JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

How Does Tomato Quality (Sugar, Acid, and Nutritional Quality) Vary with Ripening Stage, Temperature, and Irradiance?

HÉLÈNE GAUTIER,*,† VICKY DIAKOU-VERDIN,† CAMILLE BÉNARD,§ MARYSE REICH,# MICHEL BURET,# FRÉDÉRIC BOURGAUD,§ JEAN LUC POËSSEL,⊥ CATHERINE CARIS-VEYRAT,# AND MICHEL GÉNARD†

INRA, UR1115 Plantes et Systèmes de culture Horticoles, Domaine St. Paul, Site Agroparc, F-84914 Avignon, France, ENSAIA, UMR1121 Agronomie et Environnement, 2 Avenue Foret de Haye, F-54500 Vandoeuvre, France, UMR408 Sécurité et Qualité des Produits d'Origine Végétale, INRA, Université d'Avignon, F-84000 Avignon, France, and INRA, UR1052 Génétique et d'Amélioration des Fruits et Légumes, Domaine Saint-Maurice, F-84143 Montfavet, France

The objective of this study was to understand the respective impact of ripening stage, temperature, and irradiance on seasonal variations of tomato fruit quality. During ripening, concentrations in reducing sugars, carotenes, ascorbate, rutin, and caffeic acid derivates increased, whereas those in titratable acidity, chlorophylls, and chlorogenic acid content decreased. Fruit temperature and irradiance affected final fruit composition. Sugars and acids (linked to fruit gustative quality) were not considerably modified, but secondary metabolites with antioxidant properties were very sensitive to fruit environment. Increased fruit irradiance enhanced ascorbate, lycopene, β-carotene, rutin, and caffeic acid derivate concentrations and the disappearance of oxidized ascorbate and chlorophylls. Increasing the temperature from 21 to 26 °C reduced total carotene content without affecting lycopene content. A further temperature increase from 27 to 32 °C reduced ascorbate, lycopene, and its precursor's content, but enhanced rutin, caffeic acid derivates, and glucoside contents. The regulation by light and temperature of the biosynthesis pathways of secondary metabolites is discussed.

KEYWORDS: Ascorbate; carotene; fruit temperature; irradiance; phenolics; *Solanum lycopersicum*; sugars; tomato

INTRODUCTION

Fresh tomatoes are produced year-round in the greenhouse under contrasting environmental conditions, triggering seasonal variations in their gustative and nutritional quality (*1*–*5*). Fruit gustative quality fluctuates with the sugar/acid ratio, which generally increases during summer and decreases during winter. Little is known about the environmental regulation of tomato metabolites that are responsible for variations in fruit nutritional quality (*6*). Tomato contains carotenes (mostly lycopene and β-carotene, a precursor of vitamin A), vitamin C, in its reduced form, ascorbic acid (AA) and its oxidized form, dehydroascorbic acid (DHA), and several phenolic compounds such as flavonoids (quercetin and kaempferol derivatives including rutin, and naringenin chalcone) and hydroxycinnamic acid derivatives [caffeic, ferulic and *p*-coumaric acid derivatives including chlorogenic acid (*7*)]. Several factors can affect these antioxidant concentrations, such as the ripening stage, cultivation practices (water availability, mineral nutrients), and climatic environment (mostly light and temperature (*8*)). Antioxidants play an important role by scavenging oxygen-active species generated during ripening (*9*), under excessive radiation (*10*) and cold or heat stress (*11*). Ascorbic acid and some phenolics tend to accumulate from the green to midripe stage, whereas the total carotenes increase constantly during the ripening process (*8*). Seasonal changes in phenolic contents (*12*) or ascorbate (*13*) have generally been related to light environment, probably due to their role in photoprotection (*14*). The soluble phenol content of plants grown under high light is approximately double the content of low-light plants (*12*). Similarly, transferring plants from shade to sunshine leads to increases of 66% of the ascorbic acid content of ripe fruits (*15*). However, ascorbate may also decrease with increased fruit solar exposure, probably due to increased ascorbic acid degradation with elevated temperatures (*16*). The effects of temperature on phenolics and ascorbate have not yet been properly assessed (*8*). Carotene biosynthesis is

* Author to whom correspondence should be addressed [telephone +33 (0)4.32.72.23.45; fax 33 (0)4.32.72.22.82; e-mail gautier@avignon.inra.fr].

† INRA, UR1115 Plantes et Systèmes de culture Horticoles.

§ ENSAIA, UMR1121 Agronomie et Environnement.

UMR408 Sécurité et Qualité des Produits d'Origine Végétale, INRA.

⊥ INRA, UR1052 Génétique et d'Amélioration des Fruits et Légumes.

10.1021/jf072196t CCC: $40.75
Published on Web 02/01/2008

affected by temperature (*17*), but β-carotene and lycopene show differential sensitivity; temperatures above 32 °C specifically inhibit lycopene accumulation (*18*). Increased fruit irradiance (*19*) leads to carotene accumulation at conducive temperatures.

Consequently, secondary metabolites are likely to vary with ripening stage and fruit environment. Nevertheless, it is difficult to correlate fruit composition and fruit environment. Fruit temperature can considerably differ from air temperature because it depends on fruit irradiance, fruit size, and transpiration. Consequently, the impact of increased irradiance can be misunderstood if the temperature impact is not properly taken into account. The experimental environment should therefore be carefully monitored because strong climatic heterogeneities (both irradiance and temperature) may exist among fruits within a plant due to differential exposure to radiation.

Using off-vine fruit ripening provides a convenient tool to study these environmental regulations. This is due to the fact that tomato fruit contains its own photoreceptors (*20*) and is able to ripen when harvested after the mature green stage. Moreover, during off-vine fruit ripening, both fruit temperature and irradiance can be monitored and separately modulated to determine their specific effects on primary and secondary metabolites.

The present study thus aimed to discriminate the impact during ripening of a range of temperatures and/or fruit irradiances on the gustative and nutritional qualities of cherry tomato fruit. Our objectives were to determine (1) how fruit quality was affected by changing fruit environment during ripening, (2) whether or not the accumulation of secondary metabolites during the ripening process was further enhanced by light and temperature conditions, and (3) the regulatory steps of these secondary metabolites pathways by light and temperature, with special emphasis on the carotene biosynthesis pathway.

MATERIALS AND METHODS

Growth Conditions. Cherry tomato plants (*Solanum lycopersicum* L. cv. Cervil) were grown in a greenhouse in Avignon (southern France, 44° N). On January 23, 2004, seeds were sown in pots (30 × 20 cm) containing potting soil (H21 Tref, Tref EGO Substrates B.V., Moerdijk, The Netherlands), and seedlings were transplanted on February 3 in 7 cm diameter pots containing the same substrate. Plants with five growing leaves were transplanted on February 25 in 4 L pots containing potting soil (P3 Tref, Tref EGO Substrates B.V.) in a multispan Venlo-type greenhouse, N−S oriented. The plants were arranged in N−S-oriented double rows of 39 plants, which created a density of 2.9 plants m^{-2} in half a (5.9 × 30.3 m^2) compartment of a multispan, Venlo-type, N−S-oriented greenhouse. Plant nutrition and chemical pest and disease control were in accordance with commercial practices. Water was supplied to the plants using a drip irrigation system to replenish potential evapotranspiration as calculated from the Penman−Monteith equation and to maintain 20−30% drainage. Biological crop protection against whitefly was provided by growing *Macrolophus* (mirical, Koppert, France) throughout the plant growing period. Flowers were mechanically pollinated every 2 days. Inflorescences were each pruned to 12 flowers after anthesis to obtain 12 fruits per truss and limit fruit size heterogeneity among trusses. All plant side shoots were removed as they appeared. Old leaves were removed every 20 days, up to the youngest orange truss (color index 3−4, OCDE).

Experiment 1. Experiment 1 was performed to determine the time course of changes in sugars, acids, and secondary metabolites during ripening as fruit external coloration changes from green immature to red. Vine-ripened tomatoes were harvested at different ripening stages on truss 2 and divided into seven classes of fruits (from A to G, **Figure 1**) according to external fruit coloration. At each ripening stage, fruits were randomly chosen to create three batches of 15 fruits and subjected to physical and chemical analyses to determine the influence of ripening stage on fruit quality.

Experiments 2 and 3. In experiments 2 and 3, different temperatures and light regimens were applied during off-vine ripening of mature green fruits to determine their impact on fruit quality.

Mature green fruits were harvested and divided into 13 batches, corresponding to the control (mature green fruit at harvest, **Figure 1**) and the 12 treatments described in **Table 1**. These can be summarized as follows: two light regimens (D, darkness, PAR < 5 μmol m^{-2} s^{-1}; or L, irradiation, PAR = 200 μmol m^{-2} s^{-1}); two temperatures (21 and 26 °C for experiment 2 or 27 and 32 °C for experiment 3); three ripening times (3, 6, and 8 days for experiment 2 or 1, 3, and 6 days for experiment 3, which ended earlier to avoid over-ripening of fruits).

Each replicate treatment consisted of 15 fruits, plus 4 fruits for fruit temperature measurement by thermocouples that were not further analyzed. Two growth chambers were used to control fruit temperature environment. Fruits were placed individually on trays on a table located 1.5 m from the light source (HQI, Osram, France). The light was left on, and irradiance was measured at the fruit level with Li-190SB quantum sensors (LI-COR Biosciences, Lincoln, NE; **Table 1**). Fruit temperature was measured every minute on four fruits per treatment with very thin thermocouples (0.2 mm copper/constantan) inserted in the middle of the fruit, then averaged, and stored every 30 min on a delta logger (Delta-T DL2e, Delta-T Devices Ldt, Cambridge, U.K.; **Table 1**).

Fruit Analyses. The physical and chemical analyses were performed as previously described (*21*). The fruits were first evaluated individually for physical traits, including fresh weight (FW). External color was characterized near the pistil scar, the pedoncular scar, and at the equatorial zone by a Minolta Chroma meter (CR 300, Minolta, France SA) using the Hunter color coordinates *L*, *a*, and *b*; (*L* = lightness, *a* ranging from green to red, *b* ranging from blue to yellow). Changes in fruit coloration at the three locations show similar patterns, and no heterogeneous fruit coloration was observed; therefore, only measurements near the pistil scar were retained. Fruits were then frozen in a ventilated freezer at −30 °C and stored at −80 °C until they were ground in liquid N_2 and submitted to various biochemical analyses. Dry matter (DM) content was estimated on a sample fraction by the difference in weight before and after lyophilization. Reducing sugar content was measured by colorimetry (with neocuproin), after extraction by boiled water (*22*), by measurement at 460 nm with a colorimeter (Technologie Diffusion, France); titratable acidity was analyzed, after extraction by boiled water, by pH end point at pH 8.1. The pH and refraction index were measured on fruit juice obtained after filtering of unfrozen sample fractions.

In the first experiment, pigments were extracted with acetone and petroleum ether; chlorophylls, β-carotene, and lycopene contents were quantified by spectrophotometry (*23*) with a Shimadzu UV-1605 spectrophotometer. A more precise and complete quantification including different carotene precursors was made in experiments 2 and 3 at harvest and after 6 ripening days under controlled conditions. Carotenes were extracted from 10 g of frozen tomato powder with 100 mL of solvent mixture (hexane/acetone/ethanol, 2:1:1, v/v/v). The mixture was stirred for 30 min in the dark. Water was added (20 mL), and the hexanic phase containing the carotenes was extracted, washed three times with water (3 × 20 mL), filtered on anhydrous sodium sulfate, and evaporated under vacuum. The dry extract was dissolved in 250 μL of methylene chloride/methanol/*tert*-butylmethyl ether (50:25:25, v/v/v) in order to be analyzed by HPLC [HP1050 apparatus equipped with a diode array detector (*24*)]. The calibration curves for β-carotene (Sigma) and lycopene (extracted from tomato oleoresin kindly provided by Naturex, Avignon, France) were obtained from dilution series of a standard solution. Lycopene content was expressed in milligrams of lycopene per kilogram of FW; other carotenes were expressed as equivalents of β-carotene in milligrams of β-carotene per kilogram of FW. The repeatability of the method was assessed with three replicate analyses of a frozen powder obtained from tomato fruit.

Vitamin C content was determined using the method of Deutsch and Weeks (*25*), as automated by Egberg et al. (*26*), with a fluorometer (fluorometer 6200, Jenway). Total and oxidized ascorbate forms were obtained when coal-based activated carbon (Norit, Le Blanc Mesnil, France) was either added or not, respectively, before fluorometric measurement.

Classes	*Fruit external coloration*	Corresponding pictures
A	"Immature green": corresponding to fruit n+2 where fruit n is at the green mature stage (n corresponds to flower appearance order on the truss).	1
B	"Mature green": green external coloration or very beginning of yellow spot.	2
C	"Breaker stage": green with orange locular tissue and orange spots	3–4
D	Orange dominant with green parts	5
E	Orange to deep orange.	6,7
F	Red	8,9
G	Deep red.	10,11

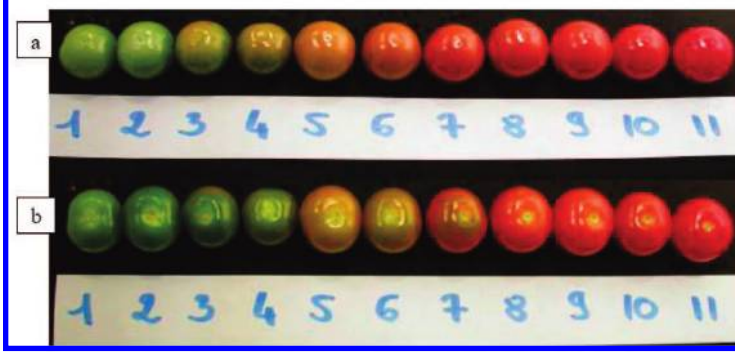

Figure 1. Distribution of vine-ripened tomato fruits into seven classes (from A to G) according to external fruit coloration (**a**, pedoncular zone; **b**, pistillar zone).

Table 1. Fruit Microclimate and Sampling Dates during off-Vine Experiments

	experiment 2		experiment 3	
off-vine experiment	irradiated fruits	shaded fruits	irradiated fruits	shaded fruits
irradiance at fruit level (μmol m^{-2} s^{-1})	210	<5	195	<5
fruit temperature (°C)	21.2 25.8	20.9 25.6	27.1 32.5	27.6 32.5
sampling dates (days after mature green stage)	3 6 8	3 6 8	1 3 6	1 3 6

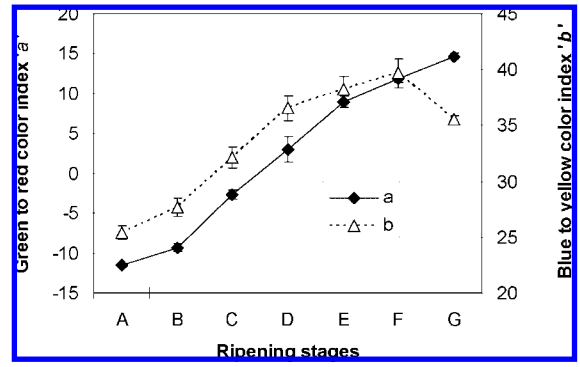

Figure 2. Changes in tomato fruit coloration during ripening. Data are means (±confident interval at 95%) of chromameter measurements of 90 fruits near the pistil scar.

Phenolic compounds were analyzed at harvest (experiments 1 and 3) and after 6 ripening days under controlled conditions (experiment 3). The extraction method employed (*27*) was modified as follows. All steps were carried out under cold conditions (4 °C): in a cold chamber or on ice. One hundred and twenty-five milligrams of DM was extracted three times with 2.5 mL of 70% cold (–20 °C) aqueous ethanol. Fifty microliters of taxifolin (2 mg mL^{-1} methanol, Extrasynthèse, Lyon, France) was added as an internal standard. The mixture was blended for 1 min and homogenized for 15 min the first time and then for 5 min twice. After centrifugation, supernatants were pooled to constitute the raw extract. The extract (total volume = 7.5 mL) was evaporated to dryness under vacuum. The residue was dissolved in 0.5 mL of methanol and filtered through a 0.45 μm filter (Minisart RC 4, Sartorius) prior to injection (20 μl) into the HPLC apparatus. Extractions were made in triplicate. Samples were analyzed using a Beckman System Gold instrument consisting of an autosampler 508 and a diode array detector (module 168, screening = 240–350 nm), set at 330 and 280 nm for the quantifications. Chromatographic separations were performed on a Lichrospher RP-18 end-capped column (4 × 250 mm, 5 μm, Merck, Darmstadt, Germany) fitted with a Lichrospher RP-18 guard column (5 μm, Merck). The mobile phase was a binary solvent system consisting of (A) water adjusted to pH 2.6 with orthophosphoric acid and (B) methanol. The gradient (from 3 to 60% of B in 180 min) was eluted at a flow rate of 0.5 mL min^{-1} at room temperature. All compounds were correctly separated, and quantification was based on peak area, referred to as a standard curve. Rutin content was expressed in milligrams of rutin per kilogram of FW; other compounds were expressed as the equivalent of chlorogenic acid in milligrams of

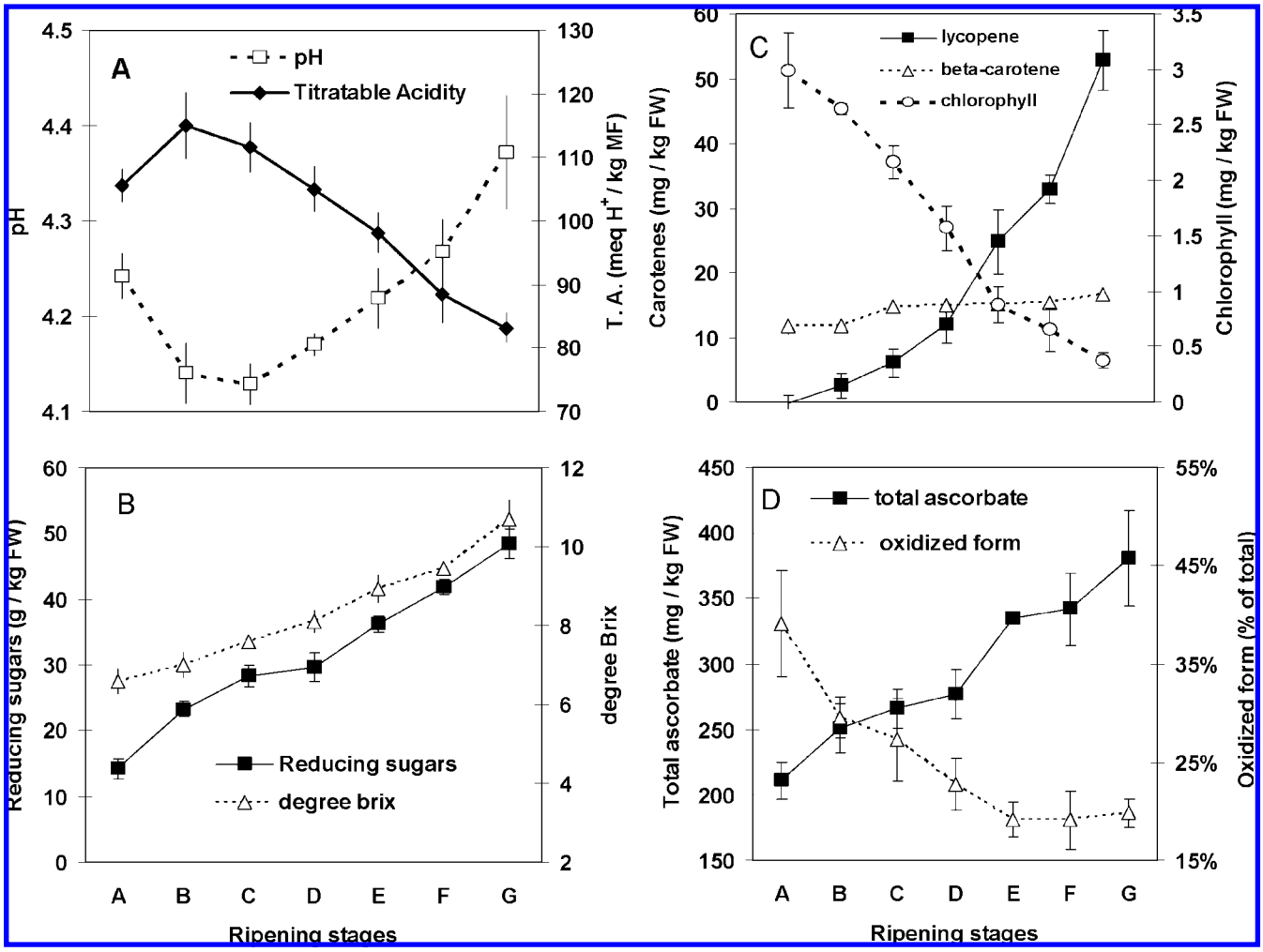

Figure 3. Changes in tomato fruit biochemical composition during ripening: **A**, pH and titratable acidity; **B**, reducing sugars and degrees Brix; **C**, carotenes and chlorophylls; **D**, ascorbate. Data are means (± standard deviation, SD) of six batches of 15 fruits.

chlorogenic acid per kilogram of FW. Rutin and chlorogenic acid standards were purchased from Sigma (Saint Quentin-Fallavier, France). Caffeic derivative compounds were identified by their absorption spectrum in UV (maximum of absorption at 325 nm, see **Figure 4**).

Statistical Analyses. The results of experiment 1 were subjected to one-way analysis of variance considering the factor "ripening stage" (XLstat, version 2007.1, Addinsoft, France). In experiments 2 and 3, the significant effects of temperature, light, ripening time, and the interaction between them were determined from a repeated measures analysis of variance using the GLM procedure from the SAS statistical package (SAS Institute, Inc., Cary, NC). The α level was set at 0.05. Treatment means and standard errors are shown on the curves.

In experiments 2 and 3, fruit contents in different secondary metabolites during ripening were compared after 6 ripening days using a two-way analysis of variance considering the effects of temperature, light, and the interaction between them.

RESULTS

During ripening (experiment 1), both color indices, *a* (which goes from green to red) and *b* (which goes from blue to yellow), increased (**Figure 2**). Titratable acidity (TA) was maximal in mature green fruit (stage B, **Figure 3A**) at 115 mequiv H^+ kg^{-1} of FW, then decreased during ripening, and was minimal in deep red fruit (stage G) at 83 mequiv of H^+ kg^{-1} of FW. The pH of tomato fruit juice followed a mirror-like curve with a minimal value at the green mature and breaker stages (B and C) and a maximal value at stage G (deep red fruit). Reducing sugars and the refractometric index (expressed in °Brix) concomitantly increased during on-vine fruit ripening (**Figure 3B**) by 3.4- and 1.6-fold, respectively, compared to immature green fruit. During fruit ripening, chlorophyll content decreased and carotene content increased because chloroplasts are transformed into chromoplasts. Immature green fruit (stage A) contained no lycopene. It did, however, contain 11.7 mg of β-carotene kg^{-1} of FW, which represents around two-thirds of its final concentration (**Figure 3C**). Deep red fruit contained the greatest amount of both lycopene (52.8 mg kg^{-1} of FW) and β-carotene (16.7 mg kg^{-1} of FW). Total ascorbate content increased throughout fruit ripening from 211 mg at the green immature stage to 380 mg kg^{-1} of FW in deep red fruit (**Figure 3D**). This was mostly due to reduced ascorbate form accumulation (data not shown). In contrast, the oxidized form rapidly decreased from 39% in green immature fruits to 20% of total ascorbate in orange and red fruits (**Figure 3D**).

In deep red tomato, chlorogenic acid, rutin, and a caffeic acid glucoside (CAG) were identified from their absorption spectra (**Figure 4**), plus two caffeic acid derivates (CAD1, CAD2), which showed absorption spectra close to the chlorogenic acid spectrum (see maximum absorbance and spectrum shape, **Figure 4**). For immature green tomato, chlorogenic acid represented the major peak of the chromatogram at 330 nm, which decreased during ripening (**Figure 4**; **Table 3**). In contrast, the contents of rutin,

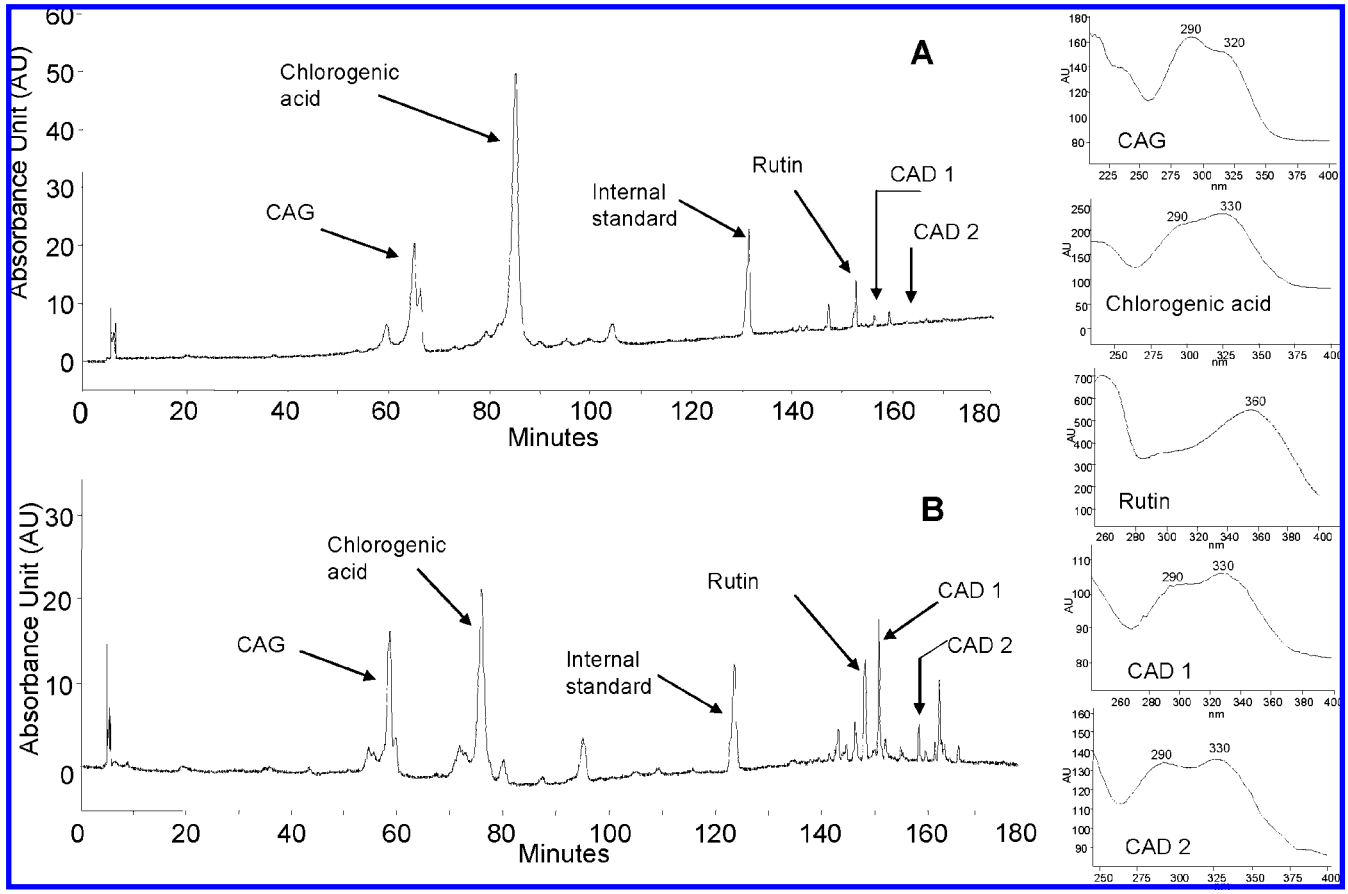

Figure 4. Chromatogram and UV spectra of phenolics found in immature green (**A**) and deep red (**B**) cherry tomatoes. Detection was at 330 nm.

Table 2. Impact of Light and Temperature for Six Ripening Days on Carotenoid Content Compared to Initial Fruit Content (Green Mature Fruit)[a]

	phytoene	phytofluene	ξ-carotene	neurosporene	lycopene	γ-carotene	β-carotene	total carotenes
				Second Experiment				
green mature stage	0.9a	0.6a	0.0a	0.0a	0.5a	0.0a	1.9a	3.9a
light, 21 °C	18.1c	10.8c	2.9b	1.4c	44.8c	1.0c	9.2c	88.1c
dark, 21 °C	10.8b	7.2b	2.4b	0.9b	29.7b	0.9bc	8.1bc	60.2b
light, 26 °C	11.8b	7.0b	2.3b	1.1b	41.3c	0.8bc	7.8bc	72.1bc
dark, 26 °C	8.1b	5.2b	2.2b	0.7b	35.1b	0.7b	6.1b	58.2b
light effect	***	***	***	***	***	***	***	***
temperature effect	*	**	NS	*	NS	*	*	NS
light × temperature effect	NS	*	NS	NS	NS	NS	NS	NS
				Third Experiment				
green mature stage	0.3a	0.5a	0.0a	0.0a	0.4a	0.0a	1.7a	2.8a
light, 27 °C	14.4d	11.1c	4.2c	3.3c	35.7e	3.0b	9.2c	81.0e
dark, 27 °C	11.1c	8.6b	4.4c	2.9bc	22.0c	2.6b	7.1b	58.7c
light, 32 °C	10.2bc	8.6b	3.2b	2.7bc	30.3d	2.9b	10.2c	68.1d
dark, 32 °C	8.3b	7.0b	3.0b	2.4b	17.0b	2.6b	6.8b	47.2b
light effect	**	**	NS	NS	***	*	**	***
temperature effect	***	**	***	*	**	NS	NS	**
light × temperature effect	NS	NS	NS	NS	NS	NS	NS	NS

[a] Carotene contents are expressed as equivalents of β-carotene in milligrams of β-carotene per kilogram of fresh weight (FW), except for lycopene, which is expressed as milligrams of lycopene per kilogram of FW. For each experiment, a two-way analysis of variance was made considering light, temperature, and the interaction between them. NS, not significant, i.e., $P > 0.1$; *, $P < 0.1$; **, $P < 0.01$; ***, $P < 0.001$. Means were compared using an SNK test with a confidence interval of 95%. Different letters per column indicate significant differences.

caffeic acid derivates (CAD1 and CAD2), and CAG increased during ripening between immature green to deep red fruit.

During experiment 2, increasing fruit irradiance enhanced red fruit coloration and chlorophyll degradation (**Figure 5A−D**). Small differences were also observed depending on temperature: increasing fruit temperature from 21 to 26 °C enhanced red fruit coloration and chlorophyll degradation, but it was no more significant after 6 ripening days. In contrast, increasing fruit temperature from 27 to 32 °C reduced red fruit coloration and chlorophyll degradation.

Reducing sugars rapidly increased during off-vine ripening (**Figure 5E,F**), in agreement with observations made during on-vine ripening (**Figure 3**), and light and temperature had no significant effects on final sugar content. Sucrose content did not vary with temperature, irradiance, or ripening duration (data not shown). Titratable acidity decreased during off-vine fruit

Table 3. Changes in Phenolic Contents of Tomato Fruits during On-Vine Ripening from Immature Green to Deep Red Fruit (Experiment 1) and during Green Mature Fruit Ripening at Different Fruit Temperatures and Light Exposures (Experiment 3)[a]

	CAG	chlorogenic acid	rutin	CAD1	CAD2
	First Experiment				
immature stage	34.9a	95.7c	8.4a	0a	0a
breaker stage	37.7a	79.0b	10.2a	7.7b	5.8b
orange	41.4a	67.1a	20.9b	14.9c	8.6c
deep red	44.5a	57.3a	24.2c	16.6d	11.0d
stage effect	*	***	***	***	***
	Third Experiment				
green mature stage	58.9a	211.3b	37.7a	6.4a	4.8a
light, 27 °C	50.0a	121.8a	77.2b	24.8c	17.5c
dark, 27 °C	64.9a	119.0a	48.3b	22.0c	15.5c
light, 32 °C	67.9a	108.8a	107.7c	21.0c	13.8bc
dark, 32 °C	68.8a	115.3a	62.1b	17.1b	10.7b
light effect	NS	NS	**	*	*
temperature effect	*	NS	*	**	**
light × temperature effect	NS	NS	NS	NS	NS

[a] Phenolic contents are expressed as equivalents of chlorogenic acid in milligrams of chlorogenic acid per kilogram of fresh weight (FW), except for rutin content, which is expressed as milligrams of rutin per kilogram of FW. Data are means ± SD. Compounds studied: caffeic acid glucoside (CAG), chlorogenic acid, rutin, and caffeic acid derivates (CAD1 and 2). For each experiment, a two-way analysis of variance was made considering light, temperature, and the interaction between them. NS, not significant, i.e., $P > 0.1$; *, $P < 0.1$; **, $P < 0.01$; ***, $P < 0.001$. Means were compared using an SNK test with a confidence interval of 95%. Different letters per column indicate significant differences.

ripening (**Figure 5G**,**H**) as was previously shown for fruits that ripened on the vine (**Figure 3**). Ripening at 26 °C compared to 21 °C led to a decrease in TA. This effect was stronger on shaded fruits than on irradiated ones. However, increasing fruit temperature from 27 to 32 °C had no effect. At 21 °C, irradiance led to the reduction in titratable acidity, compared to darkness.

Total ascorbate content increased during off-vine ripening (**Figure 6A**,**B**), in agreement with on-vine observations (**Figure 3D**). Ascorbate increased most in irradiated fruits (**Figure 6A**,**B**). Increasing fruit temperature to 32 °C reduced total ascorbate accumulation for irradiated and shaded fruits (**Figure 6B**). The ratio between oxidized and total ascorbate decreased during ripening (**Figure 6C**,**D**), in agreement with on-vine ripening (**Figure 3D**). This decrease was quicker for irradiated fruit at 26, 27, and 32 °C (**Figure 6C**,**D**); however, after 6 ripening days, the oxidized ascorbate ratio was similar, regardless of light and temperature (**Figure 6C**,**D**).

During the second and third experiments, increasing fruit irradiance during ripening significantly increased total carotene content by 45 and 42% at 21 and 32 °C, respectively, and by 26% for intermediate temperatures (**Table 2**). Carotene content decreased when temperature increased from 27 to 32 °C (third experiment). The highest carotene content was found for fruits kept at 21 °C under light (88 mg kg^{-1} of FW) and the lowest for fruits kept at 32 °C under darkness (47 mg kg^{-1} of FW). Regardless of the experiment, phytoene, phytofluene, lycopene, γ-carotene, and β-carotene contents significantly increased with irradiance (**Table 2**). In the third experiment, neurosporene and ζ-carotene were not sensitive to fruit irradiance.

Fruit ripening at 26 °C compared to ripening at 21 °C reduced β-carotene and precursor content, except for lycopene and ζ-carotene (**Table 2**). Increasing ripening temperature from 27 to 32 °C reduced lycopene precursor content and lycopene but neither β- nor γ-carotene content.

Phenolic contents showed a similar evolution during on-vine (first experiment) and off-vine ripening (third experiment): chlorogenic acid content decreased, and rutin, CAD1, and CAD2 increased (**Table 3**), except that the increase in CAG was not significant in experiment 3 compared to experiment 1. Darkness lowered rutin accumulation because shaded fruit had 34−54% less rutin than irradiated ones. In addition, fruit exposed to darkness had less CAD (12−27%), regardless of the temperature, whereas caffeic acid glucoside (CAG) content tended to increase (20−40%). Increasing fruit temperature from 27 to 32 °C reduced CAD content (to 18% in irradiated fruits or to 26% in shaded fruits) but enhanced CAG content (from 9 to 27%) and rutin content of irradiated fruits (by 40%).

DISCUSSION

Changes in Primary and Secondary Metabolites during Ripening. Tomato fruit content in primary metabolites was subject to considerable changes during ripening. Titratable acidity strongly decreased, probably due to reduced malic and citric acid content from the breaker to the deep red stage (*5*). Concomitantly, reducing sugars increased due to starch degradation and glucose and fructose accumulation (*28*). Secondary metabolites also showed strong variations during fruit ripening. Total ascorbate, lycopene, β-carotene, rutin, and caffeic acid derivates reached maximum levels in the deep red tomatoes. In contrast, chlorogenic acid content decreased to 60% of the content of mature green fruit during fruit ripening. This considerable decrease in chlorogenic acid is in agreement with previous works (*29*–*31*). In addition, phenylalanine ammonia-lyase (PAL) is a key enzyme involved in the phenylpropanoid pathway, and its activity was reported to decrease during fruit ripening (*32*), without being a limiting factor for the synthesis of phenolic compounds. The present results on the decreasing content of chlorogenic acid are consistent with a possible decrease in PAL activity and the use of chlorogenic acid as a precursor of other phenolics. Chlorogenic acid may be further catabolized to produce other phenolics such as caffeic acid derivates (CAD1 and CAD2) or caffeic acid glucoside (CAG), which accumulate during ripening.

During ripening, increased AA content and redox status were also observed by Jimenez et al. (*9*), in combination with increased enzymatic activities involved in the antioxidative system. This confirmed that ascorbate plays a key antioxidant role during fruit ripening.

Carotene content increased from 14- to 28-fold during ripening, mainly due to the accumulation of lycopene and, to a lesser extent, phytoene, phytofluene, and β-carotene. This was in agreement with the regulation of carotene biosynthesis described in ripening tomato fruits (*33*) and summarized in **Figure 7**. During ripening, expression of several genes coding for proteins involved in carotenogenesis is modified; in particular, mRNA levels of Psy-1 and Pds increased and mRNAs of lycopene cyclases disappeared (*33*), triggering the accumulation of lycopene and, to a lesser extent, β-carotene.

Light Responses during Fruit Ripening. In the present study, fruit irradiance during ripening did not affect final fruit hexose content, despite a higher transient rate of reducing sugars accumulation for irradiated fruits. This accumulation was probably not due to increased fruit photosynthesis because there was no difference in final fruit sugar content. Moreover, fruit sugar accumulation was probably very low at the mature green stage due to the start of chlorophyll degradation (**Figure 5C**,**D**) and the low fruit photosynthesis capability because sucrose synthase activity was reduced at the end of fruit expansion (*34*).

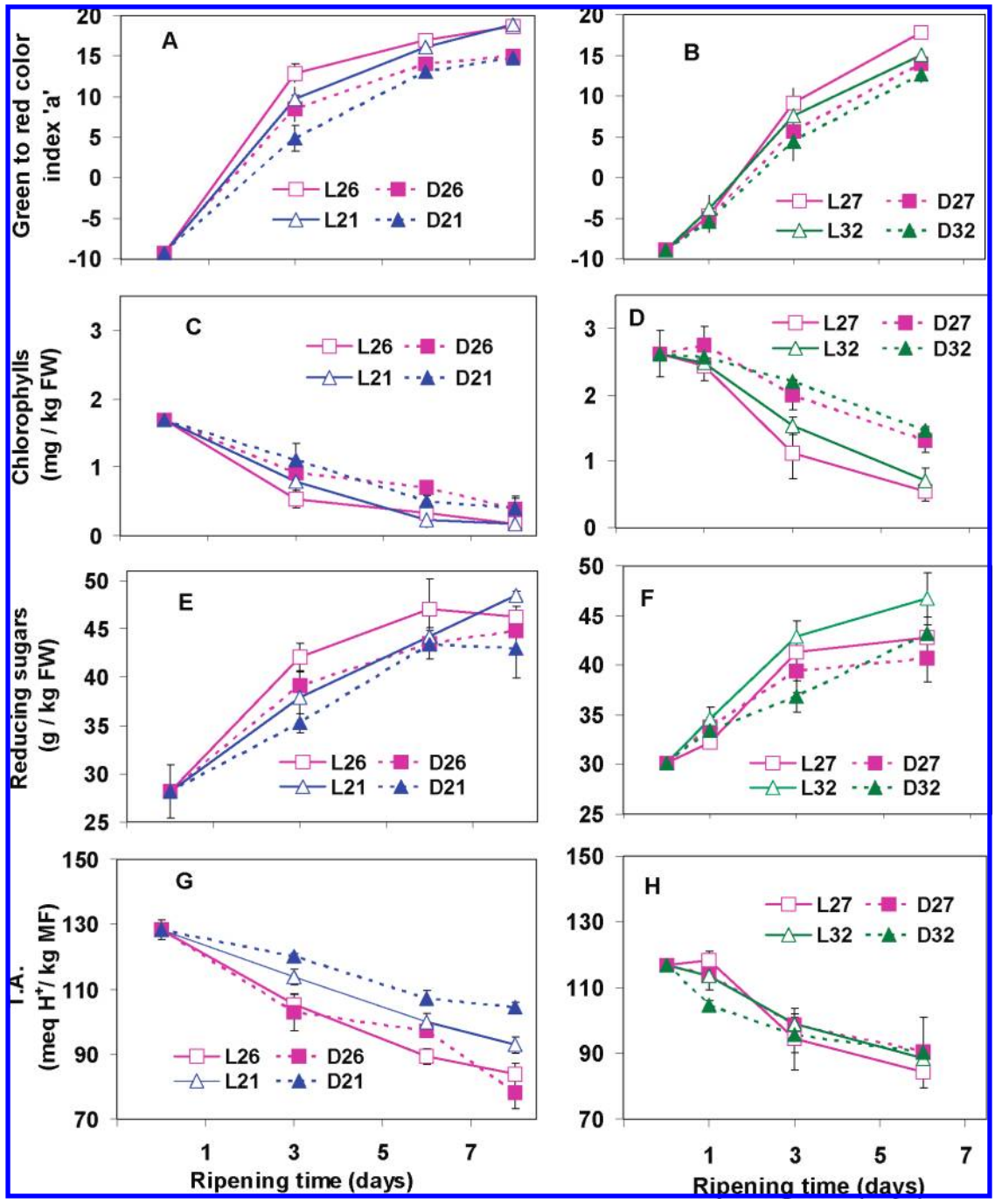

Figure 5. Impact of light and temperature during off-vine fruit ripening on external fruit coloration (**A**, **B**), chlorophyll content (**C**, **D**), reducing sugars (**E**, **F**) and titratable acidity (TA; **G**, **H**). Data are means (±SD) of measurements made on three batches of 15 fruits. **A**, **C**, **E**, **G**, second experiment; **B**, **D**, **F**, **H**, third experiment. L, irradiated fruits (open symbols); D, shaded fruits (solid symbols); 21, 26, 27, 32, fruit temperature expressed in °C.

Light treatment during ripening did not affect fruit carbon source, but it is interesting to speculate that light could influence the transcription of fruit ripening genes or enzymatic activities related to primary or secondary metabolism (*35*).

The present enhancement of carotene accumulation with irradiance is in agreement with the reduced lycopene and β-carotene content observed under selective fruit shading (*36*) and confirms that carotene content of tomato fruit depends on the light intercepted by the fruit itself. Tomato fruits possess different photoreceptors (phytochromes and a blue light photoreceptor, also called cryptochrome), which have been involved in the regulation of carotene biosynthesis. It is well established that the phytochrome regulates light-induced lycopene accumulation (*20*) because red light enhances the first step of carotenogenesis by modulating the phytoene synthase (PSY) activity, which is an important control step of carotene biosynthesis (*37*). Ascorbate content decreased with fruit shading during ripening, in agreement with the data reported by Venter (*13*) or El-Gizawy et al. (*38*) of reduced fruit ascorbate content following plant shading. This confirms that light reaching the fruit itself during ripening significantly increases fruit total ascorbate content (*13*). This could be due to a lower ascorbic acid oxidation under increased irradiance or to enhanced ascorbate synthesis. The latter has been described in *Arabidopsis* leaves, where increasing light enhanced ascorbic acid synthesis by stimulating L-galactose dehydrogenase activity (*39*). In our study, increased ascorbate content was primarily due to an increase of the reduced ascorbate form. However, different

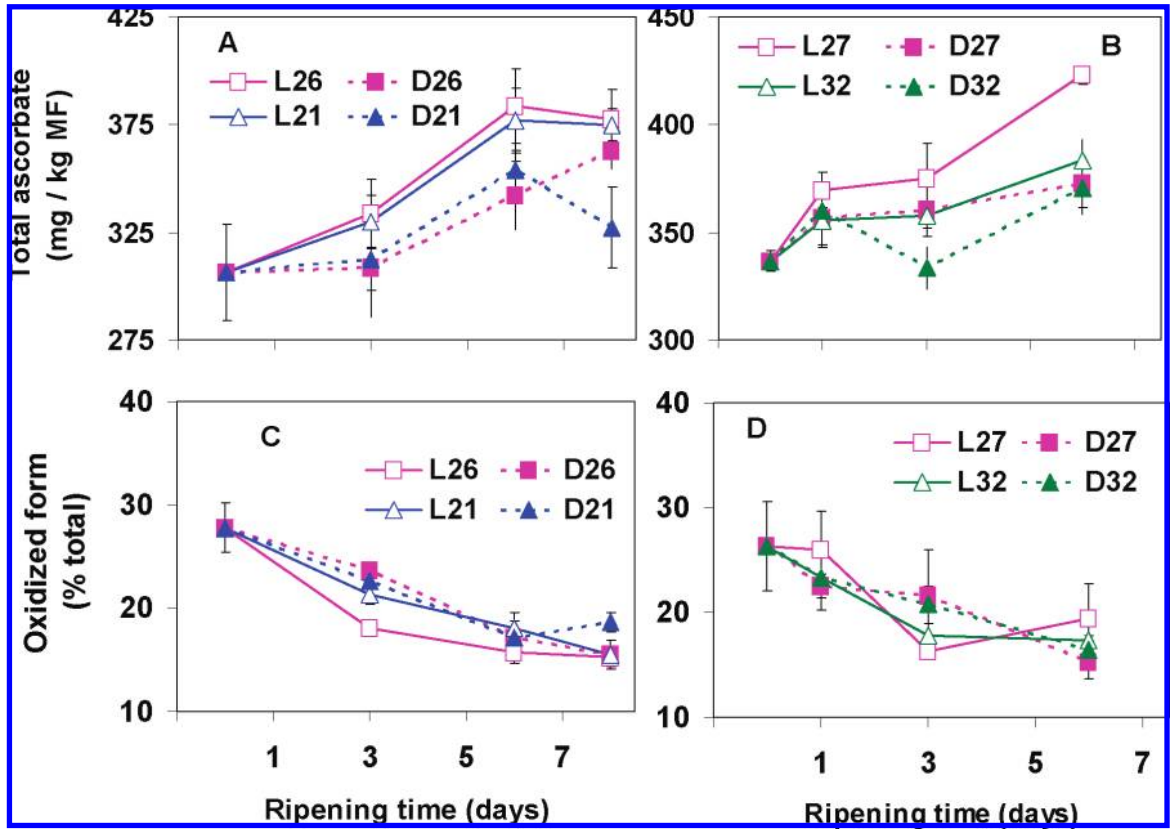

Figure 6. Impact of light and temperature during off-vine fruit ripening on total ascorbate content (**A**, **B**) and oxidized ascorbate ratio (**C**, **D**). Data are means (±SD) of measurements made on three batches of 15 fruits. **A**, **C**, second experiment; **B**, **D**, third experiment. L, irradiated fruits (open symbols); D, shaded fruits (solid symbols); 21, 26, 27, 32, fruit temperature expressed in °C.

enzymes involved in ascorbate recycling (ascorbate peroxidase, dehydroascorbate reductase, and monodehydroascorbate reductase) showed stronger activity with increased light exposure (*16*), consequently regulating the equilibrium between reduced and oxidized ascorbate forms.

We observed that rutin content was increased by 40% for irradiated fruits (**Table 3**); this is consistent with Wilkens et al. (*12*), who found that the concentration of rutin was twice as high for tomato plants growing in high- compared to low-light environments. It has been shown that the general phenolic pathway is strongly affected by light. As an example, chalcone synthase (CHS), a key enzyme catalyzing flavonoid synthesis (i.e., rutin) is up-regulated by light at the transcription level (*40*). In addition, light spectral quality can play a role in phenolic accumulation: red light via phytochrome (*29*) and blue light via cryptochrome (*41*) up-regulate the expression of CHS and, consequently, the formation of flavonoids (*29*).

Temperature Responses during Fruit Ripening. Increased fruit temperature did not affect final hexose content, but when increased from 21 to 26 °C, it reduced TA up to 25%. This could be linked to decreased vacuolar malic acid accumulation as Lobit et al. (*42*) predicted a 50% decrease in malate accumulation when temperatures increased from 15 to 25 °C. Consequently, increasing temperature may improve fruit gustative quality because it increases the fruit sugar acid ratio.

Secondary metabolites were also sensitive to increased temperature. The inhibition of carotene accumulation with increased temperature may explain the seasonal decrease in carotene content observed by Rosales et al. (*43*). In the latter case, defective pigmentations of the fruit were observed when temperature and radiation peaked. Our study allows for the discrimination of radiation and temperature effects and shows that increasing fruit radiation by itself did not inhibit carotene synthesis but promoted it instead.

A strong reduction in lycopene precursor content (mostly phytoene, phytofluene, and neurosporene) was observed when temperatures increased from 21 to 26 °C or from 27 to 32 °C (**Table 2** and **Figure 7**). Increasing fruit temperature from 21 to 26 °C had no effect on lycopene content and reduced β-carotene content. In contrast, increasing fruit temperature from 27 to 32 °C had no effect on β- and γ-carotene, but it strongly reduced precursor contents such as those of phytoene, phytofluene, ζ-carotene, lycopene, and, to a lesser extent, neurosporene. These data corroborate that lycopene synthesis was inhibited above 32 °C, whereas β-carotene synthesis was not (*18*), but they also reveal that the accumulation of some carotenes was already inhibited at 26 °C compared to 21 °C. During fruit ripening, the lycopene β-cyclase is down-regulated at the breaker stage so that lycopene starts to accumulate, and a residual lycopene cyclase activity could be responsible for increased β-carotene (*44*). The fact that β-carotene content was not reduced, whereas total carotene content was reduced up to 24% at 32 °C, could be linked to the reactivation of the lycopene β-cyclase by increased temperature or to a lower catabolism of β-carotene. Consequently, the lowest temperature studied at 21 °C was the most conducive for carotene accumulation; this was probably due to either a higher rate of carotene synthesis via PSY activation or a lower carotene catabolism.

Fruit ascorbate content was found not to be very sensitive to temperature increases from 21 to 26 °C, despite a slightly lower content observed after 8 ripening days in darkness at 21 °C (**Figure 6A**); however, increasing fruit temperature from 27 to

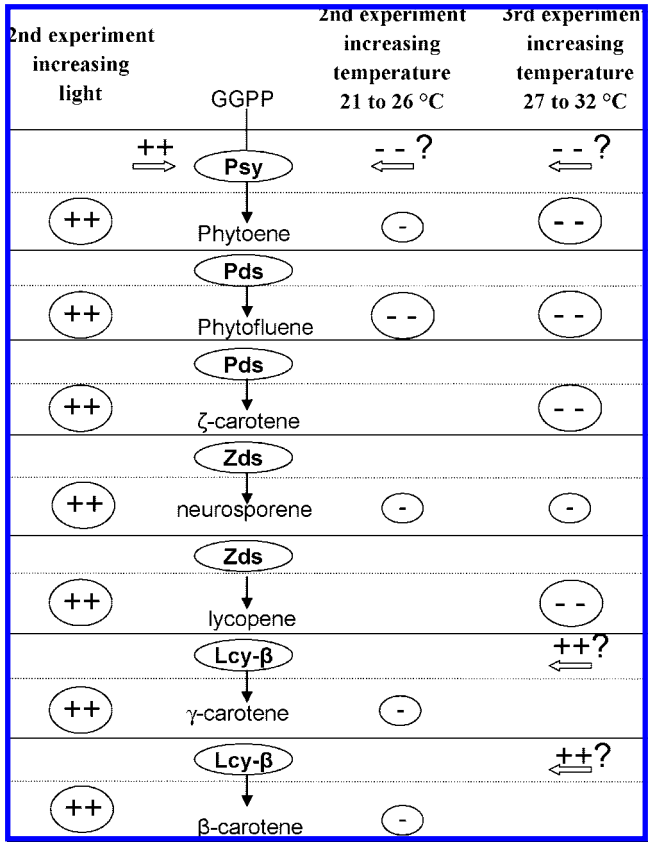

Figure 7. Schematic effect of light and temperature on lycopene and β-carotene metabolism. Horizontal arrows indicate hypothetical changes in gene expression. Plus or minus signs indicate significant changes in carotenoid content: (++) or (− −), significant at $P < 0.01$; (−), significant at $P < 0.1$. GGPP, geranylgeranyl-pyrophosphate; Psy, phytoene synthase; Pds, phytoene desaturase; Zds, ζ-carotene desaturase; Lcy-β, lycopene β-cyclase.

32 °C inhibited ascorbate accumulation (**Figure 6B**). Ascorbate content resulted from ascorbate synthesis, recycling, and degradation, but temperature regulation of these different steps is poorly understood. Rosales et al. (*43*) reported increased oxidation of reduced ascorbate as a result of temperature and irradiance increase during the summer. In the present study, the discrimination between light and temperature effects shows that increasing irradiance promoted ascorbate accumulation, whereas increasing fruit temperature (to 32 °C) limited its accumulation. Therefore, increased temperature could reduce ascorbate synthesis and/or favor ascorbate degradation due to oxidation. The present study did not show any clear effect of temperature on the recycling between oxidized and reduced forms, except a transient reduction in the oxidized form (**Figure 6B**).

Rutin and caffeic acid glucoside are the only phenolic compounds studied the contents of which were significantly improved when fruit temperature was increased from 27 to 32 °C (**Table 3**). This observation is fully consistent with that of Rivero et al. (*11*), who found a 2-fold increase in total phenols for plants grown at temperatures under 35 °C instead of 25 °C.

In conclusion, the present study confirms that fruit quality is strongly modified during fruit ripening: both primary and secondary metabolite contents showed wide variations. Acid content decreases in combination with increased sugar content so that fruit gustative quality improves during fruit ripening. Some secondary metabolites such as chlorogenic acid decreased during fruit ripening, whereas lycopene and ascorbate strongly accumulated after the green mature stage. Thus, considerable differences in both gustative and nutritional quality should be expected according to fruit ripening stage.

Changing fruit temperature and irradiance during ripening had only a small effect on primary metabolites. Fruit irradiance by itself did not affect sugar or acid content. In contrast, increasing fruit temperature from 21 to 26 °C was effective for improving the fruit sugar acid ratio because sugar content was transiently increased and titratable acidity was reduced. Changing fruit temperature and irradiance earlier during fruit development may have a stronger impact on primary metabolites. Indeed, Rosales et al. (*45*) observed lower sucrose content and higher hexose content linked to higher sucrolytic activity in fruits developing under high irradiative and temperature conditions. This could be linked to the effect of temperature and irradiance on phloem flux earlier during fruit development. In the present experiment, temperature and irradiance were modified at the end of fruit development during ripening, when fruit growth had almost stopped and fruit had already accumulated large amounts of carbohydrates.

Changes in fruit environment were more effective on secondary metabolites. Fruit irradiance during ripening stimulates the synthesis of lycopene, β-carotene, ascorbate, and flavonoids known for their antioxidant or photoprotective activity (such as rutin). Increased fruit temperature had contrasting effects on the accumulation of secondary metabolites. Rutin content was considerably increased (+40%) at 32 °C, but lycopene and ascorbate contents were reduced and, consequently, fruit nutritional quality could be altered. Increasing fruit temperature from 21 to 26 °C or from 27 to 32 °C reduced several carotene contents; this could have been due to a down-regulation of the phytoene synthase gene (PSY), which plays a key regulatory role in carotene biosynthesis (**Figure 7**), or to the enhancement of carotene catabolism. Thus, limiting increased fruit temperature during the summer could be useful for improving lycopene and ascorbate content, but this would subsequently reduce the rutin content.

Because fruit temperature and fruit irradiance are closely correlated, a compromise must be found between increased fruit irradiance and increased fruit temperature to monitor fruit antioxidant contents. Before harvest, plant density or leaf area could be modulated according to the season to optimize fruit irradiance and fruit temperature and, consequently, fruit antioxidant content at harvest. The present study underlines different hypotheses of up- or down-regulation of genes involved in carotene, ascorbate, or phenolic pathways by light or temperature. It will be interesting to confirm these hypotheses in a subsequent study and establish threshold response curves to the environment.

ACKNOWLEDGMENT

We thank E. Caroti and F. Pfeiffer for technical assistance.

LITERATURE CITED

(1) Anza, M.; Riga, P.; Garbisu, C. Effects of variety and growth season on the organoleptic and nutritional quality of hydroponically grown tomato. *J. Food Qual.* **2006**, *29*, 16–37.

(2) Toor, R. K.; Savage, G. P.; Lister, C. E. Seasonal variations in the antioxidant composition of greenhouse grown tomatoes. *J. Food Compos. Anal.* **2006**, *19*, 1–10.

(3) Raffo, A.; Malfa, G. L.; Fogliano, V.; Maiani, G.; Quaglia, G. Seasonnal variations in antioxidant components of cherry tomatoes (*Lycopersicon esculentum* cv. Naomi F1). *J. Food Compos. Anal.* **2006**, *19*, 11–19.

(4) Grierson, D.; Kader, A. A. Fruit ripening and quality; physiology and biochemistry of ripening. In *The Tomato Crop, a Scientific*

Basis for Improvement; Atherton, J. G., Rudich, J., Eds.; Chapman and Hall: London, U.K., 1986; pp 241–259.
(5) Davies, J. N.; Hobson, G. E. The constituents of tomato fruit − the influence of environment, nutrition and genotype. *Crit. Rev. Food Sci. Nutr.* **1981**, *15*, 205–280.
(6) Giovannucci, E. Tomatoes, tomato-based products, lycopene, and cancer: review of the epidemiologic literature. *Exp. Biol. Med.* **2002**, *227*, 860–863.
(7) Moco, S.; Bino, R. J.; Vorst, O.; Verhoeven, H. A.; de Groot, J.; van Beek, T. A.; Vervoort, J.; de Vos, J. H. R. A liquid chromatography−mass spectrometry-based metabolome database for tomato. *Plant Physiol.* **2006**, *141*, 1205–1218.
(8) Dumas, Y.; Dadomo, M.; Di Lucca, G.; Grolier, P. Effects of environmental factors and agricultural techniques on antioxidant content of tomatoes. *J. Sci. Food Agric.* **2003**, *83*, 369–382.
(9) Jimenez, A.; Creissen, G.; Kular, B.; Firmin, J.; Robinson, S.; Verhoeyen, M.; Mullineaux, P. Changes in oxidative processes and components of the antioxidant system during tomato fruit ripening. *Planta* **2002**, *214*, 751–758.
(10) Smirnoff, N. Ascorbate biosynthesis and function in photoprotection. *Philos. Trans. R. Soc. London, B* **2000**, *355*, 1455–1464.
(11) Rivero, R. M.; Ruiz, J. M.; Garcia, P. C.; Lopez-Lefebre, L. R.; Sanchez, E.; Romero, L. Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. *Plant Sci.* **2001**, *160*, 315–321.
(12) Wilkens, R. T.; Spoerke, J. M.; Stamp, N. E. Differential responses of growth and two soluble phenolics of tomato to resource availability. *Ecology* **1996**, *77*, 247–258.
(13) Venter, F. Solar radiation and vitamin C content of tomato fruits. *Acta Hortic.* **1977**, *58*, 121–127.
(14) Toor, R. K.; Savage, G. P. Antioxidant activity in different fractions of tomatoes. *Food Res. Int.* **2005**, *38*, 487–494.
(15) Somers, G. F.; Kelly, W. C.; Hamner, K. C. Influence of nitrate supply upon the ascorbic content of tomatoes. *Am. J. Bot.* **1951**, *38*, 472–475.
(16) Torres, C. A.; Andrews, P. K.; Davies, N. M. Physiological and biochemical responses of fruit exocarp of tomato (*Lycopersicon esculentum* Mill.) mutants to natural photo-oxidative conditions. *J. Exp. Bot.* **2006**, *57*, 1933–1947.
(17) Türk, R.; Seniz, V.; Ozdemir, N.; Zuzen, M. A. Changes in the chlorophyll carotenoid and lycopene contents of tomatoes in relation to temperature. *Acta Hortic.* **1994**, *368*, 856–862.
(18) Baqar, M. R.; Lee, T. H. Interaction of CPTA and high temperature on carotenoid synthesis in tomato fruit. *Z. Pflanzenphysiol.* **1978**, *88*, 431–435.
(19) McCollum, J. P. Effect of light on the formation of carotenoids in tomato fruits. *Food Res.* **1954**, *19*, 182–189.
(20) Alba, R.; Cordonnier-Pratt, M. M.; Pratt, L. H. Fruit-localized phytochromes regulate lycopene accumulation independently of ethylene production in tomato. *Plant Physiol.* **2000**, *123*, 363–370.
(21) Saliba-Colombani, V.; Causse, M.; Langlois, D.; Philouze, J.; Buret, M. Genetic analysis of organoleptic quality in fresh market tomato. 1. Mapping QTLs for physical and chemical traits. *Theor. Appl. Genet.* **2001**, *102*, 259–272.
(22) Bittner, D.; Manning, J. Automated neocuproin glucose method: critical factors and normal values. *Automation in Analytical Chemistry*; Technicon: Emeryville, CA, 1967; pp 1–6.
(23) Lime, B. J.; Griffiths, F. P.; O'Connor, R. T.; Heinzelman, D. C.; McCall, E. R. Spectrophotometric methods for determining pigmentation − β-carotene and lycopene − in Ruby red grapefruit. *J. Agric. Food Chem.* **1957**, *5*, 941–944.
(24) Caris-Veyrat, C.; Schmid, A.; Carail, M.; Böhm, V. Cleavage products of lycopene produced by in vitro oxidations: characterization and mechanisms of formation. *J. Agric. Food Chem.* **2003**, *51*, 7318–7325.
(25) Deutsch, M. J.; Weeks, C. E. Microfluorometric assay for vitamin C. *J. Assoc. Off. Agric. Chem.* **1965**, *48*, 1248–1256.
(26) Egberg, D. C.; Potter, R. H.; Geroff, J. C. Semi-automated method for the fluorometric determination of total vitamin C in food products. *J. Assoc. Off. Agric. Chem.* **1977**, *60*, 126–131.
(27) Fleuriet, A. Evolution des composés phénoliques au cours de la croissance et de la maturation des fruits de tomates "cerise" (*Lycopersicum esculentum* var. *cerasiforme*). *Fruits* **1976**, *31*, 117–126.
(28) Ho, L. C. Metabolism and compartmentation of imported sugars in sink organs in relation to sink strength. *Annu. Rev. Plant Phys.* **1988**, *39*, 355–378.
(29) Hunt, G. M.; Baker, E. A. Phenolic constituents of tomato fruit cuticles. *Phytochemistry* **1980**, *19*, 1415–1419.
(30) Fleuriet, A.; Macheix, J. J. Quinyl ester and glucose derivatives of hydroxycinnamic acids during growth and ripening of tomato fruit. *Phytochemistry* **1981**, *20*, 667–671.
(31) Winter, M.; Herrmann, K. Esters and glucosides of hydroxycinnamic acids in vegetables. *J. Agric. Food Chem.* **1986**, *34*, 616–620.
(32) Fleuriet, A.; Macheix, J. J. Tissue compartmentation of phenylpropanoid metabolism in tomatoes during growth and maturation. *Phytochemistry* **1985**, *24* (5), 929–932.
(33) Bramley, P. M. Regulation of carotenoid formation during tomato fruit ripening and development. *J. Exp. Bot.* **2002**, *53*, 2107–2113.
(34) Smillie, R.; Hetherington, S.; Davies, W. Photosynthetic activity of the calyx, green shoulder, pericarp, and locular parenchyma of tomato fruit. *J. Exp. Bot.* **1999**, *50* (334), 707–718.
(35) Adams-Phillips, L.; Barry, C.; Giovannoni, J. Signal transduction systems regulating fruit ripening. *Trends Plant Sci.* **2004**, *9*, 331–338.
(36) Gautier, H.; Rocci, A.; Buret, M.; Grasselly, D.; Dumas, Y.; Causse, M. Effect of photoselective filters on the physical and chemical traits of vine ripened tomato fruits. *Can. J. Plant Sci.* **2005**, *85*, 439–446.
(37) Schofield, A.; Paliyath, G. Modulation of carotenoid biosynthesis during tomato fruit ripening through phytochrome regulation of phytoene synthase activity. *Plant Physiol. Biochem.* **2005**, *43*, 1052–1060.
(38) El-Gizawy, A. M.; Abdallah, M. M. F.; Gomaa, H. M.; Mahamed, S. S. Effect of different shading levels on tomato plants. 2. Yield and fruit quality. *Acta Hortic.* **1993**, *323*, 349–354.
(39) Gatzek, S.; Wheeler, G. L.; Smirnoff, N. Antisense suppression of L-galactose dehydrogenase in *Arabidopsis thaliana* provides evidence for its role in ascorbate synthesis and reveals light modulated L-galactose synthesis. *Plant J.* **2002**, *30*, 541–553.
(40) Feinbaum, R. L.; Ausubel, F. M. Transcriptional regulation of the *Arabidopsis thaliana* chalcone synthase gene. *Mol. Cell. Biol.* **1988**, 1985–1992.
(41) Lin, C.; Ahmad, M.; Cashmore, A. R. *Arabidopsis* cryptochrome 1 is a soluble protein mediating blue light-dependent regulation of plant growth and development. *Plant J.* **1996**, *10*, 893–902.
(42) Lobit, P.; Génard, M.; Soing, P.; Habib, R. Modelling malic acid accumulation in fruits: relationships with inorganic acids, potassium, and temperature. *J. Exp. Bot.* **2006**, *57*, 1471–1483.
(43) Rosales, M. A.; Ruiz, J. M.; Hernández, J.; Soriano, T.; Castilla, N.; Romero, L. Antioxidant content and ascorbate metabolism in cherry tomato exocarp in relation to temperature and solar radiation. *J. Sci. Food Agric.* **2006**, *86*, 1545–1551.
(44) Pecker, I.; Gabbay, R.; Cunningham, F. X.; Hirschberg, J. Cloning and characterization of the cDNA for lycopene b-cyclase from tomato reveals decrease in its expression during fruit ripening. *Plant Mol. Biol.* **1996**, *30*, 807–819.
(45) Rosales, M. A.; Rubio-Wilhelmi, M. M.; Castellano, R.; Castilla, N.; Ruiz, J. M.; Romero, L. Sucrolytic activities in cherry tomato fruits in relation to temperature and solar radiation. *Sci. Hortic.* **2007**, *113*, 244–249.

Received for review July 23, 2007. Revised manuscript received December 11, 2007. Accepted December 21, 2007. This work was supported by INRA and region PACA (Provence Alpes Côte d'Azur: project 2004_05706) and region Lorraine (Ph.D. grant to C. B.).

JF072196T