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Improving crop sustainability and fresh and processed fruit quality through integrated analyses along the food chain

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ABSTRACT

While million people lack access to food or some nutrients, up to one-third of food is never consumed, especially fruit and vegetables, naturally rich in phyttonutrients. Fruit and vegetables waste and loss arise at all steps of the food chain from production to consumption. The access to phyttonutrients is also threatened with climate changes that impact both yields and the composition of harvested organs. Finding trade-offs between yield and quality along the food chain appears necessary to improve crop sustainability and to limit losses.

Processing tomato is a good target to address these challenges: it is a major crop and an important source of phyttonutrients notably phenolic compounds and carotenoids, and it represents an intensive production in terms of water use. Two examples of integrated analyses will be given.

First, we will focus on pre-postharvest relationships and the ability of tomatoes to be processed into purees. We investigated fruit quality in response to water supply, genotypes and ripening stage, and we assessed their impact on puree obtained from hot break and cold break processes. We found that fruit growth and quality were weakly impacted by moderate water insufficiency during growth. A reduction of water supply from 100% to 60% of the evapotranspiration strongly impacted plant growth but had little impact on fresh fruit yield and increased the water use efficiency by 20%.

Second, we address the accumulation of carotenoids in ripe tomato in response to water insufficiency in two genotypes. A medium water deficit (-0.5 MPa soil water potential while permanent wilting point is estimated at -1.5 MPa) at the beginning of fruit development impacted the fruit composition at maturity. The dry matter contents increased up to 23% while an interaction between genotype and water regime was found for carotenoid contents which may affect tomato health value.

Keywords: fruit quality, carotenoids, drought, process

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1.0 INTRODUCTION

Tomato is one of the most important crops in the world with about 180 million tons of fresh fruit produced per year (FAOSTAT, 2019). A part of this production is stabilized by processing as paste, purees, juices or tomato powders which limits wastes and losses. In addition, tomato and tomato product consumption is associated with health benefits. Indeed, tomatoes are major source of phytomicronutrients (phenolic compounds, carotenoids), and mainly carotenoids, have been shown to protect against chronic diseases (Cheng et al., 2019; Landrier et al., 2018). In line with this, this study is focused on this class of metabolites.

Processing tomatoes are grown under a large range of climatic conditions and practices that affect their ability to be processed, and product quality traits (Arbex de Castro
Vilas Boas et al., 2017). For example, in Mediterranean regions water requirements during the growing season for industry-type tomatoes varied from 400 to 900 mm (Saadi et al., 2015). With the foreseen climate changes in these areas, frequency of drought episodes will increase. Drought impairs yields (Casa and Rouphael, 2014), and also increases the fruit contents in dry matter and in some carotenoids depending on the genotype, the intensity, and the duration of water shortage (Constantinescu et al., 2016; Ripoll et al., 2014). These changes associated with water deficit (WD) may be beneficial for processing, by facilitating the dehydration/concentration step and may modify the viscosity and color of final products. Yet very few studies provide an integrated view of the management of tomato product quality from field to processed product. Insights into interactions between factors that drive fruit quality during the growing season and those that operate during processing will be gained. In addition, the impact of these factors, occurring along the food chain, on the nutritional efficacy of fresh and processed fruit (depending on bioactive compound amount and bioaccessibility) is not documented.

Carotenoids are hydrophobic compounds with a C40 carbon skeleton (Britton, 1995) that are synthesized and sequestered in plastids. During fruit ripening, with the dismantlement of the photosynthetic machinery, chloroplasts are converted into chromoplasts (Egea et al., 2011). While chlorophylls are degraded, large amounts of carotenoids (mainly lycopene and beta-carotene) are formed.

Water deficit (WD) may impact the metabolism and storage capacity of carotenoids. The effect of WD on carotenoid metabolism may be direct; through a reduction of net photosynthesis and supply of metabolites for the synthesis of precursors, and/or indirect; via drought-exacerbated oxidative stress/oxidative signaling (Fanciullino et al., 2014). Moreover, WD may influence the metabolism of carotenoids by hastening fruit development. Another hypothesis is that subcellular compartments dedicated to the deposition of apolar carotenoids exert a sink strength/deposition sink capacity for their accumulation. This sink strength depends on sink size and activity. Total cell number, cell size and storage compartment density determine the sink size; in tomato, WD modulates this organization throughout the three phases of fruit development, namely cell division, cell expansion and ripening (Baldazzi et al., 2013, 2016). While there are increasing lines of evidence showing that the deposition of high contents of specific metabolites is influenced by cell structures (Cazzonelli and Pogson, 2010), few studies have focused on this process, and few data are available on the effect of environmental conditions or crop management on this storage capacity during fruit development. Finally, fruit structure has been shown to modify carotenoid bioaccessibility and bioavailability (fraction of carotenoid reaching the blood system). How WD can affect carotenoid bioaccessibility and bioavailability (carotenoids from fresh or processed fruit) is not documented.

In this context, we propose integrative research focused on industry-type tomatoes that highlights the role of the fruit matrix (cell number, cell size, cell wall density and composition and plastid density) in tomato and on tomato product quality.

2.0 MATERIALS AND METHODS

2.1 Field experiments

Five field experiments were conducted near Avignon, France (44°11’22.4"N 4°48’11.7”E), in 2016, 2017, 2019, 2020, and 2021 on eight industry-type (determinate) cultivars of Solanum lycopersicum to evaluate the impact of different water regimes on fruit and puree quality (see for example the experimental design set up in 2017 on 4 cultivars and detailed in figure 1). For each cultivar-water regime-block combination, three
microplots of 4 to 6 plants, representative of the culture, were defined to determine yield and fruit and puree quality traits. At least 15 ripe fruit from each microplot were used for quality analyses and processing experiments.

Figure 1. The experimental design developed in field in 2017. Each block was 7 m wide and 90 m long. The four cultivars namely ‘H1015’, ‘H1311’, ‘Miceno’, and ‘Terradou’ and the two irrigation regimes (control in blue and WD in red) were randomly distributed within the two blocks. All plants were grown under identical field conditions: 900 plants per genotype were transplanted in May 2017 at a density of 3.3 plants m\(^{-2}\). Insects and diseases were controlled according to current practices. The water irrigation was supplied by a drip irrigation system. Irrigation was scheduled daily to compensate the evapotranspiration loss from tomato crop (ETM). ETM was determined daily based on reference evapotranspiration (ET0) estimated from the climatic data from a local weather station and the Penman-Monteith equation and considering crop coefficient (Kc). During the reproductive period, two levels of irrigation were targeted: (1) water deficit (50% replacement of ETM) and (2) well-watered to match 100% replacement of ETM. To mimic current production practices, irrigation was stopped one week before harvest.

2.2 Process and quality analyses
Tomatoes were transformed by cold break (CB) or hot break (HB) treatment according to a laboratory scaled method described by (Page et al., 2012).

The color of the purees was measured with a Minolta CR.400 using a specific cuvette for measurement of liquid or paste color and calibrated against a white background. Color results were expressed in the CIE L* a* b* color space. Color coordinates were used to calculate the hue angle (H°), which identifies the color at a 360 ° angle (McGuire, 1992). The dry matter content was determined by weighting around 3 g of fruit puree before and after drying for 3 days at 70°C. The soluble solid content (SSC) was measured by refractometry with an ATAGO PR-1000 digital refractometer with automatic temperature compensation at 25 °C and results were expressed in degree Brix. The viscosity was calculated from a steady state measurement performed on an Anton Paar MCR 301 viscosimeter (Graz, Austria), with a double ribbon impeller (with an inner radius of 11 mm, a pitch of 45 mm, a length of 45 mm and an outer stationary cup with an outer radius of 14.46 mm). A flow curve was registered between 0.1 and 100 s\(^{-1}\), 50 points and 5 seconds per point.

2.3 Glasshouse experiments
Two determinate tomato genotypes (‘M82’ and ‘H1311’) and eighteen plants per genotype were grown in 4 L pots filled with compost (substrate 460, Klasmann, Champety, France) under glasshouse conditions near Avignon (43°54’N 4°52’E), France. Day-night temperature controls were set at 25 – 15°C. Flowers were pollinated three times a week using an electrical bee. Plants were supplied daily with a nutrient solution (Liquoplant Rose, Plantin, Courthézon, France). This solution was diluted between 0.4‰ and 0.8‰ according to the plant developmental stage, which corresponded to an average electro-conductivity of 1.8 mS dS cm⁻¹. First, all plants were irrigated in order to match 100% replacement of evapotranspiration. At the beginning of the reproductive period (anthesis of the first flowers or trusses, 60 days after sawing), half of the plants (9 plants per genotype) were subjected to soil water deficit (volumetric soil water content (VWC) of 0.2 m³ m⁻³ which corresponded to a soil water potential of -0.5 MPa) whereas all the other pots were kept under controlled conditions (more than 0.5 m³ m⁻³ or a soil water potential of -0.05 MPa) until 100 days after sawing. During the WD treatment, from March to May 2017, leaf water potential and leaf conductance were monitored on control and WD plants. Measurements of leaf conductance were conducted between 9 and 10 am (solar time) using an AP4 porometer (Delta-T Devices Ltd, Cambridge, England), while measurements of water potentials were performed between 12 and 13 pm (solar time) using a pressure chamber (Scholander et al., 1965). During the water deficit treatment, leaf conductance decreased up to 60% and leaf water potentials at midday by 50%.

2.4 Physical and chemical analyses

Under WD, fruit were harvested at three developmental stages from ‘M82’ and ‘H1311’ plants: 20, 42, and 52 days post anthesis (DPA). Three to five fruit per genotype, water regime and developmental stages were harvested for all analyses. Fruit color, dry matter, starch, soluble sugar, organic acid and carotenoid contents were analyzed. For biochemical analyses, pieces of fruit pericarp were immediately frozen and kept at -80°C. Soluble sugars, starch and organic acids were extracted according to the method described by (Gomez et al., 2002) and analyzed by HPLC (Waters 410, Part WAT070390, Milford, U.S.A.). Carotenoids were extracted according to the method described by (Sérino et al., 2009).

2.5 Microscopy analysis

The pericarp cell number was measured after tissue dissociation according to a method adapted from (Bunger-Kibler and Bangerth, 1983). Cells were counted using a microscope equipped with a camera (QImaging, Surrey, Canada) and Qcapture Pro 6.0 software (QImaging, Surrey, Canada) (Bertin et al., 2002). We exploited the fluorescence properties of chlorophylls and carotenoids to analyze plastid size and filling by confocal microscopy. Sections of fresh tomato fruit (150 to 200 µm of thickness) at three developmental stages, 20, 42 and 52 DPA, were imaged by confocal microscopy with a Zeiss LSM 880 microscope (Zeiss, Jena, Germany) fitted with a x 20 objective. Pigments were excited at 488 nm with an argon laser. The carotenoid signal was recorded in green between 498 and 579 nm whereas chlorophyll signal was in red between 650-698 nm (see Figure 4). Images were processed by Image J software.

3.0 RESULTS AND DISCUSSION

The objective of this work was to provide an integrated view of the management of tomato and tomato product quality from field to consumers. Tomato products such as purees or powders represent a strategic approach to meet nutritional needs of the population, considering that they are rich carotenoids, and are available all year, provided
that their organoleptic and nutritional properties are preserved during processes. Consequently, the objective was to evaluate if it is possible to increase the durability of the commercial tomato crop by reducing its water footprint while analyzing impacts on yield, processing characteristics and accumulation of carotenoids. The five years of in-field experiments on eight cultivars provide insights into interactions between factors that drive fruit quality during the growing season and those that operate during processing. Analyses of the impact of WD on tomato reveal that carotenoid profiles are modified along with the structure of the food matrix which may impact carotenoid bioaccessibility.

3.1 Combined effects of genotype and water-supply on yield and quality traits.

The five years experiments reveal that it is possible to improve tomato crop water use efficiency (expressed in kg m⁻³, as fresh yield to water used for irrigation ratio) by a mild WD during the reproductive phase. A reduction of water supplied during the reproductive period decreased the total fresh yield up to -50% depending on the cultivar and the year but showed a more limited effect on the total dry yield (up to -30%) since the WD improved the fruit dry matter content. For example, in 2017, WD impacted plant growth and architecture: plant width declined by about 13% and the leaf area index by 9% comparing WD to control plants. Despite these changes in plant biomass, a decrease in total fresh yield was only significant for ‘H1015’ cultivar (-20%) and was due to a drop in the number of fruit per plant whereas the average fruit weight remained unchanged (Figure 2). In addition, under WD conditions in 2017, no significant reduction in total dry yield was observed no matter the cultivar (Figure 2). On the whole, the rise in water use efficiency ranged from 30 to 50% and was genotype-dependent.
Figure 2. Impact of a reduction of water supply by drip irrigation during the reproductive phase on total fresh (A) and dry yields (B), the number of fruit per plant (C) and on the average fruit weight (D) for 4 tomato cultivars namely ‘H1015’, ‘H1311’, ‘Miceno’, and ‘Terradou’ in 2017. Values are means (n≥12) ± standard errors. Control plants are in blue and WD ones in gray. Bars marked by different letters indicate significant different values (Kruskal-Wallis test, α = 0.05).

These results are in agreements with previous research investigating the effect of moderate WD on processing tomato. According to (Patanè et al., 2016) deficit irrigation at 50% ETc from flowering does not significantly reduce the total or marketable yields, but increases water use efficiency by about 40%. Similarly, (Stikic et al., 2003) demonstrated that partial root drying (PRD) induces a significant reduction of total plant biomass without affecting fruit diameter and fresh mass. Accordingly, water use efficiency at crop level is increased by PRD treatment (Stikic et al., 2003).

In addition, our data showed that a reduction in water supply improved the fruit dry matter content and increased the contents of main soluble sugars, acids and carotenoids due to a concentration effect as already shown by (Ripoll et al., 2016). The fruit metabolism was
barely affected since metabolite contents expressed on a dry weight basis remained unchanged except for carotenoids and citric acid in some cultivars. Although these slight changes in fruit composition, WD affected the ability of fruit to be processed.

3.2 Consequences for fruit processing

Viscosity constitutes one of the main quality traits of tomato purees, which are considered as suspensions of insoluble particles (pulp) into an aqueous solution (serum). Huge variations of tomato apparent viscosity were determined through the five years experiment combining eight cultivars, two water regimes and 2 processes (from 600 to 4000 mPa.s). The cultivar and the process were the main levers of variations. In addition, whatever the cultivar or the process, WD enhanced the apparent viscosity (up to +60%). However, this rise in apparent viscosity was not entirely linked to an increase in fruit or puree soluble solid and dry matter contents as shown by the absence of correlation between those variables (Figure 3).

In tomato, fruit dry matter encompasses soluble (mainly sugars and acids) and insoluble (such as pectins and other polysaccharides) solids (Foolad, 2007). Insoluble solids are thought to determine puree viscosity (Davies et al., 1981). We propose that the effect of WD on puree rheology was also driven by changes in pectin composition, and by changes in particle sizes and interactions. These variables are related to fruit structure (cell number, cell size, cell wall density and composition and plastid density).
3.3 Production conditions and fruit structure

To analyze how WD modified the whole pericarp during the fruit developmental process, a greenhouse trial was carried out in 2017 using two industry-type tomato genotypes, 'M82' and 'H1311'. We proposed to test the hypothesis that plastid compartment density determines carotenoid accumulation and that WD affects this storage capacity. In line with this, a soil water deficit was applied during the reproductive period and fruit were collected at 20, 42 and 52 DPA for microscopy and metabolite analyses (Figure 4.)
3.4 Effect of genotype on carotenoids and carotenoid storage capacity

The two genotypes presented differing contents of total carotenoids: in ripe fruit, at 52 DPA, ‘H1311’ appeared three times more concentrated in total carotenoids than ‘M82’ (Figure 5). Concomitantly the plastid compartment density in fresh pericarp was evaluated by confocal microscopy as the area of all plastids per cell volume. Three zones that account for 90% of total pericarp were analysed: the outer epidermis, the collenchyma and the parenchyma. Accordingly, the plastid area per cell tended to be higher in ‘H1311’ pericarp when compared to ‘M82’ (Figure 5). So, carotenoid accumulation seems to be determined by plastid density as illustrated by the example of the three high-pigmented tomato mutants (hp1, hp2, hp3) with enhanced chlorophyll and carotenoid concentrations and altered chloroplast number per cell (Cookson et al., 2003; Galpaz et al., 2008; Kolotilin et al., 2007).

Figure 4. Analysis of tomato (‘M82’ genotype) pericarp at three developmental stages, 20 days post anthesis, 42 and 52 DPA. The three pictures showing cells of total pericarp from inner to outer epidermis have been obtained by confocal microscopy.
Figure 5. Comparison of two tomato genotypes ‘M82’ (in green) and ‘H1311’ (in orange) at three developmental stages, 20, 42, and 52 DPA on the basis of total carotenoid contents (A), plastid area per cell of outer epidermis (B) and plastid area per cell of parenchyma (C). Values are means (n≥3) ± standard errors. Bars marked by different letters indicate significant different values (Kruskal-Wallis test, α = 0.05).

3.5 Combined effects of genotype and drought on the fruit matrix.

The impact of a soil WD on carotenoid content and plastid density was further analyzed (Figure 6). This treatment, at the beginning of the reproductive phase, only affected carotenoid contents in ‘H1311’. Under WD, the total content in carotenoids, expressed on a fruit fresh weight basis, increased by 68%. This rise was due to an increase in dry matter content (+23%) and was also related to changes in the number of plastids per cell in the outer epidermis and the collenchyma (Figure 6). It is important to note that WD also decreased cell volumes of ‘H1311’ fruit.

Several studies have already shown an impact of WD on the amount of total and individual health compounds with strong genetic variability (Ripoll et al., 2016). This is true for carotenoids with variations up to +150 % (Ripoll et al., 2014). The impact of WD on carotenoid bioaccessibility and bioavailability has not been quantified. However, we propose that WD may modify carotenoid bioaccessibility and bioavailability. Indeed, carotenoid bioavailability is reported to depend on carotenoid amount, on food matrix (cell
and plastid structures), and on effectors of absorption (cell walls and lipids) (Castenmiller and West, 1998; Desmarchelier and Borel, 2017).

![Figure 6. Impact of water deficit (in gray) on total carotenoid contents of ripe fruit (A and B) and on the plastid number per cell of immature fruit at 20 and 42 DPA (C). Values for 'M82' are in green and for 'H1311' in orange. Values are means (n ≥ 3) ± standard errors. Bars marked by different letters indicate significant different values (Kruskal-Wallis test, α = 0.05).]

4.0 CONCLUSIONS

The following conclusions can be drawn from this research: (1) mild WD, during the reproductive phase in field, improves crop water use efficiency, increases fruit dry matter content and slightly changes the composition of the dry matter (variations for some metabolites: carotenoids, citric acid); (2) changes in fruit processing characteristics, in response to WD deficit, partly stem from variations in dry matter content. We propose that the apparent viscosity is also driven by the fruit structure.; and (3) WD affects cell size and carotenoid storage capacity. These changes may modify carotenoid bioaccessibility and bioavailability.

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6.0 LITERATURE CITED


