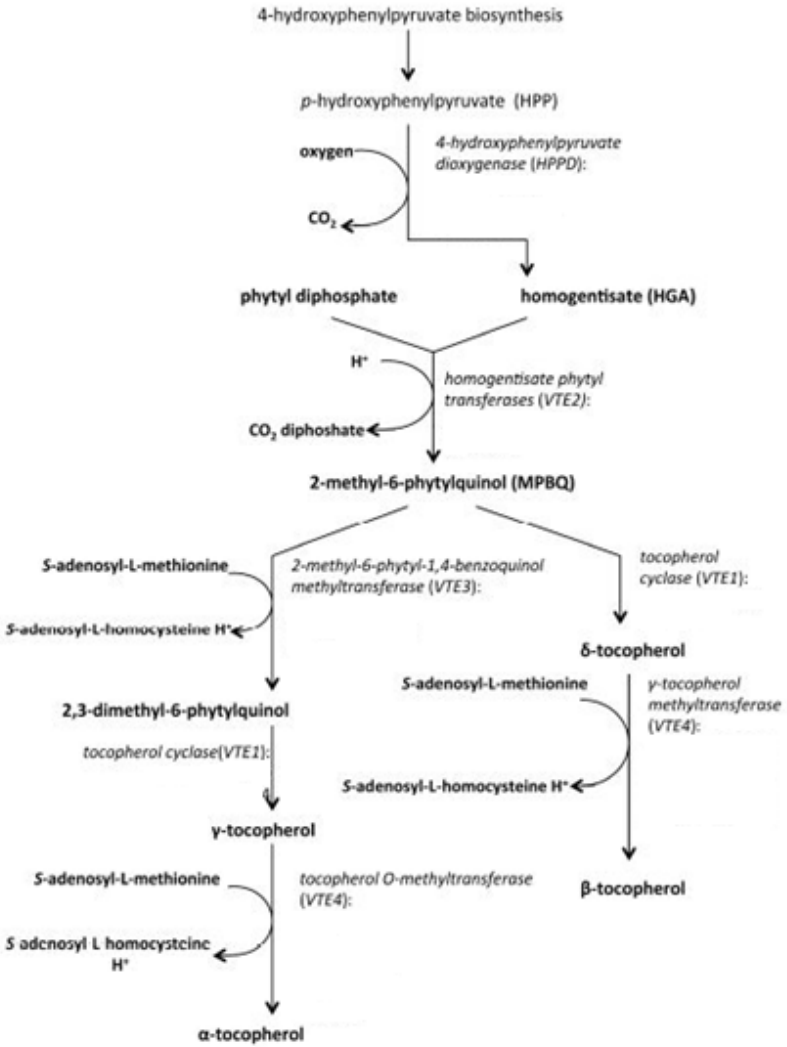

Figure 3.5a. The tocopherols biosynthetic pathway in *Solanum lycopersicum* as reported by Caspi et al., 2014 (modified).

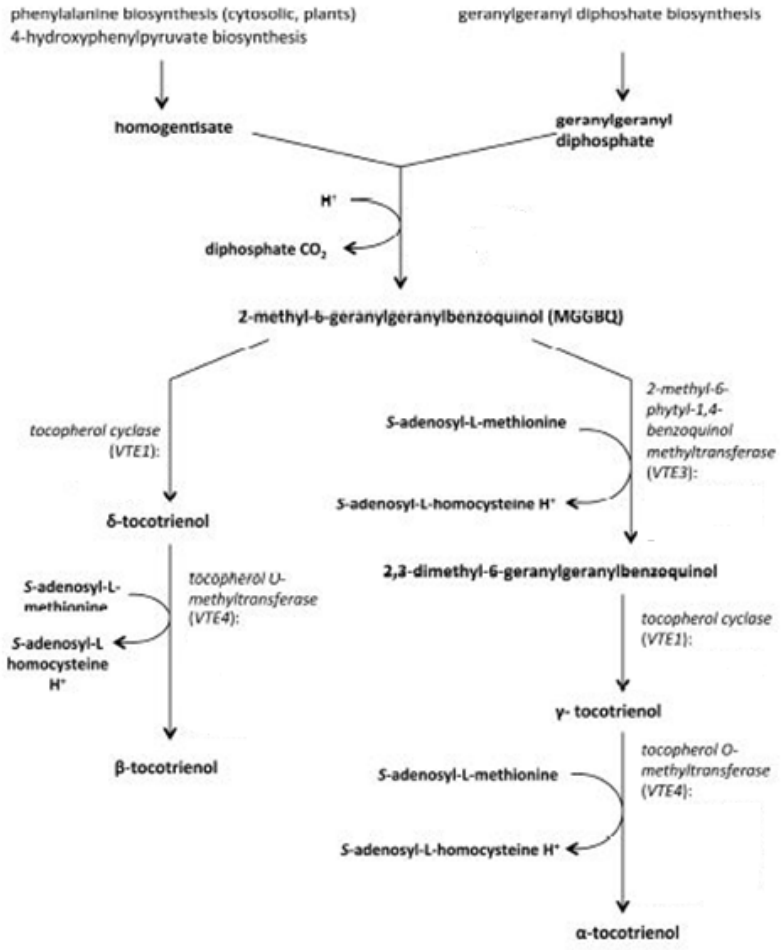

Figure 3.5b. The tocotrienols biosynthetic pathway in *Solanum lycopersicum* as reported by Caspi et al., 2014, (modified).

3.5.2 Tocochromanols role in human nutrition

Tocopherols derive for greek "tocos", birth, and "phorein", to bear and it was named for its role in maintaining rats fertility (Evans et al. 1936). Numerous researches focused on the health benefits of these compounds since the discovery of vitamin E in 1922 and nowadays, vitamin E represent an essential active nutrient in the diet of all mammals. The tocochromanols, globally know as vitamin E, consists of four tocopherols (α, β, δ, and γ), and four tocotrienols (α, β, δ, and γ). The activity of this group of lipid soluble antioxidants involves scavenging peroxyl radicals and quenching reactive oxygen species (ROS) (Lichtenthaler, 1999). Tocotrienols represent a rising important group of the vitamin E family, however, most of the vitamin E research has focused on α-tocopherols, and only 1% of vitamin E studies have investigated tocotrienols (Sen et al., 2007). Several studies stated that tocotrienols may have more powerful antioxidation and anticancer effects than tocopherols (Serbinova et al., 1991; Constantinou et al., 2008; Wada et al., 2009). On the other hand, it must be considered that tocopherols and tocotrienols are differently widespread in plant organs and considerably less widespread in plant kingdom (Horvath et al., 2006).Vitamin E can be found in high concentrations in nuts, seeds (as tomato seeds), grains or vegetable oils such as almond, safflower, canola oil, in other high-fat sources, fruits, roots, and tubers and in the green parts of the higher plants (DellaPenna, 2005; Horvath et al., 2006; Mène-Saffrané and DellaPenna, 2010). The most abundant form in leaves and most biologically active form of vitamin E is α-tocopherol, despite the dominating tocopherol form in seeds is γ-tocopherol (DellaPenna and Last, 2006;

Grusak, and DellaPenna, 1999; Shintani and DellaPenna, 1998).

According to the German Society for Nutrition (Deutsche Gesellschaft für Ernährung e.V.), the recommended daily amount of vitamin E for an adult is 14 mg/day. α-Tocopherol and γ-tocopherol are the most abundant forms and can easily found in soybean, rapeseed, corn oil, nuts fruits, roots. Among the naturally occurring α-forms, the stereoisomers RRR-α-tocopherols have the highest biological activity, and they can be stored and transported in the body due to specific selection by the hepatic α-tocopherol transfer protein (α-TTP) (Brigelius-Flohé and Traber, 1999; Dietrich et al., 2006). Despite the nutritional relevance, several studies highlighted as the recommended daily intake is often not satisfied, so recently improving vitamin E quantity and composition in crops has become a target in breeding programmes (Péter et al., 2016).

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4. Research Project and Thesis Aim

Due to its economic importance, nutritional and nutraceutical properties, tomato is among the most widely grown fruit vegetables all over the world (FAO, 2020). It is also well known for its crucial role in the human diet as a primary source of antioxidant, with ascorbic acid, lycopene, and β-carotene being among the most effective in protecting from several degenerative diseases (Bertin and Génard, 2018).
Recently flavour aspects and health-promoting compounds of fresh horticultural products are becoming important for consumers, and for tomato these characteristics nowadays play a pivotal role in influencing consumers' preferences (Wang et al., 2017; Chaudhary et al., 2018). However, over the last decades consumers have started complaining about the poor flavour and nutraceutical properties of modern tomatoes.
The aim of this study is to enhance the knowledge and application of some preharvest and postharvest techniques in order to improve tomato quality for fresh consumption. We focused on some part of the production features that, up to our knowledge, were still partially studied and affect the product quality and nutraceutical profile.

Chapter 5 focuses on composition and sensorial properties of tomato harvested at different ripening stages of an elongated (S. Marzano type) tomato cultivar for fresh consumption. Vegetable grafting is also part of the research, as a tool to improve the flavour profile in tomato fruits of plants grown onto three common rootstocks in Mediterranean greenhouse cultivation.

Chapter 6 describes how the application of a commercial, plant-based biostimulant, may affect the harvest quality of tree different cherry tomato cultivars during an off-season cultivation cycle. The attributes under investigation were yield, carpometric traits and bioactive compounds, as affected, also, by the cluster position (i.e., harvest time).

Chapter 7 aims to investigate how 3 different cherry tomato cultivars grown in a Mediterranean environment evolve their quality and nutritional profile during the postharvest storage as consequence of different thermal regimes (10 and 20 °C) and storage time (up to 14 days).

Chapter 8 is the general discussion of the results described in chapter 5-7. Physiological perspective and technical findings of this study are debated.

5. Influence of harvest stage and rootstock genotype on compositional and sensory profile of the elongated tomato cv. ‘Sir Elyan’

The following work has been already published as:

Mauro, R. P., Rizzo, V., Leonardi, C., Mazzaglia, A., Muratore, G., Distefano, M., Sabatino, L., Giuffrida, F. (2020). Influence of harvest stage and rootstock genotype on compositional and sensory profile of the elongated tomato cv.“Sir Elyan”. Agriculture, 10 (3), 82.

5.1. Introduction

In the Mediterranean Basin, tomato (*Solanum lycopersicum* L.) represents a resource of primary economic and dietary importance (Mauro et al., 2015). Where quality is concerned, organoleptic traits and health-promoting compounds of fresh vegetables are becoming increasingly important (Sabatino et al., 2019; Mauro et al., 2020), and for tomato these characteristics nowadays play a pivotal role in influencing consumers' preferences. From a nutraceutical viewpoint, tomato is a primary source of antioxidant in the human diet, with ascorbic acid, lycopene, and β-carotene being among the most effective in protecting human health from several degenerative diseases (Raiola et al., 2014).

Tomato flavor results from the interaction among taste (deriving from sugars and organic acids) and aroma (Tieman et al., 2017). Tomato aroma flows from over 400 volatile organic compounds (VOCs), although only few of them are reputed of primary importance (Wang et al., 2016; Tieman et al., 2017). It has been demonstrated that these traits involve the developmentally-regulated expression of many polygenic systems, making them strongly influenced by environmental and agronomic factors (Wang et al., 2016). Nowadays, greenhouse tomato cultivations are strongly dependent on the adoption of grafting, in order to increase their yield and resistance to biotic and/or abiotic stressors (Rouphael et al., 2018; Allevato et al., 2019; Mauro et al., 2020). It has been reported that tomato quality variables such as acidity, sugar, flavor, aroma, color, carotenoid content, and texture can be differently influenced by the rootstock-scion combinations, also because of their interaction with external factors such as climate conditions and cultural practices (Kyriacou et al.,

2017). Tomato fruits are harvested at different ripening stages, according to its destination. Fruit destined for the fresh market can be harvested from the mature-green to fully ripe stage (Wang et al., 2017), according to the consumers' demand and fruit typology. In the paste decades, breeding has prioritized yield, shelf-life, and diseases resistance, which may have contributed to compromise flavor characteristics in fresh market tomatoes. However, the lack of flavor of retail tomatoes is partly due also to harvesting fruits before their full ripening is achieved. In fact, the concentration of individual volatiles in tomato fruits depends by the ripening stage at harvest, and fruit harvested before full ripening usually do not produce the characteristic volatiles associated to high quality tomatoes (Wang et al., 2017). However, the threshold concentrations detected by humans for various aroma compounds range over many orders of magnitude. Sensory observations could be used to confirm the contribution of these compounds to odor and aroma (Saltveit, 2005). The elongated-type tomato, that represents a significant commercial niche in Italy, could be consumed at breaker or turning stage. In a survey in Italy, the first ripening stages are preferred by the 20% of fresh tomato consumers (Tirelli, 2010).

The objective of this work was to investigate the composition and sensorial properties of tomato harvested at breaker and turning stages of an elongated tomato cultivar, grafted onto three common rootstocks in Mediterranean greenhouse cultivation.

5.3Materials and Methods

5.2.1. Experimental Materials and Growth Conditions

The experiment was conducted in a 2400 m^2 greenhouse located in Southwest Sicily (36°50' N, 14°28' E; 18 m a.s.l.). Tomato plants cv. "Sir Elyan" F_1, belonging to the medium-sized, elongated type, were grafted onto 3 rootstocks, characterized by different ability to imprint vegetative vigor to the scion: "He-Man" F_1 (low-vigor) "Interpro" F_1 (medium-vigor), and "Armstrong" F_1 (high-vigor). Transplanting was effected at the end of January adopting the following distances: 1.20 m (between double rows), 0.80 m (between paired rows) and 0.70 m (within single rows) (1.43 plant m^{-2}, 2 stems $plant^{-1}$). A typical fertilization program was applied, whereas drip irrigation was provided when accumulated daily evaporation reached 25 mm. The crop was grown up to the half of July. A two-way randomized blocks design with four replications was adopted, using 6.8 × 3.6 m experimental plots, each containing 16 plants (net of borders).

5.2.2. Carpometric Determinations

On 10 May, 32 commercial tomato fruits from the third trusses per replicate were hand-harvested at 2 different ripening stages. These were breaker stage (16 fruits per replicate), i.e., when the berries started turning to red by their stylar end (rank 3 of the OECD Tomato Colour Gauge, hereafter S1); turning stage (16 fruits per replicate), i.e., when colour change regarded ~30 of the esocarp (rank 4 of OECD Tomato Colour Gauge, hereafter S2). Soon after harvest, fruits were transported in the laboratory and processed for further analysis. Fruits fresh weight was determined, whereas the fruit shape index was calculated as the ratio among longitudinal and transversal diameters. Fruit firmness was determined through a Digital Texture Analyser mod. TA-

XT2 (Stable Micro Systems, Godalming, UK) and defined as the force (N) needed to impress a 2 mm fruit deformation along its equatorial axis. Subsamples of collected fruits were kept in a thermo-ventilated oven at 70 °C (Binder, Milan, Italy) until constant weight was reached, in order to determine their dry matter content.

5.2.3. Fruit Quality Determinations

Subsamples of harvested fruit were washed with demineralized water, dried with paper and blended with a domestic food processor at room temperature. The resulting puree was centrifuged and an aliquot of the supernatant was used to determine the soluble solids content (SSC) by using a digital refractometer DBX-55A (Atago Co., Ltd., Tokyo, Japan) provided with an automatic temperature compensation system. Titratable acidity (TA) was determined using 10 g aliquots of tomato fruits poured in 50 mL of distilled water and titrated with 0.1N NaOH to an end-point of pH 8.1. TA was expressed as g L^{-1} citric acid (CA). The SSC/TA ratio was also calculated. Lycopene and β-carotene were extracted using the method described by Sharma and Le Maguer (1996) and quantified by HPLC (equipped with a C30 Acclaim column) according to Gregory et al. (1987) and Subagio et al. (1996). Ascorbic acid was extracted and quantified by HPLC (with an Ultra AQ C18 column) according to Nisperos-Carriedo et al. (1992). The antioxidant activity was determined using the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay, according to Brand-Williams et al. (2007).

5.2.4. Volatile Extraction and Analysis

Soon after harvest, fresh tomato sample were chopped and mixed. The aroma compounds identification was performed using SPME coupled with GC/MS. The fiber was chosen according to Beltran et al. (2006), i.e., a 75 μm Carboxen/PDMS (CAR/PDMS) fiber (Supelco, Bellefonte, PA, USA). Before use, the fiber was preconditioned in the GC injection port at 300 °C for 1 h, then exposed for 1 h to the headspace of a 25 mL septum-sealed glass vial containing 20 g aliquot of homogenized fresh tomato. Each vial was previously immersed in a bath water at 60 °C for 15 min. GC–MS analyses were performed using an Agilent (Palo Alto, CA, USA) 6890 N GC equipped with a 30 cm length, 0.20 mm i.d., 0.20 μm film thickness, fused silica capillary column (SUPELCOWAX™ 10, Supelco). During the analysis, the GC injection port temperature was 250 °C, with a split ratio of 5:1. Helium was used as the carrier gas at a flow rate of 1.1 mL min^{-1}. The column temperature was held at 40 °C for 5 min, then programmed to increase by 5 °C min^{-1} to 220 °C, which was held for 10 min. Mass spectrometry conditions were as follows: ion source, 230 °C; electron energy, 70 eV; multiplier voltage, 1247 V; GC/MS interface zone, 280 °C; and a scan range of 35–350 mass units. Duplicate analyses were performed for each sample. Identification of the compounds was carried out by comparison of the analytes fragmentation patterns with the spectra libraries (NIST 98, US).

5.2.5. Sensory Analysis

The UNI EN ISO 13299:2016 (2016) sensory profile method was used to measure any difference in sensory characteristics of tomatoes. Twelve trained (ISO 8586:2012) (2012)

panelists (six females and six males, 28–40 years old) with a broad expertise in vegetables were trained in 3 sessions, using both commercial and experimental samples to familiarize with scales and procedures. The panelists, using a discontinuous scale between 1 (absence of sensation) and 9 (extremely intense), evaluated the intensity of the sixteen attributes selected on the basis of frequency (≥60%): 1 for appearance (freshness); 1 for tactile hand feel (firmness); 3 for odour (herbaceous, tomato and off odours), 3 for flavour (herbaceous, tomato and off-flavours); 4 for taste (salt, sour, sweet and bitter); 4 for rheological properties (crunchy, juicy, mealy, peel thick) (**Table 5.1**). The evaluation sessions were conducted in the sensory laboratory (UNI EN ISO 8589:2014) (2014) of Di3A (University of Catania) from 11:00 a.m. to 12:00 a.m. in individual booths illuminated with a white light. Tomato samples were served on plates, coded with three-digit numbers and water was provided to panellists for rinsing between samples. The order presentation was randomized among panellists and sessions. All data were acquired by a direct computerized registration system (FIZZ Byosistemes. ver. 2.00 M, Couternon, France).

5.2.6. Statistical Procedures

Collected and calculated data attributable to ratio scales were firstly subjected to Shapiro–Wilk and Levene's test, in order to check for normal distribution and homoscedasticity, respectively, then to a factorial "rootstock × ripening stage" (R×S) analysis of variance (ANOVA), according to the experimental layout adopted in the greenhouse. Percentage data were Bliss' transformed before the ANOVA (untransformed data are reported and discussed), whereas

multiple mean comparisons were performed through Fisher's protected LSD test (p=0.05). Sensory data were subjected to a two-way non-parametric ANOVA using Friedman's test followed by the calculation of Kendall's coefficient of concordance, in order to check the independence of observations. Means separation was performed in all pairwise comparisons by using the Mann-Whitney's U-test, with an associated P-level calculated according to the Bonferroni's correction. A correlation analysis was also performed, in order to define possible relationships among volatiles concentration and sensory scores. All calculations were performed using Excel version 2016 (Microsoft Corporation, Redmond, WA, USA) and Minitab version16.1.1 (Minitab Inc., State College, PA, USA)

Table 5.1. List of evaluated sensory attributes and their definitions.

Attribute	Description
Freshness	Degree of freshness of the product by visual estimation
Firmness	Strength required to compress a food between the moles
Tomato odour	Characteristics odour of tomato perceived with the sense of smell
Herbaceous odour	Characteristics odour of herbaceous perceived with the sense of smell
Off-odour	Unpleasant odour not characteristic of the product concerned, perceived through the sense of smell
Salt	One of the four basic tastes caused by aqueous solutions of salt compounds perceived on the tongue
Sour	One of the four basic tastes caused by aqueous solutions of acid compounds perceived on the tongue
Sweet	One of the four basic tastes caused by aqueous solutions of sweet compounds perceived on the tongue
Bitter	One of the four basic tastes caused by aqueous solutions of bitter compounds perceived on the tongue
Crunchy	The sensation of muffled grinding of a foodstuff
Juicy	The amount of liquid released from the samples during first and second chew
Mealy	The amount of small particles perceived in the mouth when biting the sample
Peel thick	Resistance of the epicarp to removal
Tomato flavour	Characteristic flavour of tomato perceived by the sense of smell and mouth with the swallowing
Herbaceous flavour	Characteristic flavour of herbaceous perceived by the sense of smell and mouth with the swallowing
Off-flavour	Unpleasant flavour not characteristic of the product concerned, perceived by the sense of smell and mouth with the swallowing

5.3. Results

5.3.1. Carpometric Traits

Average fruit weight and fruit shape index were both affected by R×S interaction. Passing from S1to S2, the former variable significantly increased only in "Sir Elyan" grafted onto "Interpro" (+13.6%),whereas shape index decreased only in "Sir Elyan" grafted onto "Armstrong" (−5.3%) (**Table 5.2**). Fruit dry matter showed a similar trend in all the grafting combinations, decreasing from 7.4 (S1) to 7.0% (S2), whereas fruit firmness proved to be higher in "Sir Elyan" grafted onto "He-Man" than onto the other rootstocks, and in S1 than S2 stage (**Table 5.2**).

Variable	Ripening Stage	Rootstock			Ripening Stage Mean	$LSD_{interaction}$ (p = 0.05)
		"He-Man"	"Interpro"	"Armstrong"		
Average fruit weight (g)	S_1	88.1 ± 1.7	79.5 ± 2.3	78.7 ± 1.9	**82.1 ± 3.0 a**	7.0
	S_2	83.7 ± 2.1	90.3 ± 1.9	82.3 ± 1.9	**85.4 ± 2.8 a**	
	Rootstock mean	**85.9 ± 2.4 a**	**84.9 ± 3.6 a**	**80.5 ± 2.0 a**		
Shape index (adimensional)	S_1	1.67 ± 0.02	1.66 ± 0.02	1.70 ± 0.02	**1.68 ± 0.02 a**	0.06
	S_2	1.65 ± 0.01	1.68 ± 0.01	1.61 ± 0.01	**1.65 ± 0.02 a**	
	Rootstock mean	**1.66 ± 0.02 a**	**1.67 ± 0.02 a**	**1.66 ± 0.03 a**		
Fruit dry matter (%)	S_1	7.3 ± 0.1	7.5 ± 0.2	7.3 ± 0.1	**7.4 ± 0.2 a**	NS
	S_2	7.2 ± 0.1	6.9 ± 0.1	6.9 ± 0.1	**7.0 ± 0.1 b**	
	Rootstock mean	**7.2 ± 0.1 a**	**7.2 ± 0.2 a**	**7.1 ± 0.2 a**		
Fruit firmness (N)	S_1	13.91 ± 0.35	12.74 ± 0.47	11.81 ± 0.42	**12.82 ± 0.70 a**	NS
	S_2	13.64 ± 0.28	12.23 ± 0.46	11.77 ± 0.31	**12.55 ± 0.56 b**	
	Rootstock mean	**13.78 ± 0.32 a**	**12.49 ± 0.49 b**	**11.79 ± 0.47 b**		

Table 5.2. Carpometric traits of tomatoes "Sir Elyan" as affected by rootstock and ripening stage(mean ± standard error). Different letters among factor means (bold numbers) indicate significance at Fisher's LSD test (p=0.05). NS: not significant.

5.3.2 Taste variables

Soluble solid content (SSC) proved to be significantly higher in S2 than in S1fruits, and when "Sir Elyan" was grafted onto "Interpro" (**Table 5.3**). Differently, a significant R×S interaction was recorded for titratable acidity (TA) since,

passing from S1 to S2, it significantly decreased in "Sir Elyan" grafted onto "Interpro" and "Armstrong" (−19.7% and−12.8%, respectively) and did not change using "He-Man" as rootstock (**Table 5.3**). Both main factors significantly affected the SSC/TA ratio, as in S2 fruits it was 18.7% higher than the S1 ones, showing also a higher value in "Sir Elyan" grafted onto "He-Man" (**Table 5.3**).

Variable	Ripening Stage	Rootstock			Ripening Stage Mean	$LSD_{interaction}$ ($p = 0.05$)
		"He-Man"	"Interpro"	"Armstrong"		
SSC (°Brix)	S_1	5.66 ± 0.09	6.06 ± 0.07	5.80 ± 0.05	**5.84 ± 0.19 b**	NS
	S_2	6.04 ± 0.08	6.32 ± 0.10	5.84 ± 0.04	**6.07 ± 0.13 a**	
	Rootstock mean	**5.85 ± 0.15 b**	**6.19 ± 0.16 a**	**5.82 ± 0.03 b**		
TA (g CA L^{-1})	S_1	2.88 ± 0.08	3.61 ± 0.05	3.20 ± 0.07	**3.23 ± 0.18 a**	0.30
	S_2	2.73 ± 0.11	2.90 ± 0.09	2.79 ± 0.05	**2.81 ± 0.10 b**	
	Rootstock mean	**2.81 ± 0.10 c**	**3.25 ± 0.22 a**	**3.00 ± 0.13 b**		
SSC/TA	S_1	1.97 ± 0.01	1.68 ± 0.02	1.81 ± 0.02	**1.82 ± 0.06 b**	NS
	S_2	2.22 ± 0.03	2.18 ± 0.03	2.09 ± 0.04	**2.16 ± 0.06 a**	
	Rootstock mean	**2.09 ± 0.08 a**	**1.92 ± 0.11 b**	**1.95 ± 0.09 b**		

Table 5.3. Soluble solid content (SSC), titratable acidity (TA) and their ratio in tomatoes "Sir Elyan" as affected by rootstock and ripening stage (mean ± standard error). Different letters among factor means (bold numbers) indicate significance at Fisher's LSD test (p=0.05). NS: not significant.

5.3.3. Fruit Nutraceutical Profile

As regards the L-ascorbic acid content, the S2 fruits showed a significant decrease when "Sir Elyan" was grafted onto "He-Man" (−21.4%) while for the other rootstocks no statistical variations among ripening stages were recorded (**Table 5.4**). On the average of grafting combinations, lycopene content increased by 91.5% passing from S1 to S2, whereas showed significantly lower values in "Sir Elyan" grafted onto "Armstrong", and the highest content in "Sir Elyan" grafted onto "He-Man"(**Table 5.4**). The β-carotene

content was affected by R×S interaction since, passing from S1 to S2, the higher increase was noticed in the grafting combination "Sir Elyan"/"Armstrong" than in the other ones (**Table 5.4**). DPPH significantly decreased passing from S1 to S2 using "Interpro" and "Armstrong" rootstocks (**Table 5.4**).

Variable	Ripening Stage	Rootstock			Ripening Stage Mean	$LSD_{interaction}$ (p = 0.05)
		"He-Man"	"Interpro"	"Armstrong"		
L – ascorbic acid (μg g^{-1} FW)	S_1	126 ± 4	103 ± 3	97 ± 4	**109 ± 8 a**	14
	S_2	99 ± 3	100 ± 5	91 ± 3	**97 ± 5 b**	
	Rootstock mean	**113 ± 9 a**	**102 ± 4 b**	**94 ± 4 b**		
Lycopene (μg g^{-1} FW)	S_1	8.3 ± 0.3	7.1 ± 0.3	5.9 ± 0.2	**7.1 ± 0.6 b**	NS
	S_2	15.3 ± 0.5	13.0 ± 0.6	12.4 ± 0.3	**13.6 ± 0.9 a**	
	Rootstock mean	**11.8 ± 2.3 a**	**10.1 ± 1.6 b**	**9.2 ± 1.9 c**		
β – carotene (μg g^{-1} FW)	S_1	9.5 ± 0.3	6.6 ± 0.4	7.9 ± 0.2	8.0 ± 0.6 b	1.6
	S_2	11.4 ± 0.5	8.5 ± 0.6	10.1 ± 0.3	10.1 ± 0.9 a	
	Rootstock mean	**10.5 ± 0.6 a**	**7.6 ± 0.8 c**	**9.0 ± 1.0 b**		
DPPH (μmol TEAC g^{-1} FW)	S_1	1.90 ± 0.08	1.84 ± 0.06	1.67 ± 0.06	1.80 ± 0.18 a	0.13
	S_2	1.82 ± 0.06	1.44 ± 0.04	1.05 ± 0.04	1.44 ± 0.13 b	
	Rootstock mean	**1.86 ± 0.12 a**	**1.64 ± 0.16 b**	**1.36 ± 0.2 c**		

Table 5.4. L-ascorbic acid, main carotenoids content and antioxidant activity in tomatoes "Sir Elyan" as affected by rootstock and ripening stage (mean ± standard error). Different letters among factor means(bold numbers) indicate significance at Fisher's LSD test (p=0.05). NS: not significant.

5.3.4. Fruit Volatile Profile

Twelve volatile compounds, which were suggested to be key tomato aroma contributors (Kader et al., 1987), were identified in our study, including 4 alcohols (3-methyl-1-butanol, 1-pentanol, 1-hexanol, and3-henex-1-ol), 5 aldehydes (3-methylbutanal, hexanal, E-2-hexenal, E-2-heptenal and octanal), plus the apocarotenoids β-ionone and 6-methyl-5-hepten-2-one and the ester methyl salicylate (**Table 5.5**). Excepting methyl salicylate, the average concentration of all detected volatiles was higher in the S2

fruits (**Table 5.5**), but all these differences were rootstock-dependent. Among the alcohols volatiles, 3-methyl-1-butanol concentration increased in "Sir Elyan" grafted onto "Interpro" and "Armstrong" (+2.3 and 24.2-fold, respectively, passing from S1 to S2) and decreased when grafted onto "He-Man". "He-Man" and "Armstrong" determined a higher rise in 1-pentanol (+2.6 and 1.5-fold, respectively) than "Interpro". "He-Man" also caused the highest rise in 1-hexanol (+1.6-fold) and in 3-hexen-1-ol concentration (+1.2-fold) (**Figure 5.1**). Considering the aldehydes volatiles, "Armstrong" determined, in the S2 fruits, the strongest increase in 3-methylbutanal (+1.1-fold), "Interpro" in hexanal (+16.7-fold) and "He-Man" proved the most marked increase in E-2-hexenal (+4.3-fold), E-2-heptenal (+37.7-fold) and octanal (+5.3-fold) (**Figures 5.2 and 5.3**). Octanal significantly decreased in fruit harvested at S2 when grafted onto "Interpro" (**Figure 5.3**). Among the remaining compounds, β-ionone displayed the highest increase in the S2 fruits in "Sir Elyan" grafted onto "He-Man" (+2.1-fold), whereas 6-methyl-5-hepten-2-one proved the highest rise on "He-Man" and "Interpro" (+116.5 and 49-fold, respectively) **(Figure 5.3**). Methyl salicylate concentration peaked in the S1 fruits that, compared to the S2 ones, proved the highest concentration in the grafting combination "Sir Elyan"/"Armstrong"(+0.4-fold) (**Figure 5.3**).

Compound	Rootstock			Ripening Stage		Overall Mean	Odor Description [24,27]
	"He-Man"	"Interpro"	"Armstrong"	S_1	S_2		
Alcohols							
3-methyl-1-butanol	118.6 ± 24.5 c	336.1 ± 81.1 b	559.7 ± 118.5 a	124.0 ± 25.4 b	552.3 ± 80.3 a	338.1 ± 154.0	Whiskey, malt, burnt
1-pentanol	24.8 ± 6.3 b	14.2 ± 2.9 c	26.6 ± 5.2 a	12.0 ± 1.5 b	31.8 ± 4.2 a	21.9 ± 5.1	Green
1-hexanol	29.9 ± 5.9 b	8.0 ± 1.0 c	36.3 ± 2.9 a	17.7 ± 4.4 b	31.8 ± 6.7 a	24.7 ± 6.2	Flower, green
3-hexen-1-ol	22.3 ± 4.8 b	49.7 ± 2.8 a	15.9 ± 2.7 c	24.3 ± 6.9 b	34.3 ± 6.1 a	29.3 ± 6.6	Herbal, green
Aldehydes							
3-methylbutanal	9.9 ± 1.5 c	19.6 ± 2.7 b	50.0 ± 8.1 a	20.6 ± 4.2 b	32.4 ± 5.0 a	26.5 ± 8.4	Malt
Hexanal	635.3 ± 69.0 a	527.2 ± 91.5 b	556.3 ± 47.9 ab	379.5 ± 74.2 b	766.4 ± 74.3 a	572.9 ± 119.6	Grass, tallow, fat
E-2-hexenal	600.8 ± 84.2 a	227.7 ± 55.2 b	111.0 ± 19.9 c	144.2 ± 24.2 b	482.1 ± 64.1 a	331.2 ± 134.1	Green, apple
E-2-heptenal	106.6 ± 15.5 a	23.3 ± 2.9 c	89.5 ± 5.2 b	37.3 ± 13.5 b	108.9 ± 23.6 a	73.1 ± 29.1	Soap, fat, almond
Octanal	395.0 ± 49.4 a	236.0 ± 60.0 b	131.2 ± 19.2 c	196.3 ± 53.3 b	311.8 ± 64.4 a	254.1 ± 89.9	Soap, lemon, green, fat
Others							
β-ionone	22.6 ± 5.3 a	19.1 ± 3.9 b	17.6 ± 4.2 c	9.9 ± 0.6 b	29.6 ± 1.7 a	19.7 ± 4.3	Ripe tomato
6-methyl-5-hepten-2-one	377.0 ± 86.5 a	90.9 ± 39.2 c	145.4 ± 28.8 b	30.6 ± 15.8 b	378.3 ± 94.0 a	204.4 ± 107.5	Sweet, nutty, raspberry
Methyl salicylate	31.4 ± 3.7 b	15.6 ± 1.4 c	72.2 ± 5.1 a	48.8 ± 12.2 a	32.7 ± 8.6 b	39.7 ± 10.7	Wintergreen

Table 5.5. Peak area (×106) of volatile organic compounds detected in tomatoes "Sir Elyan", as affected by rootstock and ripening stage (main effects) (mean ± standard error). Different letters among factor's means indicate significance at Fisher's LSD test (p=0.05).

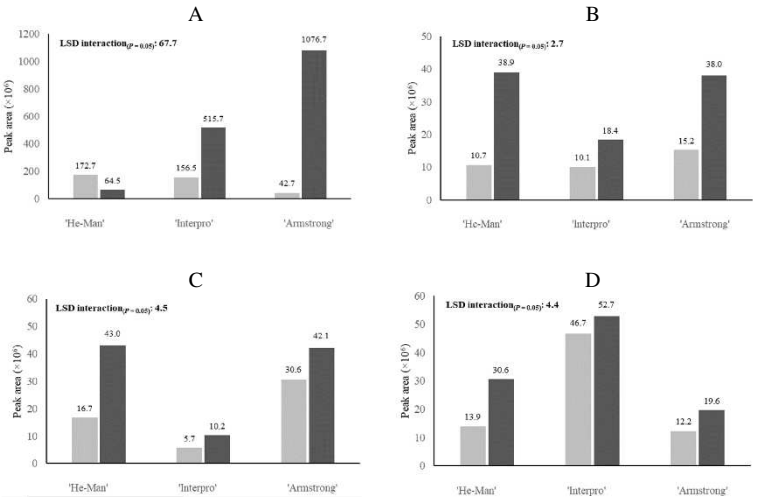

Figure 5.1.Peak area (×106) of 3-methyl-1-butanol (**A**), 1-pentanol (**B**), 1-hexanol (**C**), and 3-hexen-1-ol (**D**) in tomatoes "Sir Elyan" as affected by 'rootstock ×ripening stage' interaction. Light bars: S1. Dark bars: S2.

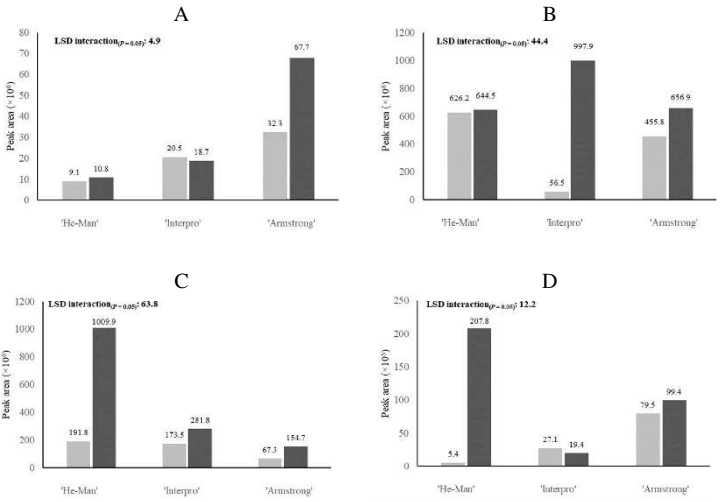

Figure 5.2. Peak area (×106) of 3-methylbutanal (**A**), hexanal (**B**), E-2-hexenal (**C**), and E-2-heptenal (**D**) in tomatoes "Sir Elyan" as affected by 'rootstock × ripening stage' interaction. Light bars: S1. Dark bars: S2

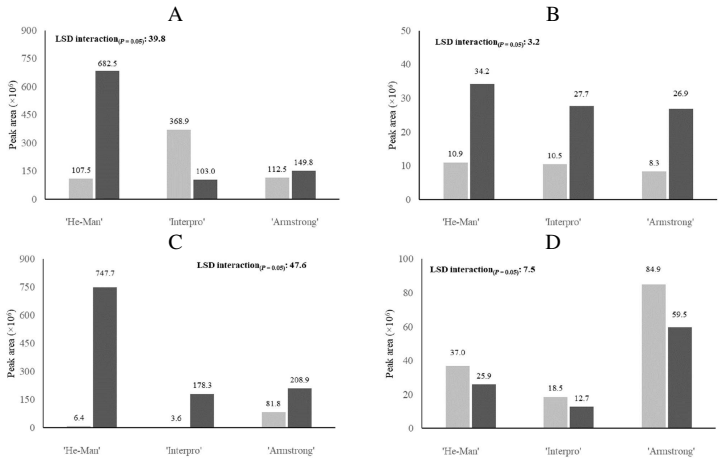

Figure 5.3. Peak area (×106) of octanal (**A**), β-ionone (**B**), 6-methyl-5-hepten-2-one (**C**), and methyl salicylate (**D**) in tomatoes "Sir Elyan" as affected by 'rootstock × ripening stage' interaction. Light bars: S1. Dark bars: S2.

5.3.5. Sensory Analysis

Tomato samples significantly differed for 11 out the 16 sensory attributes (**Table 5.6**). In particular, the bitter perception decreased in the S2 fruits only in the grafting combinations "Sir Elyan"/"Armstrong"(−21.3%); th crunchy decreased in the S2 fruits of "Sir Elyan" grafted onto "He-Man" and "Interpro" with a trend particularly evident in the latter (−32.6%). Both firmness and freshness in general dropped in the S2 fruits, particularly in "Sir Elyan" grafted onto "Interpro" (−19.2% and−6.0%, respectively).Similarly, herbaceous flavour and odour showed a general decrease in the S2 fruits with the strongest reduction in tomato grafted

onto “He-Man” (−15.6% and−18.2%, respectively). Overall, salt and sour perceptions were higher in the S1 fruits as compared to the S2 ones, with the highest differences recorded in “Sir Elyan” grafted onto “Interpro” (+34.4%) and “Armstrong” (+13.8%), respectively. Differently, the sweet perception showed a different trend passing from S1to S2 stage in the rootstock treatments. This sensory attribute increased using “He-Man” (+13.1%) and ‘Armstrong (+12.3%) rootstocks and decreased in “Interpro”. Tomato flavour and odour generally peaked in the S2 fruits, with a gradient recorded in the grafting combinations “Sir Elyan”/“Armstrong” (+18.8%) and “Sir Elyan”/“Interpro” (+24.5%).

Attribute	“He-Man”	“Interpro”	“Armstrong”	“He-Man”	“Interpro”	“Armstrong”
		S_1			S_2	
Freshness	7.36 ab	7.54 a	7.63 a	7.00 b	7.09 b	7.27 b
Firmness	7.54 ab	8.09 a	7.90 a	6.36 c	6.54 bc	7.00 b
Tomato odor	5.18 d	5.54 cd	5.81 c	6.27 b	6.90 a	6.54 b
Herbaceous odor	5.55 a	5.63 a	5.36 a	4.54 b	4.81 b	4.54 b
Off-odor	1.54 a	1.90 a	1.36 a	1.54 a	1.09 a	1.72 a
Salt	4.54 b	5.00 a	4.90 a	3.72 c	3.72 c	4.90 a
Sour	3.45 ab	3.18 b	3.72 a	3.36 b	3.00 c	3.27 b
Sweet	4.90 c	5.36 a	4.45 d	5.54 a	5.19 b	5.00 c
Bitter	1.81 b	2.09 b	2.54 a	2.09 b	2.09 b	2.00 b
Crunchy	6.18 a	7.00 a	6.36 ab	6.09 b	4.72 c	5.54 bc
Juicy	7.09 a	7.45 a	7.27 a	7.27 a	7.54 a	7.18 a
Mealy	3.09 a	2.81 a	3.18 a	2.81 a	3.09 a	2.63 a
Peel thick	5.63 a	5.81 a	5.90 a	5.90 a	5.36 a	5.63 a
Tomato flavor	6.00 c	6.27 b	5.81 d	6.81 a	6.27 b	6.90 a
Herbaceous flavor	5.27 a	5.18 a	5.18 a	4.45 b	4.45 b	4.63 b
Off-flavor	1.18 a	1.63 a	1.18 a	1.36 a	1.90 a	1.27 a

Table 5.6. Mean scores of 16 sensory attributes of tomatoes “Sir Elyan” differing for rootstock and ripening stage. Different letters within each row indicate significance at Mann–Whitney’s U-test(p=0.04885).

5.3.6. Correlation among Volatiles Concentration and Sensory Scores

Globally, 132 correlations were analysed, of which 60 (45% of total) showed significance, revealing 37 negative and 23 positive relationships (**Table 5.7**). In the case of the alcohol volatiles, 17 out of 44 correlations (39% of total) were significant, whereas they were 26 out of 55 (47%) for the aldehydes and 22 out 33 (67%) for the remaining volatiles. Among the negative correlations, the lowest *r*-values were recorded among β-ionone concentration, herbaceous flavour (−0.961 ***), firmness (−0.946 ***) and herbaceous odour (−0.932 ***), followed by that between hexanal concentration and crunchy (−0.929 ***)(**Table 5.7**). Differently, the strongest relationship in the data frame of positive correlations was found between 6-methyl-5-hepten-2-one concentration and tomato flavour (0.873 ***), β-ionone and tomato flavour (0.834 ***), methyl salicylate and sour (0.813 ***) and among β-ionone and sweet (0.798 ***)(**Table 5.7**).

Table 5.7. Pearson's product-moment correlation coefficients (r) among volatiles concentration and sensory attributes. *, ** and *** indicate significance atp≤0.05, 0.01and 0.001, respectively. NS: not significant.

Attribute	3-methyl-1-butanol	1-pentanol	1-hexanol	3-hexen-1-ol	3-methylbutanal	Hexanal	E-2-hexenal	E-2-heptenal	Octanal	β-ionone	6-methyl-5-hepten-2-one	Methyl Salicylate
Bitter	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	0.579 *
Crunchy	−0.583 *	NS	NS	NS	NS	−0.929 ***	NS	NS	NS	−0.623 **	NS	NS
Firmness	NS	−0.716 ***	NS	NS	NS	−0.788 ***	−0.695 **	−0.524 *	NS	−0.946 ***	−0.768 ***	NS
Freshness	NS	−0.644 **	NS	NS	NS	−0.709 ***	−0.742 ***	NS	NS	−0.922 ***	−0.747 ***	0.551 *
Herbaceous flavor	−0.478 *	−0.760 ***	NS	NS	NS	−0.663 **	−0.600 **	−0.539 *	NS	−0.961 ***	−0.727 ***	NS
Herbaceous odor	−0.556 *	−0.916 ***	−0.683 **	NS	NS	−0.627 **	−0.553 *	−0.672 **	NS	−0.932 ***	−0.749 ***	NS
Salt	NS	NS	NS	NS	0.511 *	−0.706 **	−0.724 ***	NS	NS	−0.723 ***	−0.668 **	0.568 *
Sour	−0.484 *	NS	NS	−0.854 ***	NS	NS	NS	NS	NS	−0.490 *	NS	0.813 ***
Sweet	NS	0.470 *	NS	0.656 **	NS	NS	0.752 ***	NS	0.628 **	0.798 ***	0.662 **	−0.827 ***
Tomato flavor	0.522 *	0.875 ***	0.558 *	NS	NS	NS	0.607 **	0.656 **	0.580 *	0.834 ***	0.703 **	NS
Tomato odor	0.586 *	0.605 **	NS	NS	NS	0.659 **	NS	NS	NS	0.788 ***	NS	NS

5.4. Discussion

Under the specific conditions of our experiment, the S1 fruits showed a higher dry matter content, consistent with their higher firmness, this last feature indicating less advanced metabolic processes when the reference ripening stage was achieved. Indeed, the decline in fruit firmness coincides with the up-regulation of several cell wall degrading enzymes, as well as with the dissolution of the middle lamella, leading to the reduction of the intercellular adhesion and cell wall depolymerization (Bertin and Génard, 2018). The grafting combination "Sir Elyan"/"He-Man" yielded the fruits with the highest firmness in both harvest stages, indicating the possibility to influence this trait by selecting the most suitable rootstock. This is an important commercial modification brought by grafting, since textural properties are implicated in fruits' shelf life and transportability, as well as on the perception of their flavor profile (Kyriacou et al., 2017). Soluble sugars (mainly glucose, fructose and sucrose) and organic acids (mainly citric and malic) are primary compounds of tomato fruits, whose amount are commonly measured through the soluble solid content (SSC) and titratable acidity (TA), respectively. From a sensorial viewpoint, their measure is linked to the perceived sweetness (SCC) and sourness (TA) of tomatoes, whereas the SCC/TA ratio describes the overall balance among them in the perceived taste (Di Gioia et al., 2010). All these variables are reputed primary contributors to the perceived flavor of tomato fruits (Di Gioia et al., 2010). In our experiment, the S1 fruits were characterized by a decreased SSC and an increased TA, overall indicating their less sweet, more acidic

taste. So our results confirm the lower sugar content characterizing tomato fruits harvested at earlier ripening stages (Raffo et al., 2018). Moreover, our results agree with previous reports of a TA increase up to breaker stage, and its subsequent decline with further ripening (Kader et al., 1977). It is generally accepted that vigorous rootstocks show a higher sink strength, competing with the fruits for photosynthates accumulation (Oztekin et al., 2009). Accordingly, we recorded a higher SCC/TA ratio when "Sir Elyan" was grafted onto "He-Man" (i.e., the least vigorous rootstock) than onto "Armstrong" (the most vigorous one), indicating a modified tendency of the fruits to accumulate sugars on the basis of the rootstock vigor When compared to other fruits, tomato shows only moderate ascorbic acid (AsA) content, but its dietary importance implies that even small variations in this micronutrient can have relevant effects for consumers (Mellidou et al., 2012). The ascorbic acid concentration we recorded showed a tendency to peak in S1 fruits. This is consistent with previous findings about the higher AsA biosynthetic capacity of younger fruits (up to 1.4-fold higher), probably to support their higher rates of cell division and expansion (Mellidou et al., 2012). Indeed, it has been reported that AsA plays an important role in plants, related to cell division and cell wall synthesis (Bertin and Génard, 2018). On the other hand, the AsA drop in the S2 fruits mirrored the decrease in fruit dry matter content, indicating a prevailing ripening-driven dilution effect in "Sir Elyan" tomatoes. For this nutritional trait, the rootstock-related differences proved to be significant only in the S1 fruits, with the grafting combination "Sir Elyan"/"He-Man" (i.e., the least vigorous one) showing the highest

concentration. Accordingly, the lowest fruit AsA content characterizing the most vigorous grafting combinations has been explained through their higher vegetative biomass, resulting in a redistribution or accumulation of this molecule in other plant fractions (Wadano et al., 1999). From a nutraceutical viewpoint, tomato is recognized as one of most important suppliers of carotenoids and phenolic acids, whose content contributes to the antioxidant capacity of this vegetable (Rizzo et al., 2016), and the shift in their concentration during ripening is one of the major changes accompanying the improvement of fruit palatability. The S1 fruits showed compositional evidences of backwarded ripening process compared to the S2 ones, resulting from a higher antioxidant activity and a lower concentration of the main carotenoids. These results agree with previous observations about the pivotal role of ripening stage in influencing these important nutraceutical traits of tomato (Raffo et al., 2002). Indeed, regarding the main tomato pigments, the carotenogenesis flows from the degradation of the photosynthetic membranes and metabolization of chlorophylls, leading to a progressive accumulation in tomato fruits of the C40 isoprenoids lycopene and β-carotene (Fanciullino et al., 2014). Regarding the rootstock effect, when grafted onto “He-Man”, “Sir Elyan” fruits showed the best nutraceutical profile, both in terms of lycopene, β-carotene, and antioxidant capacity, irrespective of the ripening stage. This is an important outcome of our experiment, demonstrating the possibility of an overall functional improvement of greenhouse tomatoes using an appropriate graft combination, also in the light of their usually poorer nutraceutical composition when compared to

field-grown tomatoes (Toor and Savage, 2005). As regards the aroma components, excepting methyl salicylate, the S1 fruits showed the lowest content for all the detected volatiles. The most severe reductions regarded the carotenoid-derived 6-methyl-5-hepten-2-one and β-ionone (−92% and −67%, respectively), the lignin-related 3-methyl-1-butanol (−78%) and the lipid-derived E-2-hexenal and E-2-heptenal (−70% and −66%, respectively). Consistent with the findings of Raffo et al. (2018), such reductions clearly indicate a disturbance in the formation of tomato key odorants induced by early harvests. Indeed, it is known that the ability of tomatoes to form lipid-derived volatiles increases as the fruits ripen (Baldwin et al., 1991), so the reduced concentration we recorded in S1 fruits in terms of C6 aldehydes and related alcohols (namely 1-hexanol and 3-hexen-1-ol) probably reflects the developmentally-induced lower expression of genes encoding for lipoxygenases and hydroperoxide lyases converting unsaturated fatty acids to 13-hydroperoxides and then to C6 aldehydes (Zhang et al., 2016). On the other hand, the increase in 6-methyl-5-hepten-2-one and β-ionone we found in the S2 fruits, is consistent with their higher concentration of precursors lycopene and β-carotene, respectively (Mathieu et al., 2009). On the contrary, methyl salicylate is typically associated to anti-herbivores defense of plant tissues (James and Prince, 2004), hence the higher concentration of this last volatile clearly indicates a less advanced ripening process in S1 fruits, whose compositional traits still refer to the seed-protecting role of the pericarp. From a sensory viewpoint, the S1 fruits were characterized as firmer and with the highest freshness, herbaceous odor and flavor. Differently, the S2 fruits were generally scored as less

acidic and with stronger typical tomato odor and flavor. Such modifications were clearly distinguishable, despite in the latter fruits a significant increase was recorded in "green" and "grassy" volatiles such as hexanal, E-2-hexenal, octanal, 3-hexen-1-ol, or 1-pentanol. This finding is only apparently contradictory, since in tomato no single compound has been found to be reminiscent of fresh ripe tomatoes, whose aroma, instead, springs from a multitude of several volatiles, blended in appropriate concentrations (Baldwin et al., 2000). In this view, the correlation analysis revealed that 1-pentanol, β-ionone, and 6-methyl-5-hepten-2-one gave the strongest contribution to tomato flavor perception, whereas β-ionone and hexanal were the volatiles most tightly linked to the increased tomato odor perception. On the other hand, the alcohol 1-pentanol, the aldehydes hexanal and E-2-hexenal, as well as the apocarotenoid β-ionone and 6-methyl-5-hepten-2-one displayed a pool of negative correlations with some traits typically associated to less ripe tomatoes (namely crunchy, firmness, freshness, and herbaceous flavor), highlighting their primary role in accompanying the organoleptic evolution of tomatoes during ripening. It is interesting to note that, consistent with its higher concentration in unripe fruits, methyl salicylate was positively correlated to the perceived sour, salt and bitter, so suggesting a possible interactive effect of this compositional trait in generating these peculiar perceptions in less ripe "Sir Elyan" fruits. As regards the studied rootstocks, the volatile composition was somewhat erratic and unpredictable on the basis of the rootstock characteristics. Irrespective of the harvest stage, the grafting combination "Sir Elyan"/"He-Man" gave the highest concentration for 6 out of the 12

volatiles detected (including the fruity volatiles β-ionone and 6-methyl-5-hepten-2-one), whereas the combinations "Sir Elyan"/"Armstrong" gave the highest concentration for 5 volatiles (including 1-pentanol, 1-hexanol and methyl salicylate, all associated to "green" notes). Only 3-hexen-1-ol showed the highest concentration when "Sir Elyan" was grafted onto "Interpro". Such diversity gave a different ability to the rootstocks to offset the fruit sensory modifications brought by the different harvest stages. Indeed, when grafted onto "He-Man" or "Interpro", "Sir Elyan" gave S1 fruits characterized by an attenuated bitter perception, consistent with their lower concentration of methyl salicylate. Moreover, the grafting combination "Sir Elyan"/"Interpro" gave less acidic S1 fruits, characterized also by improved sweetness and tomato flavor. Differently, "He-Man" and "Armstrong" were able to accentuate the perception of sweet, sour and typical tomato flavor in the S2 fruits, which, despite a lower SSC, mirrored their higher 6-methyl-5-hepten-2-one content when compared to "Interpro".
This finding is consistent with the widely accepted idea that some fruity/floral volatiles improve the perception of sweetness in tomato fruits (Baldwin et al., 2008).

5.5 Conclusions

Our findings indicate that the harvest stage significantly affected both nutraceutical and eating quality profile of "Sir Elyan" tomatoes, but a certain dichotomy emerged among the functional profile and some sensorial traits in "Sir Elyan" fruits, in relation to their harvest stage. On the other hand, the rootstock genotype influenced both the nutraceutical and

quality traits of the fruits. Despite none of the studied rootstocks was able to improve all the quality traits considered in this experiment, our results suggest the possibility to selectively improve the nutraceutical or sensorial profile of “Sir Elyan” fruits by selecting the most appropriated rootstock, provided that its interactive effects with the harvest stage are taken into account.

5.6 References

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6. Effects of biostimulant application and cluster position on quality and nutraceutical profile of cherry tomato produced in cold greenhouse

6.1Introduction

Tomato (*Solanum lycopersicum* L.) is an important vegetable crop grown both in open field and greenhouse around the Mediterranean Basin (Mauro et al., 2015), with Italy being the main producer among the European counties (5.3 out of the 22.8 Mtons produced in 2019) (Faostat, 2021). Its climacteric fruits represent a key dietary component in many countries worldwide and, consequently, a relevant dietary source for humankind of health-promoting compounds such as carotenoids, phenolics, vitamin C and E, or minerals (Vats et al., 2020). Among many product typologies available, cherry tomato is highly appreciated by consumers for its distinctive and recognizable taste characteristics (Pérez-Marín et al., 2021), so that it is among the prevailing tomatoes grown in the greenhouse systems of Southern Italy. Over the last decades, cherry tomato has been subjected to intensive breeding programs of many seed companies, in order to match the evolving standards in production, commercialization and consumption. Consequently, the currently available cultivars are characterized by different yield performances, wide compositional variability and rapid temporal turnovers (Distefano et al., 2020), making difficult to optimize the quantitative and qualitative performances of the crop, both highly influenced by the environmental conditions (Bertin and Génard, 2018; Kyriacou and Rouphael, 2018). With reference to this, in Southern Italy greenhouse tomato is primarily grown close to coastal areas, where the reduced seasonal microclimate fluctuations promote cost-effective cultivations mainly though low-tech shelters (i.e. with poor microclimate control), with transplanting in late summer and a large part of fruits production during autumn-winter months (Pardossi et al., 2004). Nonetheless, during this time span, tomato crops

experience time-course reductions of mean temperature and daily light interval (DLI), both negatively impacting actual fruit yields, along with fruit temporal continuity in terms of quality and nutraceutical traits (Iglesias et al., 2015; Bojarian et al., 2019). These last aspects are obstructing in the perspective to match the growing consumers' demand for high-quality, nutrient-dense vegetables, thus able to respond to the expected evolution of current dietary patterns (Buturi et al., 2021). In this context, plant biostimulant (PBs) have emerged as one of most interesting innovations over the last two decades (du Jardin, 2015), as they can assist the agricultural sector to face the challenge of increasing yields and improving, at the same time, the product quality in a multitude of crops (Parađiković et al., 2019). Among these, plant-derived biostimulants (PDBs), that is those derived from plant extracts, have been proven to enhance many physiological processes related to plant growth, productivity, tolerance to abiotic stressors and product compositional traits, especially when suboptimal growth conditions are concerned (Zulfiqar et al., 2020; Del Buono, 2021). These beneficial effects have been ascribed to the presence of several components of plant extracts, such as phytohormones (cytokinin, auxins, gibberellins, brassinosteriods, ethylene, and abscisic acids), amino acids, polyamine, proteins and minerals as well as antioxidants, through biochemical pathways still poorly understood (Bulgari et al., 2015; Colla et al., 2017; Yakhin et al., 2017). In tomato, the application of PDBs has been proven to enhance key agronomic and quality traits such as yield, fruit firmness and dry matter content, antioxidant activity and concentrations of secondary metabolites (e.g. carotenoids, polyphenols or ascorbic acid) (Colla et al., 2017; Francesca et al., 2020; Cozzolino et al., 2021), although with different results depending on the

genotypes and growth conditions. However, when fruit quality is concerned, these studies were conducted on tomatoes harvested only once during the crop cycle, so that there are still no available data about the effects of PDBs on tomato fruits as a function of the harvest period. Understanding to which extent PDBs effects are influenced by the time-course variations in cropping conditions, could help for a better definition of their possible contribution and limits at improving the dietary role of pivotal, long-producing vegetables subjected to seasonal variations in growth conditions (as in the case of greenhouse tomato). Due to this, the aim of the present work was to investigate the yield and compositional variables of three recently widespread cherry tomato cultivars in a Mediterranean environment, configured by the application of a PDB. For fruits composition, two harvest dates were considered (autumn vs. winter), in order to consider, also, the role of cultivation conditions in outlining the aforementioned effects.

6.2 Materials and Methods

6.2.1Chemicals

Lycopene and (*all*-E)-β-carotene standards (purity 99% or higher) were obtained from Extrasynthèse (Genay, France). Acetone, butylated hydroxytoluene and calcium carbonate were purchased Sigma-Aldrich. Methyl tert-butyl ether and methanol were from Sigma-Aldrich. Purified deionized water was prepared by a Milli-Q (Millipore, Billerica, MA, USA) or an Arium® 611 (Sartorius, Göttingen, Germany) water treatment system. All solvents and standards were of HPLC grade or higher quality.

6.2.2. Experimental Site and Plant Material

A greenhouse experiment was conducted during the 2019-2020 growing season, at the experimental farm of the University of Catania (Sicily, South Italy: 37°24′27″ N, 15°03′36″ E, 6 m a.s.l.), in an area characterized by a semi-arid/Mediterranean climate. An 800 m^2, East–West oriented, multi-aisle greenhouse was used, having a steel tubular structure with adjustable windows on the roof and along the sides, and covered with polycarbonate slabs. Three cherry tomato cultivars, namely 'Eletta' (TSI Italia srl, Foggia, Italy), 'Kaucana' (Vilmorin Italia srl, Funo, Italy) and 'Top Stellina' (TSI Italia srl, Foggia, Italy), were tested in randomized plot design with three replicates, each containing 15 plants (net of borders). On 5th September 2019, ungrafted seedlings at the stage 3 true leaves were transplanted in 5 L plastic pots (20 cm height, 19 cm width), with perlite as growing medium (particle size 2-6 mm). The studied tomato cultivars were recently spread over the reference area (South Italy) and have been chosen for their different main carpometric traits. Plant density was 3.3 plants m^{-2} (0.30 × 1.00 m). The plants were grown with a single stem up to the 8th cluster, and all the clusters were pruned leaving 14 fruits; fruit setting was allowed using bumblebees. An open soilless cultivation system was adopted, and during the cycle the crop was uniformly fertigated with a standard nutrient solution (Mauro et al., 2020), adopting a leaching fraction of 25%, in order to avoid root zone salinization (Giuffrida, et al., 2018). Mean air temperature, relative humidity (RH), global radiation and vapor pressure deficit (VPD) inside the greenhouse were recorded on an hourly basis, by means of four sets of sensors uniformly distributed over the experimental area. All sensors were connected to a CR-510 data logger (Campbell Scientific, Inc., Logan, UT, USA) (**Figure 6.1**).

Tomato plants were treated with a liquid biostimulant (Bioup TF®, Intertec s.r.l., Bibbiena, Italy), made up of plant extracts, boric acid (0.5%) and zinc sulphate (1.6%). Canopy sprays were performed early in the morning, by applying a biostimulant dose equivalent to 1 L/ha once per week, starting from the fruit setting of the first cluster (~12 days after transplanting, DAT) and 20 days after the of setting each of the 8 clusters. Clusters were harvested by hand, when all the fruits reached the full-red ripe stage. After harvest, the clusters were quickly transported to the laboratory and then processed for the analysis. Specifically, the analyses on fresh samples were performed at the University of Catania (Italy) while, the analyzes relating to the content of sugars, acids, carotenoids and tocochromanols were performed on freeze-dried samples and performed at the Geisenheim Hochschule University (Germany). The freeze-dried samples were kept at -80 °C until the analyses. All compositional variables were determined on second and sixth cluster (hereafter C_{II} and C_{VI}, respectively). The harvesting of C_{II} was effected between 77 and 80 days after transplanting (DAT), while for C_{VI} between 116 and 120 DAT.

6.2.3 Yield and carpometric analysis

After harvest, fruits were detached from rachis, selected for absence of defects and uniform appearance within each genotype, then fruit fresh weight was determined. From the fresh weight of each the 8 clusters, the yield of each genotype was calculated.

The fruit chromatic coordinates were measured on the equatorial axis of whole fruits (two measurements per fruits), through a tristimulus Minolta Chroma meter (model CR-200, Minolta Corp.) calibrated with a standard white tile (UE

certificated) with illuminant D65/10°, measuring color in terms of lightness (L*), green-red axis (a*) and blue-yellow axis (b*). Fruit color was described as tomato color index [TCI = 2000 a*/L*$(a^{*2} + b^{*2})^{1/2}$] (Distefano et al., 2020).
The dry matter was determined by gravimetric analysis. An aliquot of cherry tomato puree were placed in an oven at 70 ◦C (Thermo Fisher Scientific, Waltham, MA, USA) until the constant weight. The total soluble solids (TSS) were estimated with an Abbe refractometer 16531 (Carl Zeiss, Oberkochen, Germany) at 20 °C and the results were expressed as °Brix. Tritatable acidity (TA) was determined by titrating an aliquot of the puree sample with 0.05 N NaOH to pH 8.1. TA was expressed as g kg^{-1} of cherry tomato fresh weight (FW), as citric acid. From data obtained, the TSS/TA ratio was calculated.

6.3.4 Sugars, organic acids total phenolics

Glucose, fructose, citric and malic acid and total phenolics were determined photometrically with a Konelab 20 Xti analyzer (ThermoFisher, Dreieich, Germany), as previously reported with slight modifications (Knebel et al., 2018). Briefly, an aliquot of 500 mg of freeze-dried tomato sample was weighed into a graduated flask, then stirred for 1 hour at room temperature with 25 ml of ultrapure water. Followed a centrifugation (12850 rpm × 5 minutes) (Hettich, Tuttlingen, Germany), the extract was analysed. Concentration of glucose and fructose was analysed enzymatically (IFU method no. 55) and citric and malic acid by iodometric titration (IFU method no. 21 and 22).

6.3.5 Carotenoids analysis

Extraction of carotenoids. Tomato fruits were flash frozen (with liquid nitrogen), freeze-dried, ground with liquid

nitrogen and stored at -80°C until the extraction. Carotenoid extraction was based on a previously reported method with slight modifications (Schweiggert et al., 2012). Briefly, 20 mg of freeze-dried tomato sample was weighed into a centrifuge tube with 50 mg of $CaCO_3$ and mixed with 3 mL acetone containing 0.1% BHT. The ultrasound-assisted extraction (Elma Schmidbauer GmbH, Singen, Germany) lasted 5 minutes. Followed a centrifugation (2000 rpm × 2 minutes) (Hettich, Tuttlingen, Germany), the extraction solvent was collected and the extraction was repeated 2 times, until the solid residue was colourless, with 2 mL of the aforementioned extracting agent. The acetone extract was evaporated to dryness under a gentle N_2 steam, placing the tube in a water bath maintained at 30 °C, and stored at -20 °C until analysis.

HPLC-DAD-ESI-MS^n analysis. Prior to HPLC analysis, the dried extracts were dissolved in 900 µL of methyl tert-butyl ether (MTBE), briefly sonicated in a water bath, and 100 µL of methanol containing 0.1% BHT, membrane-filtered (PTFE, 0.2 µm) and transferred into an amber HPLC vials. An HPLC system consisting in an Accela autosampler, an Accela MS pump, and an Accela PDA diode array detector was used (all from Thermo Scientific). Separation was achieved with gradient elution using a C30 reverse phase column (150 × 3.0 mm i.d., 3 µm particle size, Phenomenex, Aschaffenburg, YMC-Schweiz, Basel, Switzeland) equipped with a YMC C30 guard column (10 × 3.0 mm i.d., 3 µm particle size, Phenomenex, Aschaffenburg, YMC-Schweiz, Basel, Switzeland). Eluent A consisted of methanol/methyl tert-butyl ether/water (90/8/2, *v*/*v*/*v*) and eluent B was methanol/methyl tert-butyl ether/water (20/78/2, *v*/*v*/*v*). The elution gradient at constant flow rate of 0.5 mL/min was as follows: 95% A for 1 min, from 95% to 0% A for 16 min,

isocratic at 0% A for 3 min, from 0% to 95% A for 5 min. Total run time was 25 min and the injection volume 10 µL. Column oven temperature was set to 23 °C. Carotenoids detection wavelengths were 286 nm for phytoenes, 348 nm for phytofluenes and 450 nm for β-carotene. UV spectra were recorded in the range of 250–600 nm. Ratios of D_{III}/D_{II} (%) and D_B/D_{II} (%) were determined according to Britton (1995). For MS^n analysis of carotenoids, the aforementioned HPLC system was coupled with a LXQ Advantage Max ion-trap mass spectrometer with an electrospray ionisation (ESI) source (Thermo Scientific). Mass spectra were recorded in the positive ion mode at a scan range of *m/z* 150/300–700. Nitrogen at 40 and 5 arbitrary units served as sheath and auxiliary gas, respectively. The source potential was 5 kV. Capillary temperature and voltage were 300 °C and 6.0 V, respectively. The normalised collision energy for MS^n experiments was set to 35%. Control of the system and data evaluation was achieved with XCalibur version 2.0 SR2 (Thermo Scientific). Compounds were assigned by comparing their retention times (RT), UV/VIS absorption, and mass spectra to those of reference standards. The carotenoids identified in tomato samples are reported in **Table 6.1**.

HPLC-DAD analysis. Carotenoids were quantified using a Surveyor (Thermo Finnigan, San Jose, CA, USA) HPLC system equipped with a Surveyor MS pump, a Surveyor autosampler and a Surveyor PDA diode array detector (all from Thermo Finnigan). Data was acquired using a XCalibur version 2.0 SR2 (Thermo Finnigan).

All HPLC parameters were set as detailed above, excepting the flow rate that was 0.6 mL/min. Phytoenes were monitored at 286 nm, phytofluenes at 348 nm and β-carotene at 450 nm. Linear calibration curves of lutein and lycopene

dissolved in light petroleum were established at concentrations ranging from 0.08 to 2.10 mg/L. LoD and LoQ were determined using signal-noise-ratios (S/N) of S/N = 10 and S/N = 15, respectively.

The stock solution concentrations were determined spectrophotometrically on the basis of molar extinction coefficients for lycopene and β-carotene as reported by Britton (1995). Quantitation of (Z)-isomers was performed using the corresponding (all-E)-carotenoids. Ratios of the molar extinction coefficients were used to quantify the carotenoid precursors phytoene and phytofluene relative to (all-E)-β-carotene (Cooperstone, Francis, & Schwartz, 2016; Maurer, Mein, Chaudhuri, & Constant, 2014).

Limit of quantitation (LOQ) was estimated on the basis of a signal to-noise (S/N) ratio of 10:1 of each calibration curve as described by Stauffer (2008). Limit of detection (LOD) was set to one third of LOQ (S/N 3:1).

Retinol activity equivalents, (RAE, according to the equivalence 12 µg (*all*-E)-β-carotene = 1 µg RAE) was calculated according to Trumbo et al. (2001).

Separation of carotenoids from cherry tomato fruits by HPLC is reported in **Figure 6.2**.

6.3.6 Tocochromanols analysis

Extraction of tocochromanols. Tomato tocopherols and tocotrienols were extracted and analysed according to Lux et al. (2020). Briefly, 100 mg of freeze-dried tomato sample was suspended in a centrifuge tube with 1.5 mL of aqueous potassium hydroxide solution (20%, *w/v*), 1.9 mL of ethanol, and 100 µL ascorbic acid aqueous solution (20%, w/w) as an antioxidant. Saponification was performed in a shaking water bath at a temperature of 70 °C for 0.5 hours. Then, the suspension was cooled on ice. In the same centrifuge tube, a

volume of 25 µL of ethanol containing BHT (1 mg/mL), 1 mL of H_2O, 0.6 mL of glacial acetic acid, and 2 mL of hexane were added. After centrifugation (3 minutes at 140 g), the organic phase was collected followed by three additional extraction steps each with 2 mL of n-hexane. The supernatants were combined and evaporated to dryness under a gentle N_2 steam. Analytes were redissolved in 300 µL of methanol, briefly sonicated in a water bath, membrane-filtered (PTFE, 0.45 µm) and transferred into amber vials for HPLC analysis.

HPLC-FLD Analyses of Tocochromanols. The Vanquish Horizon UHPLC system consisted of Vaniquish Flex F binary pump, a Vanquish Flex FT autosampler, a Vanquish Flex column oven (temperature, 40 °C), and a Vaniquish Flex fluorescence detector (all supplied by Thermo Scientific). The excitation wavelength was set at 292 nm and the emission wavelength at 325 nm. Separation was achieved using a Kinetex pentafluorophenyl column (100 × 4.6 mm i.d., 2.6 µm particle size, Phenomenex, Aschaffenburg, Germany). Methanol/ water (85/15, v/v) was used as the mobile phase with isocratic elution (22.5 min) at a flow rate of 1.0 mL/min. The injection volume was 10 µL. Chromatograms were recorded and processed by Chromeleon 7.2.10 ES software. An external calibration curves of tocopherols and tocotrienols were established within the concentration range of 0.1 and 14.00 mg/L. Total tocochromanols were represented as the sum of the aforementioned tocopherols and tocotrienols.

Detection Limit, Quantitation Limit, Linearity, and Recovery. Recoveries were performed in triplicate by standard addition experiments at low and high spike levels within the calibration range. Detection and quantitation limits were calculated from linear calibration curves based on the slope

and the standard deviation of the response according to the guidelines from the International Council for Harmonisation (ICH 22). Linearity was assessed based on correlation coefficients of the calibration curves (with a range from 0.1 to 14 mg/L). α-Tocopherol equivalents (αTE) were calculated using the following conversion factor: αTE = αT×1.0 + βT×0.5 + γT×0.1 + δT×0.03 + αT3×0.33 (NRC, 1989).

6.3.7 . Statistical procedures

All data were subjected to Shapiro–Wilk and Levene's test, in order to check for normal distribution and homoscedasticity, respectively, then to a factorial 'genotype × treatment' (fruit yield) or 'cluster × genotype × treatment' (C × G × T) analysis of variance (ANOVA) (all compositional variables), according to the experimental layout adopted in the experiment. Percentage data were Bliss transformed before the ANOVA (untransformed data are reported and discussed), whereas multiple means comparisons were performed through Tukey's honestly significant difference (HSD) test ($P \leq 0.05$). All calculations were performed using Excel version 2016 (Microsoft Corporation, Redmond, WA) and Minitab version 16.1.1 (Minitab Inc., State College, PA, USA).

6.3 Results and Discussions

6.3.1 Yield and carpometric variables

In the present experiment, the studied factors significantly affected the carpometric traits (**Table 6.2**). When fruit yield was concerned, the studied genotypes were highly diversified, also for their response to the biostimulant, since both 'Kaucana' and 'Eletta' outlined a higher yield rise (+1.25 and +0.93 kg m^{-2}, respectively) when compared to 'Top Stellina' (+0.54 kg m^{-2}) (**Figure 6.3**) Regarding the

carpometric traits, passing from C_{II} to C_{VI}, the genotypes showed an increase in fruit FW (+2.5 g, on average), DM content (up to +2.14% in 'Top Stellina') and TSS/TA (up to +0.40 in 'Eletta'), but a reduction in TCI (-2.1, on average) (**Table 6.3**). In the case of TSS/TA ratio, this outcome was related both to an increase in TSS (+10%, on average) and a decrease in TA (-10%, on average) (data not shown). These results suggest that two different effectors were involved in modifying these traits, namely the change in microclimate conditions inside the greenhouse and the progressive reduction of the plant fruit load with proceeding harvests. During the experiment, the mean air temperature and solar radiation between fruit setting and harvest, shifted from 22.5 °C and 8.6 MJ m^{-2} day^{-1} (September 22 - November 29, C_{II}) to 14.5 °C and 4.7 MJ m^{-2} day^{-1} (October 25 – January 18, C_{VI}), indicating less favourable conditions sustaining C_{VI} development. This likely was at the base of the negative trend of TCI between C_{II} and C_{VI}, a chromatic variable strongly related to lycopene content (Goisser et al., 2020), a tomato carotenoid whose synthesis is optimized by light and temperature between 22 and 25 °C (Dumas et al., 2000). For the other carpometric variables, these suboptimal growth conditions were amply compensated by the decrease in plant fruit load when C_{II} and C_{VI} were harvested, which dropped from 60 $\pm$ 4 to 36 $\pm$ 3 fruits $plant^{-1}$, respectively (data not reported). Overall, the treatment did not affect TCI, whereas promoted fruit FW, DM and TSS/TA, thought in a genotype-dependent way. Indeed, upon treatment, a higher rise in fruit FW was recorded in 'Kaucana' (+3.8 g) followed by 'Eletta' (+2.5 g), whereas 'Top Stellina' and 'Kaucana' gave the best response in terms of fruit DM (+2.14 and +1.1%, respectively); differently, for TSS/TA the highest increase was recorded in 'Eletta' (+0.14) (**Table 6.3**). The increase in

FW as well as in DM and TSS/TA due to a biostimulant application has been reported in previous experimental studies on biostimulants activity both in open-field and greenhouse conditions (Polo at al., 2018; Chehade et al., 2018; Colla et al., 2015). However, different crops and cultivars can be a determining factor in obtaining benefits using biofertilizers (Dalmastri et al. 1999; Remans et al. 2008). For example, Colla et al. (2017a) reported no difference in fruit fresh weight and dry matter content between untreated and treated plants of cv. Sir Elyan (plum shaped fruit), but a significant increase in TSS when a legume-derived protein hydrolysate was applied. On the other hand, Rouphael et al. (2017) proved that the use of a protein hydrolysate in tomato increased in cv. Sir Elyan the fruit mean weight and in cv. Akyra the number of fruits. This suggests a certain degree of specificity in the response to a biostimulant, which derives both from the chosen genotype and from the biostimulant itself, making the response dependent on different interactions within treatment and genotype.

Table 6.1. UV/Vis spectra and MS data of carotenoids from cherry tomato fruits.

No.	t_R (min)	λ_{max} (nm)	D_B/D_{II} [a] (%)	D_{III}/D_{II} [b] (%)	$[M]^{+\bullet}$ (*m/z*)	$[M + H]^+$ (*m/z*)	ESI(+)-MS^n experiment (*m/z*)	Proposed structure
1	5.4	sh274/285/sh299	-	-	544.5	545.6	[545.5]: 399.4*	Phytoene (1)
2	5.9	266 sh422/445/473	-	60	568.6	569.5 551.5 [c]	[551.5]: 533.6*, 495.3, 429.4, 411.5	(all-*E*)-Lutein [d]
3	7.2	sh275/285/sh299	-	-	544.6	545.6	[545.5]: 339.4*	Phytoene (2)
4	8.1	283 332/348/367	-	65	542.4	543.6	[542.4]: 337.4*	Phytofluene (1)
5	9.0	284 332/348/367	-	84	542.5	543.6	[542.5]: 337.4*	Phytofluene (2)
6	11.1	273 sh426/452/478	-	23	536.5	537.6	[536.5]: 445.5*, 399.4	(all-*E*)-β-Carotene [d]
7	11.5	295 sh378/401/426	-	99	540.5	541.6	[540.5]: 471.6, 403.4, 311.4*	ζ-Carotene
8	15.4	282 sh440/462/492	-	51	536.5	537.5	[536.4]: 467.5, 444.5*, 399.5	(all-*E*)-γ-Carotene [d]
9	15.6	280 sh442/464/492	-	n.d.	n.d.	n.d.	n.d.	unknown
10	16.1	297, 361 440/465/496	59	52	536.4	537.5	[536.4]: 467.6, 444.4*, 399.5	(13*Z*)-Lycopene
11	18.0	296, 361 441/467/498	14	69	536.4	537.5	[536.4]: 467.6, 444.5*, 399.5	(9*Z*)-Lycopene

Table 6.1 Cont.

12	18.2	294, 362 447/472/503	7	71	536.4	537.5	n.d.	Lycopene isomer
13	20.3	295, 363 447/472/503	7	71	536.4	537.5	[536.4]: 467.6, 444.3*, 399.5	(all-*E*)-Lycopene [d]
14	20.6	296, 365 447/472/503	7	71	536.4	537.5	[536.4]: 467.6, 444.3*, 399.5	(5*Z*)-Lycopene

t_R, retention time; λ_{max}, UV/vis absorption maxima; sh, shoulder; n.d., not detected; * base peak in the ESI(+)-MS^n experiment.

[a] D_B/D_{II}, ratio of absorption intensity at '*cis*-band' near UV maximum (D_B) to intensity at main absorption maximum (D_{II}).

[b] D_{III}/D_{II}, ratio of absorption intensity at longest wavelength maximum (D_{III}) to D_{II}.

[c] In-source elimination of water ($[M + H - H_2O]^+$).

[d] Verified by an authentic reference standard.

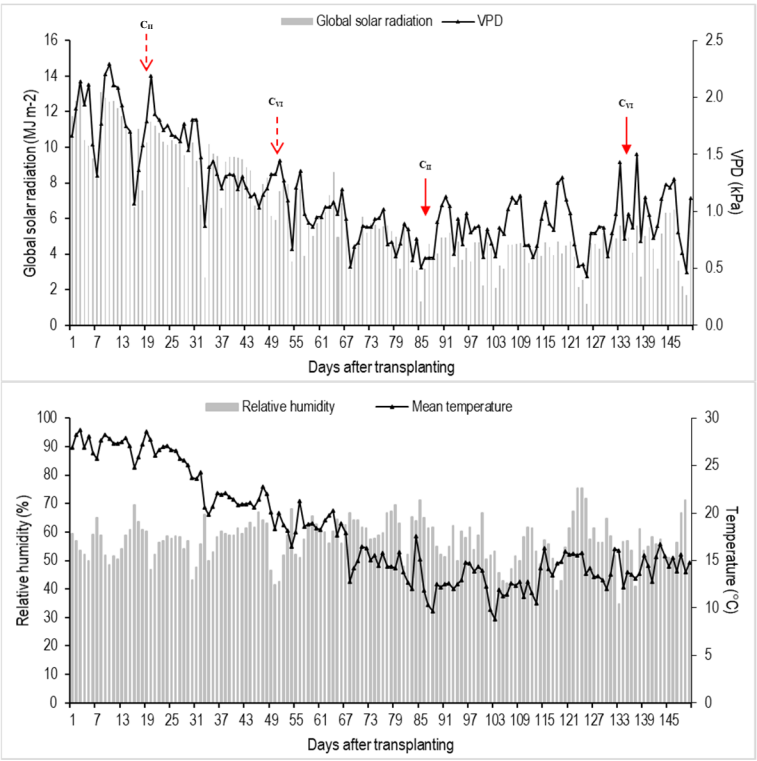


Figure 6.1. Microclimate conditions inside the greenhouse during the trial (**A**, **B**). Dashed arrows indicate the setting dates of clusters C_{II} and C_{VI} while full arrows indicate the harvest dates of clusters C_{II} and C_{VI}.

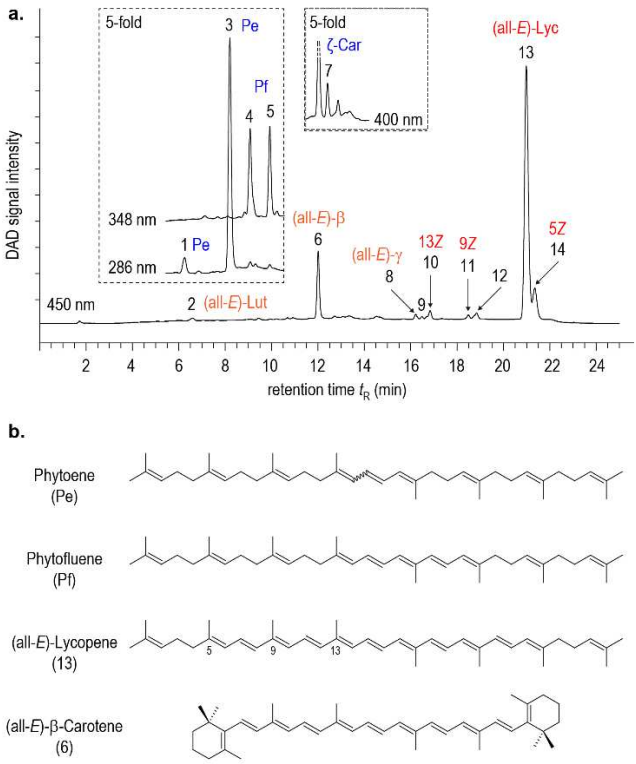


Figure 6.2. Separation of carotenoids and from cherry tomato fruits by HPLC monitored at 450 nm, 286nm, 348 nm and 400nm. For peak assignment see **Table 6.1**.

Table 6.2. *F*-values of the main factors and their first order interactions related to observed variables, with the significance resulting from the ANOVA. TCI: tomato color index; TSS: total soluble solids; TA: titratable acidity; DM: dry matter; RAE: Retinol activity equivalents; α-TEs: α-tocopherol equivalents. NS: not significant; *. ** and ***: significant at $P \leq 0.05$. 0.01 and 0.001, respectively.

Variable	Genotype (G)	Cluster (C)	Treatment (T)	G × C	G × T	C × T
Fruit yield	1289.7***	-	84.2***	-	5.7*	-
Fruit FW	1123.0***	90.1***	103.5***	NS	5.4*	NS
Fruit DM	136.6***	NS	45.1***	4.3*	4.4*	NS
TCI	37.0***	16.5***	NS	NS	NS	NS
TSS/TA	205.7***	192.6***	6.0*	6.4**	4.4*	NS
D-Glucose	208.7***	NS	16.0***	14.0***	NS	16.7***
D-Fructose	126***	9.2**	19.6***	NS	3.6*	6.8*
Total sugars	143.5***	5.8*	17.0***	5.9**	NS	9.4**
Citric acid	93.6***	NS	NS	NS	NS	NS
L-Malic acid	87.8***	7.9*	21.4***	15.5***	35.7***	NS
Total acids	113.7***	NS	NS	NS	NS	NS
Total phytoene	127.8***	16.4***	26.6***	19.1***	NS	NS
Total phytofluene	178.7***	NS	49.9***	24.5***	3.8*	6.4*
Total lycopene	108.7***	19.3**	NS	6.9**	6.7**	16.0**
(all-E)-β-Carotene	8.9**	12.4**	51.8***	NS	3.6*	7.4*
Other carotenoids	108.3***	83.8***	9.8**	NS	12.3**	24.7**
Total carotenoids	199.8***	24.9**	14.7**	NS	8.8**	24.3**
RAE	9.9**	11.9**	54.8***	NS	3.7*	8.4**

Table 6.2 Cont.

γ- tocopherol	NS	127.7***	20.3**	7.4**	10.5***	7.8*
α-tocopherol	38.2***	NS	11.9**	4.4*	6.3**	7.8*
Others tococromanols	69.7***	NS	46.8***	NS	NS	13.4**
Total tococromanols	28.0***	27.5***	19.5**	4.3*	9.2**	10.3**
α-TEs	37.7***	NS	13.2**	4.4*	6.7**	8.3**

Figure 6.3. Fruit yield of cherry tomato as affected by ‘genotype × biostimulant’ interaction.

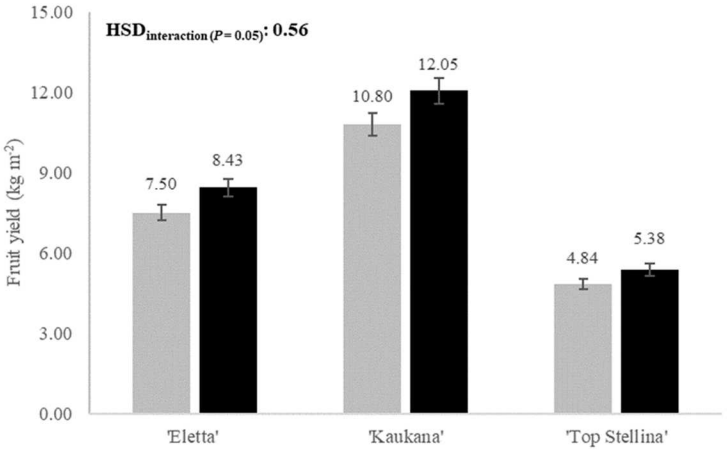


Table 6.3. Carpometric and compositional variables of cherry tomato as affected by the main factors and their first order interactions (mean ± standard deviation), with the significance resulting from the ANOVA. NS: not significant. FW: fresh weight; DM: dry matter; TCI: tomato colour index; TSS/TA: total soluble solids/titratable acidity.

	Fruit FW [g]	Fruit DM [%]	TCI	TSS/TA	D-Glucose [g/kg of FW]	D-Fructose [g/kg of FW]	Total sugars [g/kg of FW]	Citric acid [g/kg of FW]	L-Malic acid [g/kg of FW]	Total acids [g/kg of FW]
C_{II} Eletta NT	15.1 ± 0.3	8.70 ± 0.9	37.5 ± 1.6	1.40 ± 0.07	25.2 ± 2.3	29.4 ± 1.7	54.6 ± 4.3	6.05 ± 0.2	0.78 ± 0.13	6.82 ± 0.2
C_{II} Eletta T	17.3 ± 0.5	9.28 ± 0.7	38.3 ± 0.5	1.52 ± 0.03	26.5 ± 1.8	31.4 ± 3.4	57.9 ± 3.9	6.94 ± 0.2	0.78 ± 0.10	7.72 ± 0.2
C_{II} Kaukana NT	23.2 ± 1.3	6.32 ± 0.03	33.7 ± 0.5	1.31 ± 0.09	34.4 ± 0.3	42.8 ± 1.6	77.2 ± 3.3	9.05 ± 1.1	1.46 ± 0.14	10.5 ± 1.1
C_{II} Kaukana T	27.2 ± 1.1	7.28 ± 0.2	34.9 ± 0.4	1.32 ± 0.05	33.5 ± 0.3	39.6 ± 1.9	73.1 ± 2.5	9.88 ± 0.1	1.22 ± 0.10	11.1 ± 0.1
C_{II} Top Stellina NT	9.30 ± 0.5	9.70 ± 0.1	40.0 ± 1.6	0.97 ± 0.02	21.7 ± 0.6	26.4 ± 1.6	48.2 ± 5.1	6.24 ± 0.3	0.62 ± 0.06	6.86 ± 0.4
C_{II} Top Stellina T	10.5 ± 0.5	12.0 ± 0.2	39.2 ± 1.4	1.03 ± 0.03	21.4 ± 1.1	23.3 ± 1.6	44.7 ± 4.5	5.93 ± 0.4	0.48 ± 0.06	6.40 ± 0.4
C_{VI} Eletta NT	17.8 ± 0.3	7.75 ± 0.1	35.6 ± 0.5	1.79 ± 0.05	32.0 ± 1.5	37.5 ± 2.0	69.5 ± 4.0	7.24 ± 0.5	0.73 ± 0.08	7.97 ± 0.4
C_{VI} Eletta T	20.6 ± 0.6	8.72 ± 1.0	35.8 ± 0.3	1.94 ± 0.14	28.3 ± 2.8	33.6 ± 3.0	61.9 ± 5.5	5.20 ± 0.4	1.04 ± 0.14	6.24 ± 0.4
C_{VI} Kaukana NT	25.5 ± 1.1	6.73 ± 1.0	32.0 ± 3.7	1.57 ± 0.01	33.8 ± 0.7	46.1 ± 2.7	80.0 ± 1.7	10.0 ± 0.5	1.65 ± 0.14	11.7 ± 0.6
C_{VI} Kaukana T	29.1 ± 1.1	7.92 ± 0.1	32.4 ± 1.7	1.52 ± 0.01	31.0 ± 1.2	36.8 ± 2.5	67.8 ± 4.1	9.74 ± 2.1	0.74 ± 0.06	10.5 ± 2.1
C_{VI} Top Stellina NT	11.1 ± 0.2	10.3 ± 0.3	37.9 ± 1.1	1.25 ± 0.10	18.6 ± 0.8	28.6 ± 2.2	52.1 ± 3.0	4.35 ± 0.7	0.90 ± 0.18	5.26 ± 0.8
C_{VI} Top Stellina T	13.4 ± 0.5	12.3 ± 0.6	37.4 ± 1.4	1.27 ± 0.02	23.6 ± 0.9	24.9 ± 2.7	43.5 ± 2.5	5.55 ± 0.3	0.88 ± 0.08	6.43 ± 0.3

Table 6.3 Cont.

Mean values										
C_{II}	17.1 ± 6.6	8.88 ± 1.9	37.3 ± 2.5	1.26 ± 0.2	27.1 ± 5.4	32.2 ± 7.4	59.3 ± 13	7.35 ± 1.7	0.36 ± 0.89	1.97 ± 8.2
C_{VI}	19.6 ± 6.5	8.94 ± 2.0	35.2 ± 2.8	1.56 ± 0.3	27.9 ± 5.6	34.6 ± 7.3	62.5 ± 13	7.02 ± 2.4	0.34 ± 0.99	2.55 ± 8.0
'Eletta'	17.7 ± 2.1	8.61 ± 0.9	36.8 ± 1.4	1.66 ± 0.2	28.0 ± 3.2	33.0 ± 3.9	61.0 ± 6.9	6.36 ± 0.9	0.16 ± 0.83	0.78 ± 7.2
'Kaukana'	26.2 ± 2.5	7.06 ± 0.8	33.2 ± 2.1	1.43 ± 0.1	33.2 ± 1.5	41.4 ± 4.1	74.5 ± 5.4	9.68 ± 1.1	0.37 ± 1.27	1.17 ± 11
'Top stellina'	11.1 ± 1.6	11.0 ± 1.2	38.6 ± 1.6	1.13 ± 0.1	21.3 ± 2.0	25.8 ± 2.7	47.1 ± 4.8	5.52 ± 0.8	0.21 ± 0.72	0.75 ± 6.2
Control	17.0 ± 6.1	8.24 ± 1.6	36.1 ± 3.2	1.38 ± 0.3	28.5 ± 5.4	35.1 ± 7.9	63.6 ± 13	7.16 ± 2.0	0.42 ± 1.02	2.36 ± 8.2
Treated	19.7 ± 7.0	9.57 ± 2.0	36.3 ± 2.5	1.43 ± 0.3	26.6 ± 5.5	31.6 ± 6.5	58.2 ± 12	7.21 ± 2.1	0.25 ± 0.86	2.19 ± 8.1
Tukey's HSD										
Cluster (C)	0.7	NS	1.5	0.06	NS	1.7	2.7	NS	0.1	NS
Genotype (G)	0.9	0.7	1.8	0.07	1.6	2.8	4.6	0.9	0.1	0.9
Treatment (T)	0.7	0.6	NS	0.04	1.3	2.3	3.7	NS	0.1	NS
C × G	NS	0.7	NS	0.10	2.3	NS	4.7	NS	0.2	NS

6.3.2 Sugars and organic acids content

C_{VI} outperformed C_{II} in terms of D-fructose and total sugars, with 'Eletta' proving the highest rise shifting from C_{II} to C_{VI} for D-glucose and total sugars content (by 4.3 and 9.4 mg kg^{-1} FW, respectively) (**Table 6.3**). The biostimulant treatment reduced these compositional traits in both clusters, having the most depressive effect on C_{VI}, which showed a more marked drop than CII for D-glucose, D-fructose and total sugars content (by 3.8, 5.6 and 9.5 g kg^{-1} FW, respectively) (**Table 6.3**). A genotype-dependent response was recorded for D-fructose, for which the biostimulant caused a significant reduction in 'Kaucana' and 'Top Stellina' (by 6.2 and 3.4 g kg^{-1} FW, respectively) (**Table 6.3**).

When organic acids were concerned, both clusters differed only in terms of L-malic acid, whose concentration, passing from C_{II} to C_{VI}, showed an opposite trend between 'Kaucana' (from 1.34 to 1.20 g kg^{-1} FW) and 'Top Stellina' (from 0.55 to 0.89 g kg^{-1} FW) (**Table 6.3**). Moreover, the biostimulant effect on L-malic acid markedly differed among genotypes, as its concentration was promoted by the Bioup TF® in 'Eletta' (by 0.15 g kg^{-1} FW) and reduced in 'Kaucana' (by 0.57 g kg^{-1} FW) (**Table 6.3**).

Each tomato cultivar has a genetically pre-programmed maximum levels of attainable sugars content, though the external growth conditions have a relevant role in determining whether this threshold is reached or not (Beckles, 2012). The different responses of L-malic acid to the studied factors are somewhat difficult to explain, but always linked to genotype-derived interactions, thus reflecting the importance of the genetic background in modifying the fruit tricarboxylic acid cycle in response to the environmental stimuli (Etienne et al., 2015). However, it is likely that these variations will not have a significant effect

on fruit taste, given that even large variations in fruit organic acids content are not reflected in equally large variations in fruit pH (Bertin and Génard, 2018). On the other hand, the higher sugars content of C_{VI}, consistent with its higher TSS/TA ratio, reinforces the idea of a more sustained C flow toward this cluster, driven by a reduced number of fruits per plant competing for photosynthates allocation (Wardlaw, I. F., 1990). However, this points out a critical issue, consisting in a significant time-course instability of those compounds related to fruit sweetness, i.e. a trait greatly impacting consumers' acceptance for the cherry-type tomatoes (Casals et al., 2019). In this sense, the depressive effect induced by the biostimulant on fruit sugars content (mostly in C_{VI}), must be considered when overall quality of cherry tomato is concerned. Indeed this result, together with the higher fruit FW and DM content, suggest a shift in the utilization of C substrates toward non-taste related compounds, rather than a dilution effect of the fruit C pool, imposed by a higher water content. Accordingly, the non-structural carbohydrates produced by photosynthesis can serve either as energy carriers or as building blocks for anabolic processes like tissue growth (Hartmann et al., 2020). The effect we noticed seems analogous to that reported for humic substances-based biostimulants, promoting the hexoses utilization to sustain cells growth at the expense of carbohydrates content (Pizzeghello et al., 2001; Canellas et al., 2015).

6.3.3 Carotenoids content

Overall, C_{II} showed a higher concentration than C_{VI} in terms of phytoene, total lycopene and total carotenoids, while the opposite was found for *(all*-E)-β-carotene and, consequently, for RAE (**Figure 6.4**). 'Top Stellina' exhibited a slightly different behaviour, as in C_{VI} showed a slightly higher

concentration of phytoene (+211 mg 100 g^{-1} FW) and phytofluene (+169 mg 100 g^{-1} FW), along with the strongest reduction of total lycopene (which passed from 9871 to 7783 mg 100 g^{-1} FW, -21%) (**Figure 6.4**). Considering the biostimulant application, C_{VI} proved the highest responsiveness for phytoene (in which it passed from 1384 to 1696 mg 100 g^{-1} FW, +23%) phytofluene (from 889 to 1089 mg 100 g^{-1} FW, +22%), total lycopene (from 6149 to 7244 mg 100 g^{-1} FW, +18%), (all-E)-β-carotene (from 659 to 973 mg 100 g^{-1} FW, +48%), total carotenoids (from 10508 to 11503 mg 100 g^{-1} FW, +9%) and RAE (from 58 to 85, +47%) (**Figure 6.4**). On the other hand, 'Top Stellina' showed the strongest carotenoids variation in response to the Bioup TF® treatment, since its higher increase in phytofluene (from 1139 to 1367 mg 100 g^{-1} FW, +20%), total lycopene (from 8348 to 9306 mg 100 g^{-1} FW, +11%), (all-E)-β-carotene (from 683 to 1014 mg 100 g^{-1} FW, +47%), total carotenoids (from 12267 to 14198 mg 100 g^{-1} FW, +16%) and RAE (from 60 to 89, +47%) (**Figure 6.4**).

Carotenoids are C40 isoprenoids mainly associated to thylakoid membranes, where they contribute to the stability and protection of lipid bilayer matrix (Rodriguez-Concepcion et al., 2018). Environmental factors such as light and temperature are key promoters of carotenoids biosynthesis, by stimulating e.g. photosynthesis or up-regulating the transcription of key-enzymes such as phytoene synthase, i.e. one of first enzymes involved in carotenogenesis (Fanciullino et al., 2014; Lado et al., 2019). This would explain the higher phytoene and lycopene content in C_{II}, the latter being the phytonutrient most strongly associated to the differences we recorded among untreated clusters. In this sense, the strong lycopene reduction in C_{VI} probably mirrors its lower stability in response low temperature, since it has been shown that the

imposed cold stress strongly affects tomato lycopene content, probably altering the expression of the enzymatic pool acting on transformation of phytoene and phytofluene (Distefano et al., 2020). This would explain, for C_{VI}, the strong reduction of lycopene noticed in 'Top Stellina', in front of an increased concentration of both phytoene and phytofluene. Accordingly, in our experiment the number of hours with a temperature below 12 °C, i.e. the chilling threshold in tomato plants (Elizondo and Oyanedel, 2010) between fruit setting and ripening differed among C_{II} and C_{VI} (19 and 861, respectively). This represents an important temporal shift of the nutraceutical traits of the product, since lycopene is the main carotenoid of red tomatoes, characterized by an affinity for singlet oxygen and ROS scavenging activity higher than the other carotenoids, a feature configured by its peculiar acyclic polyene structure (11 conjugated double bonds) (Gruszecki and Strzałka, 2005). Differently, the enhanced β-carotene (and RAE) values in C_{VI}, could be due to a lower susceptibility of this carotenoid to chilling stress (Distefano et al., 2020) and/or its more rapid turnover after oxidation (D'Alessandro and Havaux, 2019), together with a longer time lapse required for fruit ripening in C_{VI} (85 days) than C_{II} (68 days). An interesting outcome stems from the possibility, though the use of the biostimulant, to close the gap among clusters for phytoene, phytofluene and lycopene concentration, by mainly promoting their accumulation in C_{VI}. Overall, these results are consistent with the increased lycopene content in tomato subjected to biostimulants such as seaweed extracts (Kumari et al., 2011), protein hydrolysates (Rouphael et al. (2017) or microbial-based biostimulant (Sani et al., 2020). Beyond this, our experiment, designed to measure the biostimulant effect as a function of the cluster position (i.e. harvest period), highlighted a greater

stimulatory action on C_{VI} carotenoids pool, i.e. during the least favourable period for their accumulation. Indeed, the coefficient of variation among clusters for total carotenoids concentration passed from 15.2% (control plants) to 0.1% (treated plants), indicating the possibility to reduce the nutraceutical variability of the product, by leveraging the accumulation of the most fluctuating carotenoids. Beyond lycopene, this is relevant for phytoene and phytofluene too, since the growing evidence of their health-promoting effects in human organism, along with their high bioaccessibility and persistence in human plasma and tissues (Mapelli-Brahm and Meléndez-Martínez, 2021). However, it must be pointed out the contrasting effects of the Bioup TF® application on reducing sugars and carotenoids content of C_{VI} suggesting, during the growth-limiting months, the existence of a metabolic load burdening the nutraceutical enhancement of cherry tomato, at the expense of the taste-related C pool. According to the C theory, both environmental stimuli and plant fruit load influence the C gain and its allocation in fruits, and the resulting C status (carbohydrate concentration) would configure the synthesis of primary and secondary metabolites, by acting on the whole size of plastidial equipment. More specifically, the fruit accumulation of soluble sugars during early growth would negatively affects plastids development, hence carotenoid accumulation, whereas their lower availability would promote fruit photosynthesis by boosting plastids size and density, thus enhancing the storage of carotenoids (Fanciullino et al., 2014). Consistent with this hypothesis, during early ontogeny (green stage), tomato fruits are characterized by a partially photosynthetic phase, making them able to fix up to 10-15% of C required for their growth (Bertin and Génard, 2018).

Table 6.4. Quantitation of individual carotenoids (µg 100 g-1 FW) (mean ± standard deviation) in relation to the studied factors and their first order interactions. CII: cluster 2; CVI: cluster 6; NT: control; T: treated.

	Phytoene (1)	Phytoene (2)	Phytofluene (1)	Phytofluene (2)	ζ-Carotene	(13Z)-Lycopene	(9Z)-Lycopene	(7Z)-Lycopene isomer	(all-E)-Lycopene	(5Z)-Lycopene	γ-Carotene isomer	(all-E)-Lutein	(all-*E*)-γ-Carotene	RAE
C_{II} 'Eletta' NT	132 ± 21	1804 ± 211	206 ± 5.6	868.0 ± 88.9	131 ± 33	314 ± 135	26.5 ± 6.3	193 ± 30	7713 ± 67.2	1200 ± 1147	17.7 ± 1.8	62 ± 22	98 ± 12	52.9 ± 8.3
C_{II} 'Eletta' T	137 ± 16	1762 ± 209	253 ± 19	815.2 ± 79.9	108 ± 15	197 ± 47	43.1 ± 38	121 ± 33	5383 ± 624	665 ± 571	22.5 ± 1.9	37 ± 16	79 ± 4.0	75.2 ± 17
C_{II} 'Kaukana' NT	102 ± 2.2	1202 ± 60.1	180 ± 7.2	649.5 ± 52.2	97.1 ± 6.7	148 ± 20	18.3 ± 13	103 ± 14	4481 ± 833	369 ± 291	17.5 ± 1.9	48 ± 5.7	53 ± 3.3	51.4 ± 1.2
C_{II} 'Kaukana' T	107 ± 11	1236 ± 105	200 ± 19	649.5 ± 7.77	103 ± 11	158 ± 31	26.5 ± 13	111 ± 4.7	4450 ± 903	437 ± 175	18.7 ± 1.2	50 ± 2.3	64 ± 6.4	57.5 ± 3.2
C_{II} 'Top stellina' NT	112 ± 13	1583 ± 72.8	200 ± 17	834.0 ± 12.7	131 ± 24	232 ± 46	26.8 ± 15	179 ± 17	7963 ± 1025	940 ± 455	16.9 ± 3.0	55 ± 23	76 ± 11	66.0 ± 6.2
C_{II} 'Top stellina' T	146 ± 14	2027 ± 56.7	262 ± 25	1041 ± 78.1	144 ± 10	256 ± 35	26.0 ± 13	208 ± 13	9044 ± 838	866 ± 544	21.0 ± 2.0	55 ± 4.2	85 ± 8.6	73.4 ± 5.2
C_{VI} 'Eletta' NT	132 ± 7.3	1154 ± 194	136 ± 16	681.4 ± 101	71.8 ± 112	194 ± 13	25.1 ± 20	122 ± 19	5947 ± 910	503 ± 229	21.9 ± 5.0	41 ± 10	73 ± 5.3	61.2 ± 9.2
C_{VI} 'Eletta' T	153 ± 16	1541 ± 107	218 ± 42	819.3 ± 39.0	68.7 ± 10	280 ± 60	87.7 ± 93	166 ± 5.1	6590 ± 861	962 ± 731	37.2 ± 18	39 ± 2.7	91 ± 12	77.8 ± 1.7
C_{VI} 'Kaukana' NT	88.3 ± 8.5	748 ± 65.6	98.3 ± 10	508.5 ± 49.6	54.5 ± 7.4	107 ± 16	6.85 ± 5.4	61.0 ± 10	3907 ± 319	219 ± 138	17.8 ± 2.4	32 ± 5.3	45 ± 5.3	58.4 ± 5.4
C_{VI} 'Kaukana' T	115 ± 10	1017 ± 115	138 ± 16	660.0 ± 53.8	69.4 ± 9.2	139 ± 31	11.7 ± 12	75.0 ± 2.1	4864 ± 268	346 ± 280	24.1 ± 3.3	35 ± 5.9	56 ± 9.3	74.0 ± 7.0
C_{VI} 'Top stellina' NT	230 ± 22	1799 ± 161	224 ± 20	1020 ± 46.7	89.8 ± 1.6	202 ± 12	31.9 ± 15	159 ± 6.1	6332 ± 282	630 ± 255	19.7 ± 5.8	23 ± 10	58 ± 2.6	55.0 ± 6.8
C_{VI} 'Top stellina' T	235 ± 10	2027 ± 28.4	269 ± 10	1162 ± 21.7	116 ± 5.2	217 ± 4.1	26.5 ± 18	171 ± 8.8	7211 ± 645	585 ± 217	25.0 ± 5.1	58 ± 6.0	82 ± 4.2	105 ± 13

Table 6.4 Cont.

Mean values														
C_{II}	123 ± 21	1602 ± 330	217 ± 34	809.5 ± 148	119 ± 24	218 ± 80	30.9 ± 19	152 ± 47	6506 ± 1973	489 ± 186	19.0 ± 2.7	51.1 ± 15	76.6 ± 16	62.7 ± 12
C_{VI}	159 ± 58	1381 ± 472	181 ± 65	808.5 ± 234	78.4 ± 21	190 ± 62	22.9 ± 17	126 ± 46	5808 ± 1251	471 ± 231	24.3 ± 9.5	38.1 ± 13	67.4 ± 17	71.8 ± 19
'Eletta'	138 ± 16	1565 ± 312	203 ± 49	795.9 ± 100	94.6 ± 32	246 ± 86	37.1 ± 21	150 ± 38	6408 ± 1084	481 ± 146	24.8 ± 11	44.8 ± 16	85.3 ± 13	66.8 ± 14
'Kaukana'	103 ± 12	1051 ± 216	154 ± 43	616.9 ± 76.0	81.1 ± 22	138 ± 29	15.8 ± 12	87.5 ± 22	4425 ± 658.2	343 ± 213	19.5 ± 3.5	41.2 ± 9.2	54.6 ± 9.0	60.3 ± 10
'Top stellina'	181 ± 57	1859 ± 209	239 ± 34	1014 ± 129	120 ± 24	227 ± 33	27.8 ± 13	179 ± 21	7637 ± 1222	616 ± 170	20.7 ± 4.8	47.8 ± 19	75.3 ± 13	74.7 ± 21
Control	133 ± 49	1382 ± 411	174 ± 46	760.2 ± 180	95.8 ± 33	199 ± 84	22.6 ± 14	136 ± 49	6057 ± 1649	493 ± 241	18.6 ± 3.6	43.5 ± 18	67.4 ± 19	57.5 ± 7.6
Treated	149 ± 44	1602 ± 404	224 ± 51	857.9 ± 199	102 ± 29	208 ± 61	31.3 ± 21	142 ± 47	6257 ± 1724	466 ± 173	24.8 ± 8.9	45.7 ± 11	76.1 ± 14	77.1 ± 17
Tukey's HSD														
Cluster (C)	13	121	18	NS	12	NS	NS	15	599	NS	4	10	7	7
Genotype (G)	16	148	22	61	14	NS	16	18	733	218	NS	NS	9	9
Treatment (T)	13	121	18	50	NS	NS	NS	NS	NS	NS	5	NS	7	7
C × G	22	209	32	86	NS	NS	NS	NS	1037	NS	NS	NS	NS	NS

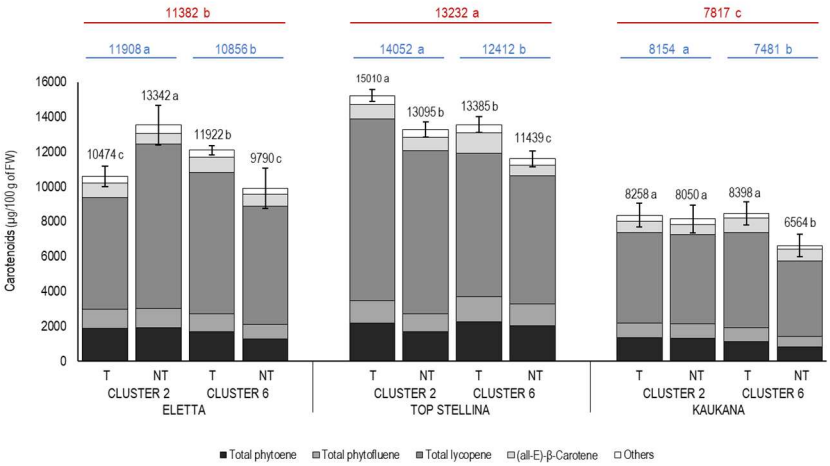


Figure 6.4. Contents of major carotenoids in cherry tomato fruits. Means separation is related to the genotype effect (in red), cluster effect (in blue) and treatment effect (in black).

6.3.4 Tocochromanols content

A key class of chemicals belonging to the group of vitamin E compound, play an important role as antioxidant from lipid peroxidation in tomatoes. These lipid-soluble molecules are tocopherols and tocotrienols, collectively known as tocochromanols or tocols. The relationship among the tocochromanols detected in our study is shown in **Table 6.5**. Tocopherols were the main representative tocochromanols (99.2% of total), whereas tocotrienols accounted for the remaining 0.8%. Among the tocopherols, α-tocopherol was the most abundant (75%, on average), followed by γ-tocopherol (23%) then by ß-tocopherol (2%) (**Table 6.5**). Most tocochromanols concentrations differed among the main factors, with C_{II} outcompeting C_{VI} mostly for γ-tocopherol, and total tocochromanols (**Table 6.5**). Moreover,

when C_{VI} was harvested, 'Top Stellina' showed the most severe reduction in γ-tocopherol (from 644 to 289 mg 100 g^{-1} FW, -55%) and total tocochromanols (from 1879 to 1469 mg 100 g^{-1} FW, -22%) (**Table 6.5**), whereas 'Eletta' did so for α-tocopherol (from 1009 to 829 mg 100 g^{-1} FW, -18%) (**Table 6.5**) and α-TES (from 1074 to 871, 19%) (**Figure 6.5**). On the other hand, C_{VI} proved the strongest positive response to the biostimulant application, since, in comparison to the untreated plants, it showed the highest increase in α-tocopherol (1045 vs. 799 mg 100 g^{-1} FW, +31%), γ-tocopherol (385 vs. 280 mg 100 g^{-1} FW, +68%), other tocochromanols (39.5 vs. 28.9 mg 100 g^{-1} FW, +37%), total tocochromanols (1469 vs. 1057 mg 100 g^{-1} FW, +39%) and α-TES (1099 vs. 833 mg 100 g^{-1} FW, +32%) (**Figure 6.5**). Among the studied genotypes, the highest responsiveness to the treatment was always recorded in 'Top Stellina', whose fruit composition was enhanced mainly in terms of a-tocopherol (1331 vs. 1000 mg 100 g^{-1} FW, +33%), γ-tocopherol (566 vs. 367 mg 100 g^{-1} FW, +54%), total tocochromanols (1945 vs. 1404 mg 100 g^{-1} FW, +39%) and α-TES (1407 vs. 1052, +34%) (**Figure 6.5**).

Tocopherols and tocotrienols, collectively known as tocochromanols, represent the main vitamin E groups of compounds, comprising thylakoid-anchored molecules through their hydrophobic prenyl tail, where they exert antioxidant functions owing to their polar chromanol head (Muñoz and Munné-Bosch, 2019). In plants they contribute to membrane lipid stability, as they participate in quenching/scavenging mainly the PSII-derived ROS, thus contributing to cell integrity both in leaves (mostly a-tocopherol) and fruits (g-tocopherol and tocotrienols) (Havaux et al., 2005; Gramegna et al., 2019). When considered as food constituents, their intake has been

correlated to health-promoting effects such as reduced risk of miscarriage, inhibition of lung cancer, delayed brain ageing and lower cholesterogenesis and incidence of Alzheimer's disease (Mène-Saffrané, 2018; Raiola et al., 2015). Environmental factors such as light intensity and temperature converge to promote the tocopherols accumulation in plants (Lushchak and Semchuk, 2012), a feature that would explain the highest tocochromanols content (mainly γ-tocopherol) in C_{II}. Indeed, this cluster ripened in a period more favourable to foster the metabolic activity of the fruit photosynthetic apparatus, hence its ROS-generating activity throughout the period preceding the conversion of chloroplast to chromoplast (Miret and Munné-Bosch, 2015). Alternatively (or concomitantly), lower temperatures, as those experienced during C_{VI} ripening, may limit the availability of phytyl diphosphate, a degradation product of chlorophyll prompting chromanol synthesis through the phytol recycling pathway (Spicher et al., 2006; Muñoz and Munné-Bosch, 2019). From a nutritional viewpoint, such compositional inconstancy represents a critical issue of Mediterranean greenhouse tomato, since its dietary importance implies that even small variations in the phytonutrient composition, can generate significant dietary effects for consumers (Mauro et al., 2020). This must be considered taking into account that converging surveys have highlighted mild to severe vitamin E deficiencies in significant proportion of populations, even in developed areas such as Europe (Mène-Saffrané, 2018; Polito et al., 2005).

In our experiment, the overall tocochromanols increase induced by the biostimulant, is consistent with those reported by Casadesús et al. (2019) and Mannino et al. (2020) subjecting tomato plants to animal-derived protein hydrolysates (Pepton®) or seaweed + yeast extracts

(Expando®), respectively. However, considering the biostimulant effect as a function of the cluster position, a clear analogy stems among fruit carotenoids and tocochromanols concentration. Indeed, the biostimulant acted to marginalize the differences among clusters, through a more marked tocochromanols accumulation in C_{VI}, thus stabilizing their concentration over time and counteracting the physiological constraints imposed by variable environmental conditions. However, even in this case, the contrasting response of soluble sugars and tocochromanols content, reinforces the idea of divergent biostimulant effects on primary and secondary pathways of C metabolism, through a low sugar-driven, enhanced size of fruit biosynthetic machinery. Accordingly, it has been reported that both plastid size and density are intimately linked to cell chlorophyll content, whereas the degradation of this pigment accompanying fruit ripening is strongly correlated to tocopherols accumulation (Fanciullino et al., 2014; Muñoz et al., 2018; Xiong et al., 2017).

Table 6.5. Quantitation of individual minor tocotrienols (µg 100 g^{-1} FW) (mean ± sd) referred as 'Others' in Figure 6.4.

Source of variation	γ-tocotrienol	α-tocotrienol	ß-tocopherol	γ-tocopherol	α-tocopherol	Total Tocopherol (T)	Total Tocotrienol (T3)	Total Tocochromanols (T3±T)	α-Tocopherol Equivalents (α-TEs)
C_{II} 'Eletta' NT	2.1 ± 0.4	5.2 ± 0.6	25 ± 1.0	567 ± 89	1082 ± 217	1674 ± 295	7.3 ± 0.2	1682 ± 295	1153 ± 224
C_{II} 'Eletta' T	2.9 ± 0.1	6.7 ± 0.9	25 ± 2.4	439 ± 25	936 ± 184	1400 ± 210	9.6 ± 0.9	1410 ± 210	994 ± 187
C_{II} 'Kaukana' NT	2.0 ± 0.2	5.9 ± 0.3	15 ± 0.5	451 ± 14	676 ± 30	1142 ± 36	7.9 ± 0.2	1150 ± 36	731 ± 31
C_{II} 'Kaukana' T	2.4 ± 0.5	8.4 ± 1.2	19 ± 2.6	544 ± 26	708 ± 44	1271 ± 71	10.8 ± 1.5	1282 ± 73	774 ± 48
C_{II} 'Top Stellina' NT	3.0 ± 0.7	11 ± 1.0	27 ± 3.8	572 ± 137	1098 ± 138	1697 ± 278	13.7 ± 1.6	1711 ± 279	1172 ± 153
C_{II} 'Top Stellina' T	4.3 ± 0.3	8.5 ± 1.5	30 ± 3.4	716 ± 57	1290 ± 53	2035 ± 77	12.8 ± 1.8	2048 ± 77	1379 ± 52
C_{VI} 'Eletta' NT	2.6 ± 0.7	9.1 ± 1.6	16 ± 0.9	276 ± 84	747 ± 67	1039 ± 151	11.7 ± 1.9	1051 ± 153	786 ± 76
C_{VI} 'Eletta' T	3.0 ± 0.4	11 ± 0.8	21 ± 1.7	334 ± 58	910 ± 78	1265 ± 84	13.5 ± 1.3	1278 ± 83	957 ± 76
C_{VI} 'Kaukana' NT	2.0 ± 0.2	6.0 ± 0.9	18 ± 1.1	252 ± 27	754 ± 119	1016 ± 145	8.1 ± 1.1	1024 ± 145	781 ± 121
C_{VI} 'Kaukana' T	1.9 ± 0.2	7.2 ± 0.4	23 ± 1.7	405 ± 28	852 ± 72	1279 ± 86	9.1 ± 0.3	1288 ± 86	906 ± 73
C_{VI} 'Top Stellina' NT	3.5 ± 0.4	8.3 ± 0.6	21 ± 1.1	161 ± 19	903 ± 58	1085 ± 73	12.8 ± 1.0	1097 ± 73	932 ± 60
C_{VI} 'Top Stellina' T	4.5 ± 0.1	14 ± 1.5	34 ± 4.4	416 ± 56	1373 ± 140	1823 ± 104	15.5 ± 1.6	1841 ± 104	1436 ± 137

Table 6.5 Cont.

Mean values									
C_{II}	2.8 ± 0.9	7.6 ± 2.1	23 ± 5.4	548 ± 112	965 ± 251	1537 ± 348	10 ± 2.6	1547 ± 349	1034 ± 262
C_{VI}	2.9 ± 1.0	9.1 ± 2.7	22 ± 6.3	307 ± 101	922 ± 232	1251 ± 299	12 ± 3.5	1263 ± 301	966 ± 241
'Eletta'	2.7 ± 0.5	7.9 ± 2.3	22 ± 4.2	404 ± 130	919 ± 179	1345 ± 295	11 ± 2.6	1355 ± 293	973 ± 190
'Kaukana'	2.0 ± 0.3	6.9 ± 1.2	19 ± 3.1	413 ± 112	745 ± 94	1177 ± 138	9.0 ± 1.4	1186 ± 139	798 ± 94.1
'Top stellina'	3.9 ± 0.7	10 ± 2.5	28 ± 5.8	466 ± 225	1166 ± 210	1660 ±393	14 ± 2.8	1674 ± 394	1230 ± 227
Control	2.6 ± 0.7	7.6 ± 2.2	20 ± 4.9	380 ± 176	875 ± 199	1276 ± 341	10 ± 2.7	1286 ± 341	926 ± 213
Treated	3.2 ± 1.0	9.1 ± 2.6	25 ± 5.8	476 ± 133	1011 ± 262	1512 ± 328	12 ± 3.3	1525 ± 330	1074 ± 268
Tukey's HSD									
Genotype (G)	0.5	1.2	2.8	NS	136	187	1.5	187	141
Cluster (C)	NS	1.0	NS	60	NS	152	1.2	153	NS
Treatment (T)	0.4	1.0	2.3	60	111	152	1.2	153	115
C × G	NS	1.7	4.0	104	193	264	2.1	264	199

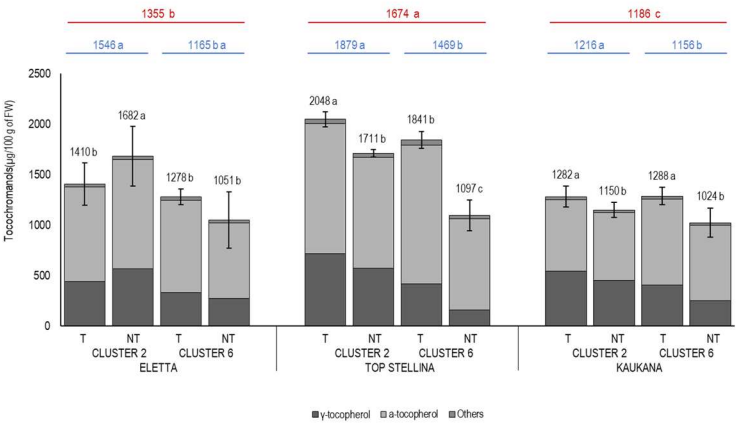


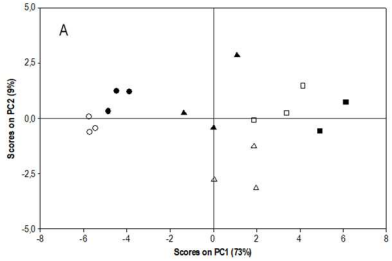
Figure 6.5. Contents of major tocochromanols in cherry tomato fruits. Means separation is related to the genotype effect (in red), cluster effect (in blue) and treatment effect (in black).

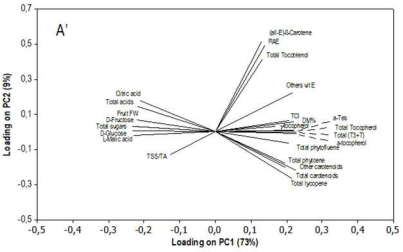
6.3.5 PCA

The PCA b-plots related to C_{II} and C_{VI} are reported in **Figure 6.6**, whereas the correlation statistics in are reported in **Table 6.6.** For both clusters the first principal component gave eigenvalue greater than 1, and together accounted for more than 80% of total variance. When C_{II} was concerned the PC1 was positively correlated to α-tocopherol, α-Tes, total carotenoids, other and total tocopherols+tocotrienols and fruit DM; and negatively correlated to citric acid, malic acid, total sugars, D-glucose and fruit FW. The PC2 was positively correlated to (all-E)-β-carotene, RAE, Total tocotrienols while was negatively correlated to total lycopene, total carotenoids, other carotenoids and TSS/TA. For the C_{VI} PC1 was positively correlated to fruit DM, total phytoene, total phytofluene, total carotenoids and total tocotrienols and negatively correlated to D-glucose, D-fructose, total sugars,

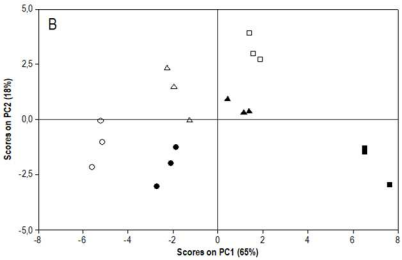
total acids. The PC2 positively contributed to TCI, total phytoene, total lycopene and total carotenoids while was negatively correlated to citric acid, total acids, (all-E)-β-carotene, REA , γ-tocopherol and total tocopherol.
Regarding C_{II} the PC1 axes (73% of total variance) separated 'Kaucana' (on the left side) from the other genotypes, which partly overlapped among them, maybe as a consequence of their similar genetic background (same seed company). On the other hand, excepting for 'Eletta', no clear separation was detectable on the basis of biostimulant application. Such separation mainly derived from the genotypic differences in terms of organic acids, sugars and fruit FW (higher in 'Kaucana').
Differently, for C_{VI} the b-plot (**Figure 6.6B**) highlighted a stronger biostimulant separation effect of PC1 along the tested genotypes, since the biostimulant-driven difference in terms of α-TEs, total tocopherols + tocotrienols, total tocotrienol ('Top Stellina'), α-TEs, total tocopherols + tocotrienols and fruit FW('Eletta'), α-TEs, total tocopherols + tocotrienols, and fruit DM ('Kaucana').

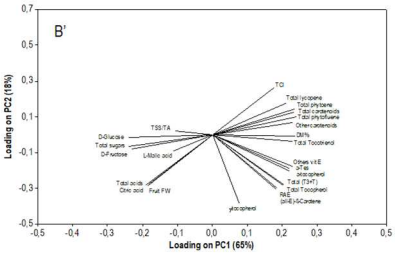
Cluster II





Cluster VI





▲ ‘Eletta’

● ‘Kaucana’

■ ‘Top Stellina’

Figure 6.6. PCA biplots (A-B) and score plots (A’-B’) calculated on the basis of all measured variables. White symbols: control plants; black symbols: treated plants. Genotype and biostimulant effects are reported separately for each cluster.

Table 6.6. Correlation coefficients for each measured trait with respect to the first two principal components, eigenvalues, relative and cumulative proportion of total variance, related to clusters II and VI.

Variable	Cluster II		Cluster VI	
	PC 1	PC 2	PC 1	PC 2
Fruit FW	-0.219	0.070	-0.181	-0.276
Fruit DM	0.220	0.055	0.237	-0.008
TSS/TA	-0.126	-0.131	-0.105	0.021
TCI	0.208	0.064	0.174	0.263
D-Glucose	-0.229	0.030	-0.238	-0.016
D-Fructose	-0.211	0.176	-0.232	-0.079
Total sugars	-0.229	-0.018	-0.238	-0.065
Citric acid	-0.232	0.006	-0.185	-0.287
L-Malic	-0.232	0.032	-0.112	-0.093
Total acids	-0.218	0.147	-0.189	-0.282
Total phytoene	0.194	-0.175	0.235	0.144
Total phytofluene	0.205	-0.061	0.240	0.102
Total lycopene	0.214	-0.261	0.210	0.174
(all-E)-β-Carotene	0.130	0.513	0.181	-0.303
Other carotenoids	0.198	-0.200	0.229	0.072
Total carotenoids	0.223	-0.216	0.233	0.125
RAE	0.138	0.493	0.183	-0.294
γ-tocopherol	0.166	0.031	0.076	-0.383
α-tocopherol	0.226	-0.007	0.221	-0.189
Total tocopherol	0.216	0.225	0.202	-0.281
Other tocopherols+tocotrienols	0.220	0.007	0.228	-0.176
Total tocotrienols	0.132	0.411	0.230	-0.034
Total tocopherols+tocotrienols	0.220	0.010	0.203	-0.279
α-Tes	0.226	-0.003	0.220	-0.202
Eigenvalue	17.486	2.080	15.673	4.269
Explained variability (%)	73	9	65	18
Accumulated variability (%)	73	82	65	83

6.4 Conclusions

The results of the present experiment highlighted the complex and variable effect on the chemical composition and bioactive properties of the tested cherry tomatoes cultivars quality parameters in response to the studied factors.

Our findings indicate that the plant-based biostimulant BioupTF® significantly affected both carpometric and nutraceutical quality of cherry tomatoes 'Top Stellina', 'Eletta' and 'Kaucana', even if with not always comparable trends and in a genotype-dependent way. On the other hand,

the cluster position influenced too both the quality traits of the fruits such as fruit FW, fruit DM, TCI, total acids, L-malic acid, glucose, fructose, total sugars, and nutraceutical traits, including α-tocotrienol, γ-tocopherol, total tocopherol, total phytoene, (all-E)-β-carotene, total lycopene, total carotenoid and RAE.

Despite the fact that fruits from C_{VI} had developed under sub-optimal conditions in terms of temperature and solar radiation compared to C_{II} between setting and harvest, shifted from 22.5 °C and 326.6 MJ m^{-2} (C_{II}) to 14.7 °C and 423.3 MJ m^{-2} (C_{VI}), the biostimulant applications were capable to increase the amount of several nutraceutical compounds such as γ-tocotrienol, α-tocotrienol, total tocotrienol, phytoene (1), (*all*-E)-β-carotene and RAE content.

In conclusion, the variable effects of the biostimulant applied, with different cluster positions, on different cherry tomato varieties mark the need for further research in order to more deeply understand how to improve cherry tomato quality in cold greenhouse during the winter cycle.

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7. Effects of Genotype, Storage Temperature and Time on Quality and Compositional Traits of Cherry Tomato

The following work has been already published as:

Distefano, M., Arena, E., Mauro, R. P., Brighina, S., Leonardi, C., Fallico, B., & Giuffrida, F. (2020). Effects of genotype, storage temperature and time on quality and compositional traits of cherry tomato. Foods, 9(12), 1729.

7.1 Introduction

Tomato (*Solanum lycopersicum* L.) is one of most important vegetable crops throughout the world, with an estimated production of 182 Mt from more than 4.8 Mha cropland (FAO, 2020). In the Mediterranean basin it is the primary field and greenhouse vegetable crop (Mauro et al., 2015), since tomato strongly characterizes the Mediterranean diet, hence its consumption is widely spread around this macro area (Capurso and Vendemiale, 2017). Fresh tomatoes commercialization is often characterized by significant temporal gaps among production and consumption. This implies the optimization of quality maintenance of the product along the distribution chain, in order to match the consumers' sensorial and nutritional demands (Cainelli and Ruperti, 2019). Indeed, fresh vegetables are perishable commodities, whose postharvest decay represents a primary matter of social concern in terms of economic (loss of capital, fuel and manpower) and environmental costs (due to landfilling), associated to losses of valuable phytonutrients (Mauro et al., 2020). In this context, temperature is a key factor to extend quality of fresh horticultural products along the distribution chain (Petric et al., 2018). Because of its sensitivity to chilling injuries (Affandi et al., 2015), the optimization of tomatoes cold storage implies a compromise between temperatures low enough to slow down the ripening process but high enough to generate either no or tolerable side effects on the main organoleptic and nutritional traits (Tadesse et al., 2015). Similarly to other plant foods (Farzaneh et al., 2018), tomato is a source of many valuable phytonutrients having potential health benefits, including

minerals, vitamins C and E, organic acids, polyphenols and carotenoids (Park et al., 2018) . Carotenoids represent by far the most studied phytochemical fraction of tomatoes (Martí et al., 2016), which are considered the main dietary source of lycopene (Story et al., 2010), i.e., the prevailing constituent conferring the typical pigmentation to red-ripe fruits. From a nutritional viewpoint, lycopene is a powerful antioxidant, whose intake has been linked to reduced frequency and severity of several types of cancer and heart diseases (Peters et al., 2007). Moreover, it has been indicated as the most effective singlet oxygen quencher among all known carotenoids (Srivastava and Srivastava, 2015). β-carotene is the second main carotenoid constituent of tomato fruits (Liu et al., 2015). It is a red-orange pigment having strong chemoprotective functions and the highest provitamin A activity in the human metabolism, and its deficiency can result in xerophthalmia, blindness, and even premature death (Gul et al., 2015). Although both carotenoids can be specifically ingested through dietary supplements, scientific evidences seem to point out stronger health benefits associated to their direct assumption from tomato matrices, likely as a consequence of synergistic effects involving other naturally occurring compounds (Basu et al., 2007). Among these, the colourless carotenoids phytoene and phytofluene have been supposed to have biological activity, as in the case of skin protection from UV-induced erythema or in the protection of human lipoproteins from oxidation (Engelmann et al., 2011). Over the last decades, cherry tomato has been intensively targeted in breeding programs of many seed companies, in order to match the evolving standards in tomato production, commercialization and consumption

(Passam et al., 2007). Consequently, the currently available cultivars are characterized by better functional profile than the past (Raiola et al., 2014), wide compositional variability (Kavitha et al., 2014) and rapid temporal turnovers. Such diversification and dynamism represent a challenging task to optimize the product management along the distribution chain, since postharvest quality modifications are strongly affected by both storage conditions and genotype (Alenazi et al., 2020). Hence, to address the growing demands for tomatoes with high quality and functional profiles, it is appropriate to in-depth the knowledge about whole patterns of change in these properties, as a function of the storage conditions applied to the emerging germplasm.

Due to this, the aim of the present work was to investigate the postharvest modifications on main quality variables of three recently widespread cherry tomato cultivars in a Mediterranean environment induced by different thermal regimes (10 and 20 °C) and storage time (up to 14 days).

7.2 Materials and Methods

7.2.1. Experimental Site and Plant Material

A greenhouse experiment was carried out from February to June 2019, at the experimental farm of the University of Catania (Sicily, South Italy: 3724027” N, 1503036” E, 6 m a.s.l.). The climate of the area is semi-arid Mediterranean, with mild winters and hot, dry summers. An 800 m2, multi-aisle cold greenhouse was used, having a steel tubular structure with adjustable windows on the roof and along the sides, and covered with polycarbonate slabs. Three cherry tomato cultivars, namely ‘Eletta’, ‘Ottymo’ and ‘Sugarland’, recently di used in the reference area, were grown in the

experiment, chosen on the basis of their different main carpometric traits (**Table 7.1**). To this end, data were previously acquired from different local farms operating in comparable growth conditions.

Table 7.1. This is a table. Tables should be placed in the main text near to the first time they are cited.

	'Eletta'	**'Ottymo'**	**'Sugarland'**
Seed company	TSI Italia srl, Foggia (FG), Italy	Vilmorin Italia srl, Funo (BO), Italy	Rijk Zwaan Italia srl, Bologna (BO), Italy
Fruit color	Deep red	Red	Deep red
Average fruit diameter (mm)	15 ± 2	18 ± 2	12 ± 1
Average fruit weight (g)	15.0 ± 1.5	20.5 ± 2.5	12.0 ± 1.0

7.2.2. Growth conditions, fruit sampling and storage

Plants were transplanted on 11th February 2019 within the greenhouse at the stage of two true leaves, in an open soilless cultivation system using 5 L plastic pots (20 cm height, 19 cm width), with perlite as growing medium (particle size 2-6 mm). Before transplanting, plantlets were selected for uniform size and health appearance, whereas pots were arranged in simple rows, adopting a 0.40 × 1.00 m rectangular format (centre to centre) and 1 plant per pot (2.5 plants m^{-2}). Plants were grown at single stem up to the 8th cluster, whereas all clusters were pruned leaving 12 fruits, whose setting was allowed by using bumblebee hives. Each net experimental unit contained 12 plants. During the cycle, the crop was uniformly fertigated with a standard nutrient solution (Mauro et al., 2020b), adopting a leaching fraction of at least 75%, to avoid root zone salinization (Giuffrida et al., 2018).

From 14 to 16 May, tomatoes belonging to the 4th cluster were harvested by hand at the red stage (stage F) according to Gautier et al. (2008). This was done to allow tomatoes to reach stage G (deep red) during postharvest, as it is usual among local growers. Soon after harvest, fruits were transported to the laboratory and processed for further analysis. Overall, 72 clusters were collected (8 clusters x 3 cultivars x 3 replicates) and divided in 3 batches for the characterization of fruits after 0 (harvest date), 7 and 14 days of storage (hereafter S_0, S_7 and S_{14}, respectively), stored either at 10 $\pm$ 0.5 (T_{10}) or 20 $\pm$ 0.5 °C (T_{20}) and 85% relative humidity (RH). The lowest thermal regime was chosen since it represents a mild stressing conditions frequently adopted during transportation and storage of cherry tomatoes, whereas T_{20} was comparatively chosen as it simulates storage at room temperature (Khairi et al., 2018). Before storage, fruits were detached from rachis, selected for absence of defeats and uniform appearance within each genotype, washed with deionized water and dried with paper for further analysis. Fifteen to twenty-two fruits per replicate were placed in common commercial trays, i.e. transparent PET trays Mod. C500/41p (190 × 115 × 41 mm) covered with a perforated PET LC32 lid (Carton Pack s.p.a., Rutigliano, Italy) for a final net weight of 250 $\pm$ 8 g, then stored at the abovementioned conditions.

7.2.3. Carpometric determinations

At each time point, fruit fresh weight was determined on 10 fruits per tray, then their firmness was determined through a Digital Texture Analyser mod. TA-XT2 (Stable Micro Systems, Godalming, UK) and defined as the force (N)

needed to impress a 2 mm fruit deformation along the polar axis, between two steel plates.

7.2.4. Cherry tomato quality variables

For each sample, ~50 g of cherry tomatoes were homogenized up to a puree in a home blender (La Moulinette, Moulinex, 2002) and immediately analyzed for: soluble solids content, dry matter, pH, total acidity (TA), reducing sugars, total polyphenols and carotenoids profile and content. The soluble solids content was estimated with an Abbe refractometer (Carl Zeiss 16531) at 20 °C and the results were expressed as °Brix. The dry matter was determined by gravimetric analysis. An aliquot of cherry tomato puree was placed in an oven at 70 °C (Thermo Scientific-Herathermoven) until the constant weight (Carli et al., 2011). The pH was measured using a pHmeter (Mettler Toledo, MP 220), and TA was determined by titrating an aliquot of the puree sample with 0.05 N NaOH to pH 8.1. TA was expressed as g kg^{-1} of cherry tomato fresh weight (FW), as citric acid (Baldwin et al., 2015).

Reducing sugars (fructose and glucose), were estimated using Fehling's method according to the official Italian method of analysis (D.M. 3.2.1989, GU n.168/1989). An aliquot of the puree sample (20 g) was transferred into a volumetric flask (50 ml) and neutralized with 1 N NaOH. Subsequently sample was cleared by the addition of 10 ml saturated sodium sulphate decahydrate and 5 ml saturated basic lead acetate. The samples were diluted up to 50 g with distilled water, mixed and centrifuged for 10 min at 10,000 rpm. The supernatant was filtered through a filter paper (Whatman No 1, Whatman International, Maidstone, UK) and used to

completely reduce in hot condition a mixed of the Fehling's solution using methylene blue solution as indicator. The Fehling solution was prepared as follow: 5 ml of each stock Fehling solution A and B were mixed with 40 ml of distilled water immediately before the determination. Results were expressed as g of reducing sugars kg^{-1} of dry weight (DW) and all analysis were conducted in triplicate.

7.2.5. Fruit chromatic coordinates

The fruit chromatic coordinates were measured as described by McGuire (1992) on the equatorial axis of whole fruits (two measurements per fruits), through a tristimulus Minolta Chroma meter (model CR-200, Minolta Corp.) calibrated with a standard white tile (UE certificated) with illuminant D65/10°, measuring color in terms of lightness (L*), green-red axis (a*) and blue-yellow axis (b*). Fruit color was described as $(a^*/b^*)^2$, Chroma [as $(a^{*2} + b^{*2})^{1/2}$], tomato color index [TCI = $2000\ a^*/L^*(a^{*2} + b^{*2})^{1/2}$] and total color difference [$\Delta E^*_{ab} = (\Delta L^{*2} + \Delta a^{*2} + \Delta b^{*2})^{1/2}$], this last describing the color deviation recoded at S_7 and S_{14}.

7.2.6. Total polyphenols content

The extraction of polyphenol compounds was performed according to Atanasova et al. (2014) with some modifications. An aliquot of cherry tomato puree sample (1 g) was mixed and shacked with 40 ml of acetone (80% solution in distilled water) and left in the dark, overnight at room temperature. After that, each sample was filtered (0.45 µm Albet) and the supernatant was collected for determination of total polyphenols content (TPC). This was determined according to Gahler et al. (2003) using the Folin-Ciocâlteu reagent and measuring spectrophotometrically the

absorbance at 725 nm using a Perkin Elmer lambda 25 Uv-Vis spectrometer. Gallic acid was used as standard (standard curve, 0.29-8.18 mg kg^{-1}; $R^2 = 1.00$) and TPC was expressed as mg gallic acid equivalents (GAE) kg^{-1} on a dry weight (DW) basis. All analyses were carried out in triplicate.

7.2.7. Carotenoids extraction and HPLC analysis

Tomato carotenoids were extracted using the method of Siracusa et al. (2018). An aliquot of the cherry tomato puree sample (0.5 g) was transferred into a vial and 5 mL of a n-hexane/acetone/ethanol (2:1:1) solution were added. The vial was left shaking for 40 min. in the dark at room temperature. Subsequently 1 ml of H_2O (HPLC grade) was added and a further 2 min. agitation was applied. The resulting heterogeneous mixtures were left decanting until phases separation. The apolar coloured layers were transferred into an amber vial and analysed.

Quantitative analyses were carried out on an HPLC (Shimadzu USA Manufactoring Company Inc., Class VPLC-10 Dvp) equipped with a DAD (Shimadzu SPD-M10Avp). The column was a Gemini NX C18 (150×4.6 mm; 3µm particle size; Phenomenex, Italy), fitted with a guard cartridge packed with the same stationary phase. The flow rate was 0.7 ml/min. and the injector volume was 20 µl. Carotenoids were eluted with the following gradient of A (Methanol: H_2O 75:25) and B (Ethyl acetate): T0 30% B; T15 82% B; T25 30% B. All reagents used were HPLC purity grade: water, methanol and Ethyl acetate were obtained from Merck. The wavelength range was 220–660 nm, and the chromatograms were monitored at 473 nm for lycopene; at 453 nm for β-carotene; at 348 nm for phytofluene and at 288 nm for

phytoene. Carotenoids were identified by splitting the peak of the carotenoids from the tomato-solution sample with a standard of β-carotene and lycopene; (P≥95% and P≥98%, Sigma-Aldrich, St. Louis, Mo., U.S.A.) and by comparing retention times and UV spectra with those of standards. Quantification of β-carotene and lycopene was performed using external calibration curves; for phytofluene and phytoene the calibration curve of β-carotene was used. Linearity was checked for β-carotene between 3.36 and 21 mg kg^{-1} (R^2=1.00) and lycopene between 2.56 and 40.0 mg kg^{-1} (R^2=1.00). All analyses were performer in triplicate, including the extraction procedure, and the results were expressed as mg kg^{-1} DW.

7.2.8. Statistical procedures

All data were subjected to Shapiro–Wilk and Levene's test, in order to check for normality and homoscedasticity, respectively, then to a factorial 'storage temperature × genotype × storage time' (T × G × S) analysis of variance (ANOVA), according to the experimental layout adopted in the experiment. Percentage data were Bliss transformed before the ANOVA (untransformed data are reported and discussed), whereas multiple means comparisons were performed through Tukey's honestly significant difference (HSD) test ($P \leq 0.05$). All calculations were performed using Excel version 2016 (Microsoft Corporation, Redmond, WA) and Minitab version 16.1.1 (Minitab Inc., State College, PA, USA).

7.3. Results

In the present study, the significance resulting from the ANOVA related to storage temperature (T), genotype (G) and storage time (S) and their first order interactions is reported in **Table 7.2** (Fisher-Snedecor F-test), whereas their effects on variable means are reported in **Tables 7.3-7.6** and **Figures 7.1-7.3**.

Table 7.2. F-test values of the main factors and their first order interactions related to observed variables, with the significance resulting from the analysis of variance. SSC: soluble solids content; TA: tritatable acidity. (T): storage temperature; (G): genotype; (S): storage time. NS: not significant; *, ** and ***: significant at $P < 0.05$, 0.01 and 0.001, respectively.

Variable	Source of variation					
	Storage temperature	Genotype	Storage time	(T)×(G)	(T)×(S)	(G)×(S)
Average fruit weight	13.6***	140.8***	65.6***	3.8*	NS	8.1***
Fruit dry matter	10.6**	88.4***	29.7***	3.5*	NS	8.7***
Fruit firmness	8.7**	29.4***	11.5***	3.9*	NS	NS
Reducing sugars content	NS	50.2***	10.2***	NS	NS	3.8*
SSC/TA	NS	265.2***	4.7*	NS	NS	12.4***
Fruit pH	NS	19.6***	NS	NS	NS	NS
$(a^*/b^*)^2$	NS	38.0***	6.3**	NS	NS	NS
Chroma	39.4***	544.7***	30.9***	NS	13.2***	NS
Tomato color index	NS	39.9***	4.9*	NS	NS	NS
ΔE^*_{ab}	8.1**	15.1***	5.7*	6.0**	NS	5.0*
Total polyphenols content	20.9***	9.5***	56.4***	17.2***	4.7*	32.5***
Phytoene content	NS	82.8***	7.5**	NS	3.6*	15.1***
Phytofluene content	NS	44.3***	6.5**	6.3**	3.9*	6.8***
Lycopene content	33.8***	1462.3***	138.5***	3.6*	9.8***	121.4***
β-carotene content	NS	17.4***	23.1***	NS	NS	11.7***

7.3.1. Carpometric traits

Average fruit weight showed a significant 'T × G' interaction since, passing from T_{10} to T_{20}, 'Ottymo' and 'Sugarland' showed the highest reduction (-9%, on average) (**Table 7.3**). Moreover, both cultivars proved the highest decline of fruit weight at the end of the storage period, as this variable was reduced by 28%, on the average of both cultivars (**Figure 7.1A**).

Fruit dry matter, proved a higher value at T_{20} than at T_{10}, reaching the highest rise among the thermal regimes in 'Ottymo' (+15%) and 'Sugarland' (+12%) (**Table 7.3**). Both genotypes highlighted the highest rise during the storage period, as their fruit dry matter increased by 44% on average, passing from S_0 to S_{14} (**Figure 7.1B**). Differently, at T_{20} fruit firmness was significantly reduced, with 'Ottymo' showing the strongest decline passing from T_{10} to T_{20} (-19%) (**Table 7.3**). For this variable, a decreasing trend was recorded along the storage period, since, by comparison with the initial value, fruit firmness was reduced by 22% at S_{14} (**Table 7.3**).

Table 7.3. Carpometric variables of cherry tomato as affected by the main factors. Different letters among factor means indicate significance at Tukey's HSD test ($P < 0.05$). Interaction values ($P = 0.05$) related to 'storage temperature × genotype' and 'storage temperature × storage time' are reported. NS: not significant.

Variable		Genotype			Storage Time			Storage Temperature Mean
		'Eletta'	'Ottymo'	'Sugarland'	S_0	S_7	S_{14}	
Average fruit weight (g)	T_{10}	13.3	17.2	12.0	15.3	14.1	12.9	14.1 [a]
	T_{20}	13.0	15.5	11.1	15.5	13.6	12.0	13.2 [b]
	Mean	13.2 [b]	16.4 [a]	11.6 [c]	15.4 [a]	13.9 [b]	12.5 [c]	
$HSD_{interaction}$			0.8			0.8		
Fruit dry matter content (%)	T_{10}	8.2	12.1	9.1	9.1	9.4	10.9	9.8 [b]
	T_{20}	8.2	13.9	10.2	9.2	10.3	12.9	10.8 [a]
	Mean	8.2 [c]	13.0 [a]	9.7 [b]	9.2 [b]	9.8 [b]	11.9 [a]	
$HSD_{interaction}$			1.1			NS		
Fruit firmness (N)	T_{10}	14.1	19.7	12.8	16.5	16.1	14.1	15.6 [a]
	T_{20}	13.6	16.0	11.6	16.6	12.9	11.7	13.7 [b]
	Mean	13.8 [b]	17.9 [a]	12.2 [c]	16.6 [a]	14.5 [b]	12.9 [c]	
$HSD_{interaction}$			2.2			NS		

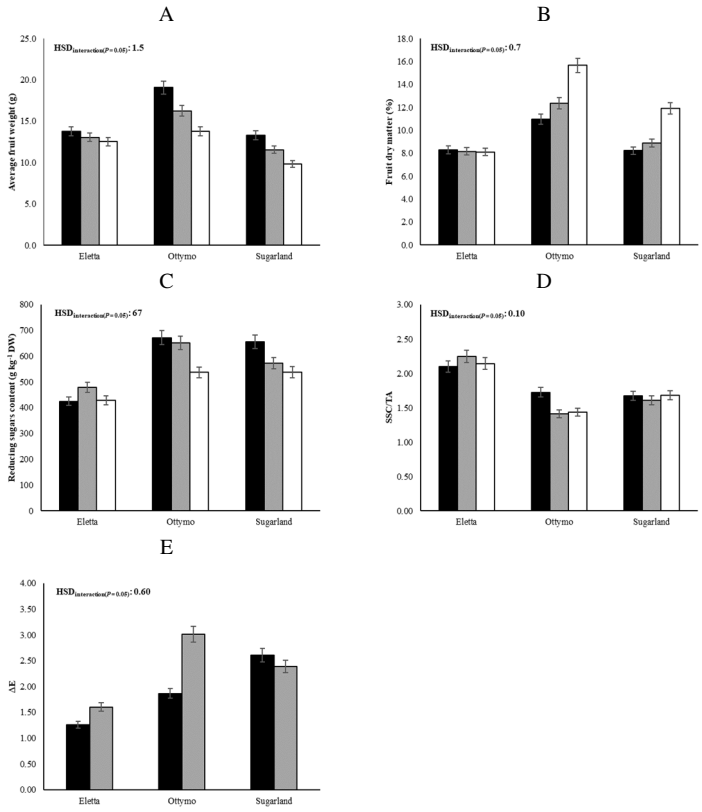

Figure 7.1. Average fruit weight (A), fruit dry matter (B), reducing sugars content (C), SSC/TA (D) and ΔE*ab (E) as affected by 'genotype × storage time' interaction. Black bars: S0; grey bars: S7; white bars S14.

7.3.2. Cherry tomato quality variables

On the average of the other factors, 'Ottymo' and 'Sugarland' proved the highest reducing sugars content (581 g kg^{-1} DW,

on average), whereas the former cultivar proved the lowest pH; differently, 'Sugarland' minimized the SSC/TA ratio (**Table 7.4**). Both reducing sugars content and SSC/TA declined passing from S_0 to S_{14} (by 14 and 4%, respectively). For the former variable, the strongest reduction along the storage period was noticed in 'Ottymo' and 'Sugarland' (-19%, on average) (**Figure 7.1C**), whereas for SSC/TA the only significant reduction within the S_0-S_{14} period was found in 'Ottymo' (-17%) (**Figure 7.1D**).

Table 7.4. Compositional variables related to fruit taste of cherry tomato as affected by the main factors. Different letters among factor means indicate significance at Tukey's HSD test ($P < 0.05$). Interaction values ($P = 0.05$) related to 'storage temperature × genotype' and 'storage temperature × storage time' are reported. NS: not significant.

Variable		Genotype			Storage Time			Storage Temperature Mean
		'Eletta'	'Ottymo'	'Sugarland'	S_0	S_7	S_{14}	
Reducing sugars content (g kg^{-1} DW)	T_{10}	434	595	552	557	555	463	527 [a]
	T_{20}	420	597	579	565	536	499	529 [a]
	Mean	427 [b]	596 [a]	565 [a]	561 [a]	545 [a]	481 [b]	
	$HSD_{interaction}$		NS			NS		
SSC/TA (adimensional)	T_{10}	2.15	1.65	1.52	1.80	1.77	1.71	1.77 [a]
	T_{20}	2.18	1.66	1.54	1.86	1.74	1.80	1.79 [a]
	Mean	2.16 [a]	1.65 [b]	1.53 [c]	1.83 [a]	1.76 [b]	1.76 [b]	
	$HSD_{interaction}$		NS			NS		
Fruit pH	T_{10}	4.59	4.21	4.33	4.37	4.35	4.42	4.38 [a]
	T_{20}	4.61	4.22	4.38	4.38	4.42	4.42	4.40 [a]
	Mean	4.60 [a]	4.21 [b]	4.36 [ab]	4.37 [a]	4.38 [a]	4.42 [a]	
	$HSD_{interaction}$		NS			NS		

7.3.3. Chromatic variables

Among the chromatic variables, Chroma and ΔE*ab showed a similar response to storage temperature, as they were both increased at T10 (by 4 and 27%, respectively) (**Table 7.5**). For Chroma, the increase under cold storage was particularly evident passing from S7 (24.1) to S14 (26.0, +8%) (**Table 7.5**). Among the studied genotypes, 'Eletta' showed the highest $(a^*/b^*)^2$ and Chroma (0.81 and 26.4, respectively) and the lowest ΔE*ab (1.43), whereas the lowest TCI was found in 'Sugarland' (31.3) (**Table 7.5**). All the chromatic variables significantly increased between S7 and S14, but for ΔE*ab such temporal rise was more prominent in 'Ottymo' (by 61%) (**Figure 7.1E**).

Table 7.5. Chromatic variables of the epicarp of cherry tomato as affected by the main factors. Different letters among factor means indicate significance at Tukey's HSD test ($P \leq 0.05$). Interaction values ($P = 0.05$) related to 'storage temperature × genotype' and 'storage temperature × storage time' are reported. NS: not significant.

Variable		Genotype			Storage Time			Storage Temperature Mean
		'Eletta'	'Ottymo'	'Sugarland'	S_0	S_7	S_{14}	
$(a^*/b^*)^2$	T_{10}	0.79	0.74	0.59	0.72	0.67	0.73	0.71 [a]
	T_{20}	0.84	0.71	0.63	0.72	0.67	0.78	0.73 [a]
	Mean	0.81 [a]	0.73 [b]	0.61 [c]	0.72 [a]	0.67 [b]	0.75 [a]	
$HSD_{interaction}$			NS			NS		
Chroma	T_{10}	26.8	26.5	21.1	24.5	24.1	26.0	24.8 [a]
	T_{20}	26.1	25.2	20.3	24.3	23.1	24.1	23.8 [b]
	Mean	26.4 [a]	25.9 [b]	20.7 [c]	24.4 [b]	23.6 [c]	25.1 [a]	
$HSD_{interaction}$			NS			0.7		
TCI	T_{10}	33.9	34.9	31.1	33.1	32.9	33.8	33.3 [a]
	T_{20}	34.1	34.2	31.6	33.1	32.6	34.0	33.3 [a]
	Mean	34.0 [a]	34.5 [a]	31.3 [b]	33.1 [ab]	32.7 [b]	33.9 [a]	
$HSD_{interaction}$			NS			NS		
ΔE^*_{ab}	T_{10}	1.37	2.65	3.11	-	2.18	2.57	2.38 [a]
	T_{20}	1.49	2.36	1.77	-	1.64	2.10	1.87 [b]
	Mean	1.43 [b]	2.50 [a]	2.44 [a]	-	1.91 [b]	2.34 [a]	
$HSD_{interaction}$			0.6			NS		

7.3.4. Total polyphenols content

Total polyphenols content (TPC) was significantly higher at T_{10} (4327 mg GAE kg^{-1} DW) that at T_{20} (4034 mg GAE kg^{-1} DW) (**Table 7.6**), but with strong interactive effects with genotype and storage time. Indeed, while 'Eletta' showed no differences among the 2 thermal regimes, TPC was strongly promoted by the lowest thermal regime in 'Sugarland' (+20%), followed by 'Ottymo' (+9%) (**Table 7.6**). As regards its temporal trend, TPC significantly increased passing from S_0 to S_7 (+17%) then sharply declined at S_{14} (-16%), with a steeper rise in the S_0-S_7 period recorded at T_{10} (+22%) than at T_{14} (+12%) (**Table 7.6**). Moreover, the studied genotypes displayed different time-courses of TPC along the storage

period, since 'Sugarland' proved the highest TPC rise passing from S_0 to S_7 (+37%) followed by the strongest decline at S_{14} (-33%) (**Figure 7.3A**).

Table 7.6 Compositional variables of cherry tomato as affected by the main factors. Different letters among factor means indicate significance at Tukey's HSD test ($P \leq 0.05$). Interaction values ($P = 0.05$) related to 'storage temperature × genotype' and 'storage temperature × storage time' are reported. NS: not significant.

Variable		Genotype			Storage Time			Storage Temperature Mean
		'Eletta'	'Ottymo'	'Sugarland'	S_0	S_7	S_{14}	
TPC (mg GAE kg^{-1} FW)	T_{10}	4087	4531	4364	3997	4869	4116	4327 ^{a}
	T_{20}	4287	4167	3647	3982	4452	3668	4034 ^{b}
	Mean	4187 ^{b}	4349 ^{a}	4006 ^{c}	3989 ^{b}	4660 ^{a}	3892 ^{b}	
$HSD_{interaction}$			303			303		
Phytoene content (mg kg^{-1} FW)	T_{10}	50.4	41.5	41.2	43.3	45.5	45.1	44.3 ^{a}
	T_{20}	53.5	38.5	41.3	43.1	41.2	48.3	44.5 ^{a}
	Mean	51.9 ^{a}	40.0 ^{b}	41.2 ^{b}	43.2 ^{b}	43.3 ^{b}	46.7 ^{a}	
$HSD_{interaction}$			NS			3.2		
Phytofluene content (mg kg^{-1} FW)	T_{10}	42.1	38.7	32.3	39.6	37.7	35.6	37.7 ^{a}
	T_{20}	46.5	34.6	33.9	39.8	33.4	41.9	38.3 ^{a}
	Mean	44.3 ^{a}	36.6 ^{b}	33.1 ^{c}	39.7 ^{a}	35.5 ^{c}	38.8 ^{b}	
$HSD_{interaction}$			3.5			3.5		
Lycopene content (mg kg^{-1} FW)	T_{10}	488	185	662	392	526	404	445 ^{b}
	T_{20}	556	207	701	416	577	484	488 ^{a}
	Mean	552 ^{b}	196 ^{c}	682 ^{a}	404 ^{c}	552 ^{a}	444 ^{b}	
$HSD_{interaction}$			35			35		
β-carotene content (mg kg^{-1} DW)	T_{10}	92.0	85.2	83.4	77.0	89.7	94.0	86.9 ^{a}
	T_{20}	96.0	81.7	77.1	76.7	85.9	92.3	84.9 ^{a}
	Mean	94.0 ^{a}	83.5 ^{b}	80.3 ^{b}	76.8 ^{c}	87.8 ^{b}	93.1 ^{a}	
$HSD_{interaction}$			NS			NS		

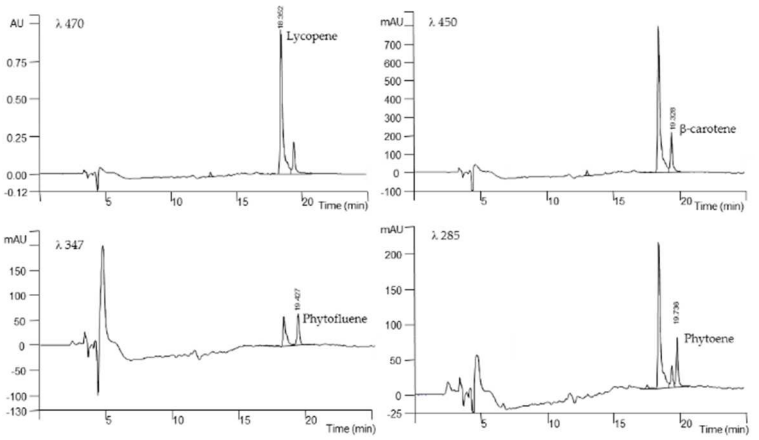

Figure 7.2. HPLC profile of carotenoids extracted from cherry tomato 'Sugarland' at harvest date (S0).

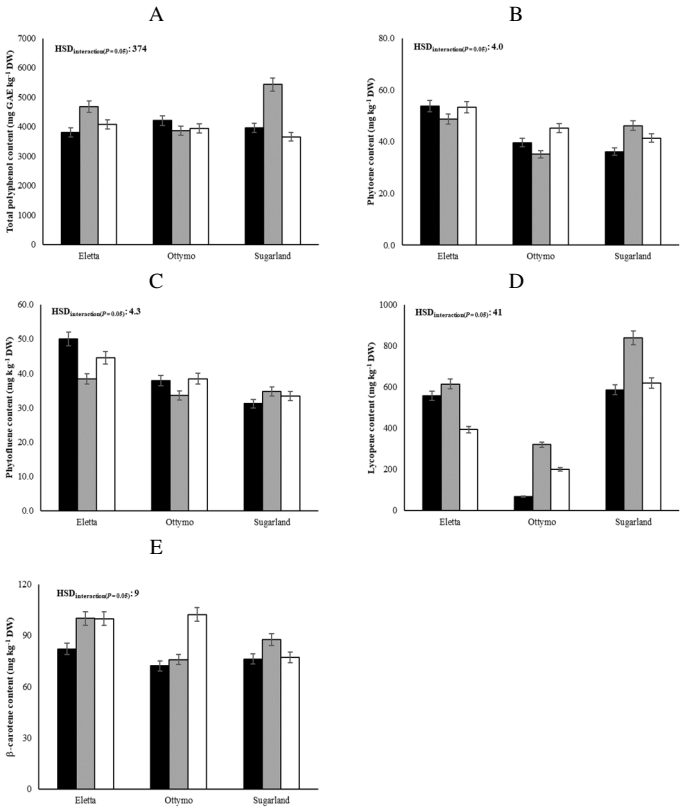

Figure 7.3. Total polyphenols (A), phytoene (B), phytofluene (C), lycopene (D) and β-carotene (E) content as affected by 'genotype × storage time' interaction. Black bars: S0; grey bars: S7; white bars S14.

7.3.5. Carotenoids content

Figure 2 shows the HPLC carotenoids profile extracted from cherry tomato 'Sugarland'. At harvest date, the level of lycopene ranging from 68.1 to 582.5 mg kg^{-1} DW in 'Ottymo'

and ‘Sugarland’, respectively, followed by β-carotene, ranging from 72.8 to 82.17 mg kg^{-1} DW, in ‘Ottymo and ‘Eletta’, respectively. Among genotype ‘Eletta’ recorded the highest levels of both phytoene and phytofluene (54.2 and 50.7 mg kg^{-1} DW, respectively). The levels determined in ‘Sugarland’ and ‘Ottymo’ varying from 31.0 to 38.2 mg kg^{-1} DW, for phytoene and from 36.1 to 39.9 mg kg^{-1} DW, for phytofluene, respectively.

The phytoene content of the studied genotypes proved different time courses among the 2 thermal regimes, as it significantly increased passing from S_7 to S_{14} when the T_{20} storage was considered (from 41.2 to 48.3 mg kg-1 DW, +14%) (**Table 7.6**). Among the genotypes, ‘Sugarland’ proved the highest phytoene rise passing from S_0 to S_7 (from 36.2 to 46.1 mg kg^{-1} DW, +28%), whereas in ‘Ottymo’ a significant increase was recorded between S_7 (35.1 mg kg^{-1} DW) and S_{14} (45.3 mg kg^{-1} DW, +29%) (**Figure7.3B**).

Regarding phytofluene, the lowest storage temperature showed a depressive effect in ‘Eletta’ (in which it was reduced by 9%) and the opposite in ‘Ottymo’ (in which it increased by 10%) (**Table 7.6**). Phytofluene content proved also wider temporal oscillations at T20, as the initial value was reduced by 6.4 mg kg^{-1} DW at S_7 (-9%), then increased by 6.4 mg kg^{-1} DW at S_{14} (+19%) (**Table 7.6**). Such temporal oscillations proved to be genotype-dependent too, since ‘Eletta’ showed the highest reduction passing from S_0 (50 mg kg^{-1} DW) to S_7 (38.4 mg kg^{-1} DW, -23%), then the sharpest rise at S14 (44.5 mg kg^{-1} DW, +16%) (**Figure 7.3C**).

Lycopene was significantly affected by the storage temperature, as it was lower at T_{10} than at T_{20} (445 vs. 488 mg kg^{-1} DW), and this reduction was more marked for

'Eletta' (-12%) and 'Sugarland' (-6%) (**Table 7.6**). Moreover, T_{20} promoted a sharper lycopene rise than T_{10} passing from S0 to S_7 (from 416 to 577 mg kg^{-1} DW, +39%) followed by a milder decrease at S_{14} (484 mg kg^{-1} DW, -16%) (**Table 7.6**). All the studied cultivars showed a significant decrease in lycopene content between S_7 and S_{14} (ranging from 119 to 221 mg kg^{-1} DW in 'Ottymo and 'Eletta', respectively), with 'Ottymo' and 'Sugarland' proving also a higher lycopene increase between S_0 and S_7 (by 252 mg kg^{-1} DW, on average) (**Figure 7.3D**).

β-carotene concentration proved to be not sensitive to the storage temperature and was higher in 'Eletta' (94.0 mg kg^{-1} DW) than in the other genotypes (81.9 mg kg^{-1} DW, on average) and, over the storage period, increased up to 93.1 mg kg^{-1} DW at S_{14} (**Table 7.6**). However, such temporal increase was more marked in 'Eletta' within the S_0-S_7 period (from 82.1 to 100.0 mg kg^{-1} DW) and in 'Ottymo' in the S_7-S_{14} one (from 75.9 to 102.3 mg kg^{-1} DW) (**Figure 7.3E**).

7.4. Discussion

The fruits stored at 10 °C showed a higher fruit weight and a lower dry matter content as compared to those stored at 20 °C, indicating that fruit transpiration and water loss were the main processes affected by storage temperature. As a consequence, at 20 °C tomatoes proved a higher loss of fruit firmness over time. The transpiration-driven softening of tomatoes during postharvest is a major problem, as it increases their susceptibility to damages along the distribution chain (Batu, 2004). Moreover, fruit firmness is considered a key indicator of tomato freshness, able to influence the purchasing behaviour of consumers (Bui et al.,

2010). However, despite cold storage is commonly practiced for reducing postharvest softening of tomatoes, the opposite effect can be found when too low storage temperatures are used, because of the tropical origin of the plant (Farzaneh et al., 2018). For this reason, storage temperature over 11-12 °C are advised for storing tomatoes, depending on fruit typology and ripening stage (Batu, 2004; Bui et al., 2010; Beckles, 2012;). Nonetheless, the differences in terms of fruit weight and firmness we found among the 2 thermal regimes showed that storage at 10 °C was a suitable way to extend these main characteristics of tomato fruits. Among the studied cultivars, both 'Sugarland' (small-fruited) and 'Ottymo' (large-fruited) showed the highest fruit weight reduction during storage, consistent with their steeper rise in dry matter content. Differently, 'Eletta' (medium-fruited) proved the highest temporal stability in relation to both variables. Hence our results suggest that the genotypic attitude of cherry tomato to retain fruit weight and firmness during postharvest, is dependent from factors other than simply the fruit size (i.e. the ratio among berry volume and its external transpiring surface) (Leonardi et al., 1999), and likely due to the functional traits of the epicarp. Indeed, it has been reported that the dynamics of fruit water loss and consequent tissue collapse are influenced by genotypic differences in structural characteristics of the cuticle, whose alteration over time is an intrinsic feature of the genetically-programmed ripening process (Saladié et al., 2007).

In tomato, the ethylene-driven ripening and senescence lead to the alteration of the carbon substrates content (Anton et al., 2017), as they are energy-requiring processes whose kinetic is influenced by the ambient temperature (Giuffrida et al.,

2018). In our experiment, reducing sugars content, the ratio SSC/TA and fruit pH were not affected by the storage temperature, proving instead to be genotype-dependent. Despite their higher increase in dry matter, 'Sugarland' and 'Ottymo' highlighted the steepest drop in reducing sugars content at the end of storage period (by 19%, on average), denoting within the 10-20 °C range a temperature-insensitive acceleration of their autocatalytic metabolism. This demonstrates that no chilling disturbance in reducing sugars metabolism occurred in the experiment (Beckles, 2012). To this end, while the cultivars did not show appreciable pH variations during storage, 'Ottymo' proved the highest SSC/TA reduction over time, denoting its lowest suitability to keep unchanged the taste peculiarities. Indeed, the SSC/TA ratio is a pivotal organoleptic descriptor, as it is related to the overall balance in the perceived sweetness (SSC) and sourness (TA) of tomatoes (Mauro et al., 2020c).

Color is one of most important and widely used parameters to define the quality of tomato and tomato products (Ganje et al., 2018). When fresh tomato fruits are concerned, it is linked to fruit ripeness and firmness and is generally associated by consumers to tomatoes eating quality. In the present experiment, we used an array of chromatic variables summarizing the main color modifications occurring in tomato epicarp. Chroma, $(a^*/b^*)^2$ and tomato color index have been related to quality traits of tomato (Anton et al., 2017; Mauro et al., 2020c), whereas ΔE^*_{ab} has been successfully used to monitor the quality maintenance of potato sticks during refrigerated storage (Licciardello et al., 2018). All these variables showed a certain variability among the studied cultivars, with two of them, namely Chroma and

ΔE*ab, increasing at T_{10}, overall indicating a higher deviation toward more vivid fruit colors. In particular, after 14 days of storage, a higher reduction of Chroma was recorded at 20 °C, a condition which matched the strongest decrease in fruit weight and firmness experienced by the studied cultivars. 'Sugarland' and 'Ottymo' proved higher ΔE*ab variations during storage. According to Dattner and Bohn (Dattner and Bohn, 2015), independently from the deviation formula, two colours can be optically distinguished if ΔE > 1. The ΔE*ab differences attained by 'Sugarland' and 'Ottymo' (2.47 units, on average) and 'Eletta' (1.43) indicate for the former cultivars a higher perceivable colour deviation along the storage period, consistent with their higher qualitative decline in terms of fruit weight and turgor.

When phytochemical composition was concerned, total polyphenols, lycopene and β-carotene contents found in our experiment were substantially in line with those reported by Fernandes et al. (Fernandes et al., 2020) for cherry tomato 'Moscatel RZ' grown in hydroponic or semi-hydroponic systems. On the other hand, phytoene and phytofluene contents were very similar to those found in cherry tomato by Mapelli-Brahm et al. (2018). Plant polyphenols are a large group of phytochemicals involved in the regulation of plant growth, reproduction and response to the environmental stressors (Sharma et al., 2019). From a nutraceutical viewpoint, they have strong antioxidant properties probably implicated in the decreased incidence of cardiovascular diseases and certain forms of cancer (Holst and Williamson, 2008). Both thermal regimes promoted a bell-shaped postharvest trend of TPC, consisting in their sharp rise at S_7, followed by a decrease at S_{14}, this last indicating the onset of

metabolic senescence processes (Mirdehghan and Valero, 2017). However, such increase was more marked at 10 °C, suggesting the occurrence of a cold-adaptive response in up-regulating the polyphenols expression during postharvest storage. Indeed, several phenolic compounds typically accumulate in plant cells subjected to cold stress, as they contribute to the homeostasis of cold-induced reactive oxygen species (ROS) and to enhance the thickness of the cell wall, so preventing lipid peroxidation and cell collapse (Sharma et al., 2019). This would explain the best retention of fruit firmness recorded at 10 °C, indicating at the same time, the improvement of tomato phenolic profile as a benefit induced by a mild cold stress. Thus, although polyphenols have not been considered a priority target in tomato breeding programs, our results suggest that they could represent a sensitive target for improving the functional profile of the tomato, mostly during postharvest cold storage.

Regarding the carotenoid fraction, we recorded variable effects, resulting from different time-course response to storage temperature and duration. Lycopene displayed a bell-shaped temporal trend too since, under both storage temperatures, this carotenoid sharply increased at S_7 then declined at S_{14}. This trend substantially differed from that of β-carotene which continuously increased until S_{14}, so confirming the higher stability of its postharvest accumulation in tomato (Brashlyanova and Pevicharova, 2009). According to Rodriguez-Amaya (1993), carotenoids accumulation can continue during postharvest transport or storage, provided that the integrity of the fruit is maintained, so preserving the enzymatic activity responsible for carotenogenesis. Lycopene plays a paramount function in

protecting the photosynthetic apparatus and plant lipid membranes, as its acyclic polyene structure (11 conjugated double bonds) increases its affinity for singlet oxygen and radical scavenging activity beyond the other carotenoids (Gruszecki and Strzałka, 2005). For this reason, it has been reported that oxidation is the main cause for lycopene degradation (Srivastava and Srivastava, 2015). This could partly explain the depressive effect on lycopene content we recorded upon storing tomatoes in a stressing, ROS-inducing environment (10 °C). In this view, it is interesting to note the contrasting effect of cold storage on tomato compositional traits, resulting in a higher polyphenols accumulation in case of a lower lycopene content. This suggests the existence of a fine tuning among different classes of compounds in response to cold stress. However, by comparing the temporal trend of lycopene with that of its colourless precursors phytoene and phytofluene, clear time-dependent temperature effects on carotenogenesis where noticeable. Indeed, at S_7, the highest lycopene content recorded at 20 °C matched the strongest reduction of both phytoene and phytofluene. In other words, the lowest the lycopene concentration the highest the accumulation of its precursors and vice versa. This implies that reduced transformation kinetics of both phytoene and phytofluene represented the earliest metabolic constraints recorded in response to the imposed cold stress. According to Dumas et al. (2003) the over-expression of phytoene desaturase (leading to lycopene synthesis by desaturating both phytoene and phytofluene) is the most important upstream metabolic step in increasing the lycopene content of tomato fruits at harvest. Our results bear this out in postharvest conditions too, as they indicate that, under mild

cold stress storage conditions, desaturation of phytoene and phytofluene represents the earliest metabolic bottleneck in lycopene synthesis of cherry tomatoes, hence a possible priority target to modulate the postharvest evolution of their nutraceutical profile. On the other hand, to which extent this implies a mid-term modification of the overall nutraceutical profile of tomato represents an interesting topic, taking into account that, despite they are not effective antiradicals as lycopene, phytoene and phytofluene are among the prevailing carotenoids found in human plasma and tissues, and their bioaccessibility following gastro-intestinal digestion of tomato juice has been found ~3-4 fold higher than that of lycopene (Meléndez-Martínez et al., 2015; Mapelli-Brahm et al., 2017).

Among the studied cultivars 'Sugarland' proved the highest lycopene and total polyphenols content, whereas 'Eletta' overcame the other cultivars for phytoene and phytofluene. Excepting β-carotene, which over time increased more sharply in 'Eletta' and 'Ottymo', these differences were still noticeable at the end of the storage period, regardless of the storage temperature. These highlights, beyond the environmental influence, the existence of a strong genetic component determining the stoichiometric relationships among lycopene and its precursors. Unravelling the possible interactive effects among these three carotenoids in generating the antioxidative health benefits (Gul et al., 2015; Mirdehghan and Valero, 2017) will allow for a better orientation of breeding programs toward the most convenient phytochemical evolution of tomatoes during refrigerated storage.

7.5. Conclusions

The results of the present experiment highlighted complex postharvest modifications of cherry tomatoes in response to the studied factors. By storing them under mild stressing conditions (10 °C) it was possible to improve the stability over time of carpometric traits (mainly fruit weight, firmness and Chroma) having commercial relevance, without alterations of compositional traits related to taste perception (reducing sugars content, SSC/TA and pH). Moreover, when compared to 20 °C, storing at 10 °C boosted the accumulation of total polyphenol and, at least in the short term (within 7 days of storage), the concentration of both phytoene and phytofluene, probably inhibiting their enzymatic desaturation leading to lycopene. This suggests their possible usefulness in modulating the nutraceutical evolution of cold stored cherry tomatoes during postharvest. This idea is reinforced by the stable varietal differences we found in terms of stoichiometric relationships among lycopene, phytoene and phytofluene. Regarding the varietal attitude to postharvest storage, the stability over time of fruit weight, dry matter content, SSC/TA and ΔE*ab proved to be highly discriminant among cultivars, indicating the lowest ability of 'Ottymo' and 'Eletta' to maintain their fruit peculiarities over time. Thus, our results suggest the use of these variables to screen for cherry tomato germplasm suited to periods of postharvest storage.

7.6 References

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8. Concluding remarks

The need of global trade and supply chain management to have a product with 'standardized' characteristics, attractive, durable, easy to manipulate, homogeneous, has sometimes contrasted with those of modern consumers, conscious of the qualitative and hedonistic aspects of food, health and sustainable production. The results from the present PhD research project report some interesting results about the possibility to improve the qualitative and functional profile of greenhouse tomato, by applying different technical solutions, both in pre- and postharvest conditions.

The first research line studied the composition and sensorial properties of tomatoes harvested at different ripening stages of an elongated tomato cultivar for fresh consumption. In this experiment, grafting technique as a tool to improve flavour compounds in tomato fruits of plants grown onto three common rootstocks in Mediterranean greenhouse cultivation was investigated. Our results pointed out that the harvest stage plays a crucial role in the nutraceutical and eating quality profile of “Sir Elyan”, whereas none of the studied rootstocks was able to improve in a consistent way the quality traits considered. Our findings underline the pivotal importance of selecting the rootstock in relation to the single variety considered and how it is possible to selectively improve the nutraceutical and sensorial profile of the elongated fruit of ‘Sir Elyan’ by selecting the most appropriated rootstock and considering the harvest stage effect.

The second line of research provided a deeper knowledge about the effects of a plant-based biostimulant on quality and functional composition of three different cherry tomato cultivars during the off-season cultivation cycle focusing on

two different clusters, namely 2nd and 6th. The results highlighted how the use of the biostimulant and the cluster position influenced the carpometric and biochemical characteristics of cherry tomatoes. In general, the fruits of the 2nd cluster underwent better environmental conditions (especially in terms of temperature and solar radiation) than the fruits of the 6th cluster. However, the use of the biostimulant significantly contributed to improving the performance of the fruits belonging to the 6th cluster compared to the untreated ones, in terms of biosynthesis of secondary metabolites. This work, confirmed that biostimulants could affect cherry tomato plants grown during the cold cycle greenhouse and improve fruit nutraceutical quality, modulating the effect of cluster position.

Finally, the third line of research addressed the effects of two storage temperatures, namely 10 and 20 °C, on the main quality and functional traits of three cherry tomato cultivars 'Eletta', 'Sugarland' and 'Ottymo', after 0, 7 and 14 days of storage. Tomato fruits stored at 10 °C showed improved stability during storage of carpometric traits, mostly in terms of fruit weight, firmness and Chroma. Moreover, the initial phase of storage boosted the accumulation of total polyphenol, phytoene and phytofluene, perhaps inhibiting or slowing down the enzymatic desaturation leading to lycopene. These observations could be useful in modulating the nutraceutical evolution of cold-stored cherry tomatoes during postharvest. Moreover, the varietal attitude to postharvest storage was highly discriminant in terms of fruit weight, dry matter content, SSC/TA and ΔE^*_{ab}, suggesting the use of these variables to screen for cherry tomato germplasm suited to periods of postharvest storage.

As a whole, the results of this doctoral thesis encourage to continue the investigations on agronomical techniques to

improve the quality of fresh tomatoes, increase yields, reduce the non-marketable fraction, maintain quality in the post-harvest phase and ameliorate the nutraceutical profile.