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Isoprenoid, Lipid, and Protein Contents in Intact Plastids Isolated from Mesocarp Cells of Traditional and High-Pigment Tomato Cultivars at Different Ripening Stages

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ABSTRACT: This study reports quali-quantitative analyses on isoprenoids, phospholipids, neutral lipids, phytosterols, and proteins in purified plastids isolated from fresh fruits of traditional (Donald and Incas) and high-pigment (Kalvert and HLY-18) tomato cultivars at four ripening stages. In all of the investigated cultivars, lycopene, β-catotene, lutein, and total carotenoids varied significantly during ripening. Chromoplasts of red-ripe tomato fruits of high-pigment cultivars accumulated twice as much as lycopene (307.6 and 319.2 μg/mg of plastid proteins in Kalvert and HLY-18, respectively) than ordinary cultivars (178.6 and 151.7 μg/mg of plastid proteins in Donald and Incas, respectively); differences in chlorophyll and α-tocopherol contents were also evidenced. Phospholipids and phytosterols increased during ripening, whereas triglycerides showed a general decrease. Regardless of the stage of ripening, palmitic acid was the major fatty acid in all cultivars (ranging from 35 to 52% of the total fatty acids), followed by stearic, oleic, linoleic, linolenic, and myristic acids, but their relative percentage was affected by ripening. Most of the bands detected on the SDS-PAGEs of plastid proteins were constantly present during chloroplast-to-chromoplast conversion, some others disappeared, and only one, with a molecular weight of ~41.6 kDa, was found to increase in intensity.

KEYWORDS: *carotenoids, chloroplast−chromoplast conversion, fatty acids, lipids, Lycopersicon esculentum (Mill.), tocopherols, tomato ripening*

INTRODUCTION

Plastids are plant cell distinctive organelles that have evolved a wide morphological, metabolic, and functional plasticity. They can be categorized into different interconvertible types (chloroplasts, amyloplasts, chromoplasts, leucoplasts, and elaioplasts) arising, during ontogeny, from embryonic proplastids. Plastid biogenesis, differentiation and interconversion, require a coordinated expression of genes encoded in the nuclear and plastid genomes.[1,2]

Chloroplast-to-chromoplast conversion is a typical plastid transition that occurs during the ripening process of many fruits and the development of flower petals. This transition is visible by the progressive disappearance of the green color and the progressive pigmentation of the tissue in the yellow-red color spectra. The fall in the expression of photosynthetic nuclear genes, the degradation of chlorophylls and photosynthetic proteins, the disassembling of thylakoid membranes,[3] and the simultaneous marked increase of gene expression involved in carotenoid biogenesis[4] are the major events that characterize this transition.

Ripening of tomato [*Lycopersicon esculentum* (Mill.)] fruits is a very complex developmental process involving several drastic changes, including the chloroplast-to-chromoplast transition. The red color of the pericarp tissue of ripening tomato fruits is mainly due to the synthesis and storage of enormous quantities of the red carotenoid lycopene.[5] Several chromoplast types have been described; this morphologic heterogeneity likely reflects the high variety of carotenoids showing a different solubility and the possibility to form crystals during their storage.[6]

Besides photosynthesis, chloroplasts, well-recognized as evolutionary ancestors of all the other plastids,[1] are the site of important plant metabolic pathways. Among these, the 2-*C*-methyl-D-erythritol 4-phosphate (MEP) pathway has a fundamental role in the biosynthesis of isoprenoids required as precursors for several photosynthesis-related compounds (carotenoids, chlorophylls, phylloquinones, plastoquinones, and tocopherols), hormones (gibberellins, strigolactone, and abscissic acid), isoprene, monoterpenes, and some sesquiterpenes.[7−12] In addition, the mevalonic acid (MVA) pathway, through which the isopentenyl diphosphate (IPP) and its isomer dimethylallyl diphosphate (DMAPP) are synthesized, occurs with the involvement of the cytosol, endoplasmic reticulum, and chloroplasts as well as other plastids. Because in plant cells both MEP and MVA pathways are functional, very complex modulation mechanisms of the isoprenoid metabolic pathway operate in the transition from chloroplast to chromoplast during tomato ripening.[12−14]

Increasing interest has been devoted to carotenoid content and composition of food crops because of their important role in human health.[15] Red-ripe tomatoes and tomato-based products are the major dietary sources of lycopene. So far, several attempts have been made to increase carotenogenesis in

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tomato fruits. The carotenoid biosynthetic pathway has been well established, and progress in gene cloning has also been made.[9,10,15] Simultaneously, natural high-pigment tomato mutants have been exploited to develop cultivars with increased nutritional value.[16−19] These pigment mutations have already been introgressed into different commercial tomato cultivars that are sold on the market as lycopene-rich tomatoes.[17,20−23] Investigations performed on these mutants have suggested that plastid biogenesis is the major determinant in driving the increase of the fruit phytonutrients.[19,24−26]

Although several investigations have monitored the amount of carotenoids and, in general, isoprenoids during ripening of tomato fruits,[27−30] little attention has been given to the changes of these compounds in plastids during the transition from chloroplast to chromoplast. The aim of this study was to explore these aspects by monitoring carotenoids (lycopene, β-carotene, and lutein) at different ripening stages in purified plastids isolated from fruits of two traditional (Donald and Incas) and two high-pigment (Kalvert and HLY-18) tomato cultivars. A more detailed analysis on plastid isoprenoid contents, as well as on the protein pattern change during tomato ripening, was performed on Donald and HLY-18 cultivars. At all ripening stages, phospholipid, sterol, and neutral lipid (triacylglycerols, diacylglycerols, and free fatty acids) contents were also chemically characterized in purified plastids of all the investigated cultivars. The characterization of these parameters has a remarkable meaning in the light of a potential use of purified plastids as superior quality ingredients in functional foods and goods.

MATERIALS AND METHODS

Plant Culture. Tomato plants were cultivated in an open field in the province of Lecce (southern Italy) during the 2007 and 2008 growing seasons (April−August). Four tomato cultivars with a determinant growth habit were used in these experiments: two traditional (Donald and Incas) cultivars and two tomato cultivars claimed to be high-lycopene (HLY-18 and Kalvert). Seeds of Donald and Incas cultivars were from Nunhems (Nunhems SRL, BO, Italy), whereas seeds of HLY-18 and Kalvert cultivars were from COIS' 94 Srl (Belpasso, CT, Italy). Sowing was carried out in alveolar boxes at the beginning of April. One-month-old tomato seedlings were transplanted in an open field with a spacing of approximately 50 cm within the row and 100 cm between rows, matching a density of about 20000 plants/ha, and grown to maturity. About 1500 plants per cultivar were arranged in 15 consecutive rows of 100 individuals for a total cultivated area of roughly 0.3 ha. Standard agronomical techniques were used for plant nutrition and pathogen prevention as previously described.[31] Drip irrigation ran for 1−2.5 h, at 1−2 day intervals, depending on potential evapotranspiration, climate data, and crop coefficient.

Fruit Sampling. Tomato fruits of each cultivar were hand harvested randomly from the 50 m rows and from the middle of the plant at different maturity stages and quickly delivered to the laboratory. From these, intact tomato fruits were visually sorted according to the U.S. color standard for classifying tomato ripeness in four ripening stages corresponding to green [fruit surface completely green, varying from light to dark green; approximately 20 days post anthesis (DPA)], green-orange (first appearance of external change in color on not more than 10% of fruit surface; approximately 35 DPA), orange-red (over 30% but not more than 60% red; approximately 40 DPA), and red-ripe (over 90% red surface; desirable table ripeness; approximately 45 DPA) fruit color.[32] Sampling was repeated three times during the last three months of the growing season for two consecutive years. The tomato samples were immediately used for plastid isolation and purification.

Plastid Isolation and Purification. Plastids were isolated and purified accordingly to the protocol of Fraser et al.[27] slightly modified. Tomatoes (at least 2 kg) were cut open; the peel, the gelatinous material containing seeds (placental tissue), and the columella were discarded. All subsequent steps were carried out in a cold room at 4 ± 1 °C. The remaining mesocarp was sliced, weighed, diluted 1:1 by weight with homogenization buffer [50 mM Tris-HCl buffer (pH 8.0), containing 0.4 M sucrose, 1 mM EDTA, and a cocktail of protease inhibitors (Roche S.p.A, Milan, Italy)], and homogenized (three cycles of 10 s) in a mixer (Waring Laboratory and Science, Torrington, CT). Each homogenate was filtered through gauze and, sequentially, through one layer of Miracloth (pore size = 22−25 μm; Merck Chemicals Ltd., Nottingham, U.K.).

Intact chloroplasts were precipitated by centrifugation (5000*g*, 15 min, 4 °C) of the filtrates prepared from tomatoes at the green ripening stage, using a Beckman model J2-21 centrifuge (Beckman Coulter Inc., Brea, CA). The pellet was washed twice with homogenization buffer (5 mL) and recovered by centrifugation (10000*g*, 15 min, 4 °C).

The filtrates prepared from tomatoes at the green-orange, orange-red, and red-ripe stages of ripening were centrifuged at 2000*g* for 5 min; the supernatants were then centrifuged at 10000*g* for 15 min to yield the dedifferentiating and differentiated chromoplast fractions. The pellets were washed twice with homogenization buffer (5 mL) and recovered by centrifugation (10000*g*, 15 min, 4 °C).

The washed plastids (chloroplast and dedifferentiating/differentiated chromoplasts fractions) were suspended with 2 mL of homogenization buffer and layered onto a stepwise sucrose gradient (9 mL) formed with 0.45, 0.84, and 1.45 M sucrose solutions in 50 mM Tris-HC1 buffer (pH 7.6), containing 1 mM dithiothreitol (DTT). Following centrifugation at 62000*g* for 1 h in a swing-out rotor (L8-55 ultracentifuge with SW 32 Ti rotor, Beckman Coulter Inc.) intact plastids were obtained at the interface between 0.84 and 1.45 M sucrose. Plastid integrity was verified by phase contrast microscopy [Leiz Orthoplan microscope, Leica microsystems GmbH (former Ernst Leitz Wetzlar GmbH), Wetzlar, Germany].

Plastids were recovered from the interface and washed five times with 5 mL of Hepes buffer [40 mM Hepes−NaOH buffer (pH 7.5), containing 10 mM imidazole, 1 mM benzamidine, 5 mM 6-amino-hexanoic acid, 10 mM DTT, and 1 mM phenylmethanesulfonyl fluoride] by centrifuging at 25700*g* for 10 min to remove excess sucrose.

Plastids were immediately frozen with liquid nitrogen, freeze-dried (Christ ALPHA 2-4 LSC freeze-dryer, Martin Christ Gefriertrocknungsanlagen GmbH, Osterode am Harz, Germany) for 24 h and stored at −80 °C.

Protein Analysis. Aliquots (5 mg) of freeze-dried plastids were suspended in 400 μL of Hepes buffer and delipidated according to the method of Hori and Elbein.[33] Delipidated proteins were dissolved in 200 μL of Hepes buffer and precipitated with 80% acetone at −20 °C (three times). Total protein concentration was quantified by the biuret method[34] using the Bio-Rad (Richmond, CA) protein assay and bovine serum albumin as standard. Protein contents were expressed as percent of variation during tomato chloroplast-to-chromoplast dedifferentiation with respect to the green stage of ripening. Proteins were analyzed by SDS-PAGE according to the method of Laemmli[35] on a 13% polyacrylamide gel. Gels were stained with Coomassie brilliant blue R-250.

It is well-known that chloroplast-to-chromoplast transition is associated with important structural, metabolic, and molecular reorganization, including the disruption of the photosynthetic machinery and the reduction in the levels of proteins and mRNAs associated with photosynthesis;[3] therefore, we decided to normalize isoprenoid and lipid contents to the amount of plastid proteins, as an easily and accurately quantifiable reference parameter.

Determination of Carotenoid Contents. Lycopene, β-carotene, and lutein contents were determined according to the method of Sadler et al.[36] as modified by Perkins-Veazie et al.[37] on triplicate aliquots (5 mg) of freeze-dried purified plastids. Freeze-dried plastids were resuspended into 400 μL of Hepes buffer and subjected to carotenoid extraction. Carotenoids were extracted with 0.05% (w/v) butylated hydroxytoluene (BHT) in acetone and 95% ethanol (1:1 by vol). Lycopene, β-carotene, and lutein were separated by partition into hexane and directly assayed. A Dionex HPLC (Dionex s.r.l., Milan, Italy)

with an AD 25 UV−vis detector was used, and the separation was performed at 31 °C on an Acclaim HPLC column C_{18} (5 μm, 250 × 4.6 mm). The separation was performed by using a linear gradient of acetonitrile (A), hexane (B), and methanol (C) as follows: from 70% A, 7% B, 23% C to 70% A, 4% B, 26% C within 35 min, with a flow rate of 1.5 mL/min. Concentration of standard solutions was calculated using the molar extinction coefficients 17.2×10^4 for lycopene, 13.9×10^4 for β-carotene, and 14.3×10^4 for lutein in hexane. Peaks were detected at 503 nm, and results were expressed as micrograms per milligram of plastidial proteins.

Determination of Isoprenoid Contents. Isoprenoids were extracted and analyzed as described by Long et al.[38] Briefly, triplicate aliquots (2 mg) of freeze-dried plastids were suspended into 100 μL of methanol and mixed for 5 min at 4 °C. Tris-HCl (50 mM, pH 7.5), containing 1 M NaCl, was added (100 μL) and a further incubation at 4 °C for 10 min carried out. Chloroform (400 μL) was then added to the mixture and incubated on ice for 10 min. A clear partition was formed by centrifugation at 3000*g* for 5 min at 4 °C. The lower phase was removed and the aqueous phase re-extracted with chloroform (400 μL). The pooled chloroform extracts were dried under a stream of nitrogen. Dried residues were dissolved in ethyl acetate and analyzed by HPLC.

Chromatography was carried out on a Waters system (Watford, Hertfordshire, U.K.) consisting of a no. 616 pump, a no. 996 diode array detector, and a no. 717 autosampler. Throughout chromatography, the eluate was monitored continuously from 200 to 600 nm. Column temperature was maintained at 25 °C by a no. 7955 column oven (Jones Chromatography, Hengoed, Mid-Glamorgan, U.K.). A reverse-phase C_{30} column (5 μm, 250 × 4.6 mm) coupled to a 20 × 4.6 mm C_{30} guard (YMC Inc., Wilmington, NC) was used with mobile phases consisting of methanol (A), 0.2% ammonium acetate in water/methanol (20:80 by vol) (B), and *tert*-methyl butyl ether (C). The gradient elution was 95% A/5% B isocratically for 12 min and a step to 80% A/5% B/15% C at 12 min, followed by a linear gradient to 30% A/5% B/65% C by 30 min. Flow rate of 1 mL/min was used. Peak areas were determined at the wavelength providing maximum absorbance using the Waters Millennium software. Isoprenoid contents were expressed as micrograms per milligram of plastidial proteins and micrograms per milligram of plastid freeze-dried matter (f-dm).

Lipid Determinations. Total lipids were extracted using the Bligh and Dyer method[39] starting from triplicate 10 mg aliquots of freeze-dried purified plastids.

Neutral lipids were separated using 20 × 20 cm TLC silica gel 60 glass plates (Merck Sharp & Dohme Spa, Rome, Italy) in hexane/diethyl ether/acetic acid (70:30:1 by vol). Tripalmitin, dipalmitin (approximately 50% 1,2- and 50% 1,3-isomer) and palmitic acid (Sigma-Aldrich Srl., Milan, Italy) were used as external standards for triacylglycerols, diacylglycerols, and free fatty acids, respectively. TLC plates were sprayed with cupric acetate (3% w/v) and phosphoric acid (8% by vol.) and incubated at 150 °C for 1 h. The quantification of neutral lipids was made densitometrically by a Kodak Electrophoresis Documentation and Analysis System 290 using Kodak ID 3.6 software (Kodak, New Haven, CT).

Phospholipid phosphorus was assayed according to the Nakamura method[40] starting from aliquots (1−2 mg of the total lipid extract).

For phytosterol and free fatty acid determinations, aliquots (2 mg) of the total lipid extract were emulsified with 300 μL of distilled water and saponified with ethanolic KOH for 90 min at 90 °C. Phytosterols were extracted by partitioning in 500 μL of low-boiling petroleum ether. The extract was then evaporated and the residue dissolved in 2-propanol. Phytosterols were quantified using a kit (SGM Italia, Rome, Italy) based on the colorimetric/enzymatic Trinder method.

The ethanolic phase remaining after phytosterol extraction was acidified with 3.5 M HCl, and free fatty acids were extracted with 500 μL of petroleum ether. Their corresponding methyl esters were prepared by transesterification with methanolic BF_3 (170 g/L) at 65 °C for 30 min. Fatty acid methyl esters (FAMEs) were analyzed in an Agilent HP 6890 gas chromatography system (Agilent Technologies, Santa Clara, CA). Helium carrier gas was used at a flow rate of 1 mL/min. FAMEs were separated on a 30 m × 0.25 mm Omegawax 250 capillary column (Sigma-Aldrich Srl). The injector and detector temperatures were maintained at 250 °C. The column was operated isothermally at 150 °C for 4 min and then programmed to 250 °C at 4 °C min^{-1}. Peak identification was performed using known standards (FAME mix, Sigma-Aldrich Srl), and relative quantitation was automatically carried out by peak integration. Data were expressed as micrograms per milligram of plastidial proteins.

Statistical Analysis. Unless differently reported, results are presented as the mean value ± standard deviation of at least three independent replicated experiments for each of the two years considered in this study ($n = 6$). Statistical analysis was based on a one-way ANOVA test. The posthoc method by Holm−Sidak was applied to establish significant differences between means ($p = 0.05$). All statistical comparisons were performed using SigmaStat version 3.11 software (Systat Software Inc., Chicago, IL).

RESULTS

From a statistical point of view, the protein content of plastids isolated from tomato fruits at the green stage of ripening did not vary significantly among cultivars ($p = 0.051$), showing an average value of 240 μg/mg f-dm. Nevertheless, in all tested cultivars, a marked differential decrease in total plastid protein content was evidenced (Table 1) as chloroplast dedifferentiated to chromoplast during tomato fruit ripening.

Table 1. Variation in the Amount of Proteins during Tomato Chloroplast-to-Chromoplast Dedifferentiation[a]

	variation (%) stage of ripening			
cultivar	green	green-orange	orange-red	red-ripe
Donald		−38	−52	−76
Incas		−16	−71	−71
Kalvert		−35	−46	−55
HLY-18		−47	−51	−67

[a]Mature and dedifferentiating plastids were isolated from mesocarp cells of traditional (Donald, Incas) and high-pigment (Kalvert, HLY-18) tomato cultivars harvested at four different ripening stages.

In all investigated tomato cultivars, the amounts of lycopene, β-carotene, lutein, and total carotenoids varied significantly during fruit ripening ($p < 0.001$) (Table 2). At the green stage, the amount of lycopene, expressed as micrograms per milligram of plastid proteins, was undetectable in all four cultivars (Table 2). A sharp increase in lycopene content occurred in dedifferentiating and fully differentiated chromoplasts isolated from tomato fruits during the transition from the green-orange to the red-ripe stage of ripening. Although the general patterns of lycopene biosynthesis and accumulation in plastids were similar in the four cultivars, it was quantitatively different among them during ripening. These differences were remarkably evident when the traditional and high-pigment cultivars were compared. At the final stage of ripening (red-ripe), the amount of lycopene in chromoplasts of high-pigment (Kalvert and HLY-18) cultivars was approximately twice that in the traditional (Donald and Incas) ones.

The amount of β-carotene was low at the green stage. It reached values of approximately 1.1 and 1.4 μg/mg of plastid proteins for Donald and Incas cultivars, respectively, and was slightly higher (2.4 μg/mg of plastid proteins) for both Kalvert and HLY-18 cultivars. The highest amount of β-carotene was recorded at the green-orange stage of tomato ripening and corresponded to 5.0 and 6.4 μg/mg of plastid proteins for Donald and Incas cultivars and 9.5 and 16.1 μg/mg of plastid proteins

Table 2. Carotenoid Content in Purified Plastids Isolated from the Mesocarp of Traditional (Donald, Incas) and High-Pigment (Kalvert, HLY-18) Tomato Cultivars at Four Different Ripening Stages[a]

	carotenoid content stage of ripening							
	green		green-orange		orange-red		red-ripe	
cultivar/carotenoid	μg/mg	%	μg/mg	%	μg/mg	%	μg/mg	%
Donald								
lycopene			10.4 ± 1.0aA	49.8	39.7 ± 3.1bA	89.6	178.6 ± 5.1cB	96.5
β-carotene	1.1 ± 0.5aA	22.9	5.0 ± 0.1dA	23.9	2.1 ± 0.8bA	4.7	4.2 ± 0.3cB	2.3
lutein	3.7 ± 0.2bA	77.1	5.5 ± 0.7cA	26.3	2.5 ± 0.4aA	5.7	2.3 ± 0.6aB	1.2
total	**4.8 ± 0.7aA**	**100.0**	**20.9 ± 1.8bA**	**100.0**	**44.3 ± 4.3cA**	**100.0**	**185.1 ± 6.0dB**	**100.0**
Incas								
lycopene			32.5 ± 1.9aBC	70.7	102.6 ± 2.1bC	95.1	151.7 ± 15.0cA	96.6
β-carotene	1.4 ± 0.3aA	25.9	6.4 ± 0.7 dB	13.9	2.6 ± 0.3bA	2.4	4.2 ± 0.2cB	2.7
lutein	4.0 ± 0.8cAB	74.1	7.1 ± 1.0 dB	15.4	2.7 ± 0.1bA	2.5	1.2 ± 0.4aA	0.7
total	**5.4 ± 1.1aA**	**100.0**	**46.0 ± 3.6bB**	**100.0**	**107.9 ± 2.5cC**	**100.0**	**157.1 ± 15.6dA**	**100.0**
Kalvert								
lycopene			35.4 ± 2.9aC	63.3	92.5 ± 2.2bB	92.7	307.6 ± 7.6cC	98.1
β-carotene	2.4 ± 0.3aB	33.3	9.5 ± 0.3cC	17.0	2.5 ± 0.3aA	2.5	3.5 ± 0.2bA	1.1
lutein	4.8 ± 0.3aB	66.6	11.0 ± 0.5cC	19.7	4.8 ± 0.4aB	4.8	2.5 ± 0.3bB	0.8
total	**7.2 ± 0.6aB**	**100.0**	**55.9 ± 3.7bC**	**100.0**	**99.8 ± 2.9cB**	**100.0**	**313.6 ± 8.1dC**	**100.0**
HLY-18								
lycopene			25.1 ± 10.2aB	43.7	274.6 ± 6.1bD	95.8	319.2 ± 1.1cD	96.0
β-carotene	2.4 ± 0.4aB	27.9	16.1 ± 1.2dD	28.0	4.7 ± 1.9bB	1.7	7.8 ± 0.6cC	2.4
lutein	6.2 ± 1.1abC	72.1	16.3 ± 0.1cD	28.3	7.2 ± 0.9bC	2.5	5.3 ± 0.8aC	1.6
total	**8.6 ± 1.5aB**	**100.0**	**57.5 ± 11.5bC**	**100.0**	**286.5 ± 8.9cD**	**100.0**	**332.3 ± 2.5dD**	**100.0**

[a]Data are the mean ± standard deviation of six independent replicates. Lower case letters indicate mean separation among stages of ripening within cultivar by the Holm−Sidak test, $p < 0.05$. Upper case letters indicate mean separation among cultivars within stage of ripening and carotenoid by the Holm−Sidak test, $p < 0.05$.

for Kalvert and HLY-18 cultivars, respectively. At the orange-red stage, the amount of β-carotene tended to decrease in all cultivars, whereas it increased again at the red-ripe stage. Similarly to β-carotene, also lutein showed the highest content at the green-orange stage, but its amount progressively decreased during fruit ripening. This occurred in traditional as well as in high-pigment cultivars (Table 2).

By using the methodological approach described by Fraser et al.,[41] the simultaneous separation of carotenoids, xanthophylls, chlorophylls, tocopherols, and plastoquinones in a single chromatography run was performed on plastids isolated at the four tomato ripening stages from Donald and HLY-18 cultivars, representative of traditional and high-pigment cultivars, respectively. In this case, the amount of each isoprenoid was expressed as micrograms per milligram of plastid proteins and micrograms per milligram of plastid f-dm (Table 3). At the green stage, phytoene and lycopene were not detected in either cultivar. During fruit ripening, the total amount of both acyclic carotenoids (phytoene plus lycopene) progressively increased in both cultivars with a more marked increase of lycopene content. These values were always higher in HLY-18 cultivar than in Donald cultivar when referred to milligrams of plastid proteins or to plastid f-dm. Other than phytoene and lycopene, no other acyclic carotenoids, namely, phytofluene, ζ-carotene, and neurosporene, were detected in all ripening stages.

In both cultivars, the total amount of cyclic carotenoids, including δ-carotene, lutein, γ-carotene, β-carotene, and violaxanthin, detected in tomato fruit plastids at the four different ripening stages showed a more complex qualitative and quantitative pattern (Table 3). In Donald cultivar, the highest amount of total cyclic carotenoids, referred as micrograms per milligram of plastid f-dm, was recorded at the green-orange stage (2.6) and the lowest at the green stage (1.2) with intermediate values at the orange-red (1.3) and red-ripe (1.6) stages., whereas in HLY-18 cultivar, the highest amount was recorded at the green stage (2.5) and the lowest at the green-orange (1.5) with intermediate values at the orange-red (2.1) and red-ripe (2.3) stages. When these values were referred to milligrams of plastid proteins, in Donald cultivar the highest amount was recorded at the red-ripe (28.0) stage, followed by the green-orange (24.3), orange-red (10.5), and green (4.3) stages of ripening. In HLY-18 cultivar a marked increase of the amount of cyclic carotenoids was observed during the transition from green (11.5) to red-ripe (45.3) stage.

In Donald cultivar, the ratio between total acyclic/cyclic carotenoids increased from the green-orange (0.57) to the orange-red (3.93) and red-ripe (6.83) stages, whereas in HLY-18 cultivar, the increase was more marked from green-orange (2.44) to orange-red (8.31) stages and remained approximately constant at the red-ripe (8.11) stage.

Besides differences in carotenoid contents, the traditional and high-pigment cultivars also differed in the amount of other isoprenoid compounds such as chlorophylls (*a* plus *b*) and α-tocopherol. The total amount of chlorophylls (*a* plus *b*), expressed as micrograms per milligram of plastid f-dm, similarly decreased in both cultivars during the green to green-orange stages of ripening and then disappeared at the last stages (orange-red and red-ripe). On the contrary, when these values were referred to milligrams of plastid proteins, increases of approximately 55% in Donald cultivar and 119% in HLY-18 cultivar were detected from the green to the green-orange stage. Obviously, no chlorophylls were detected at the orange-red and red-ripe stages. In HLY-18 cultivar, the total amount of chlorophylls was higher than in Donald cultivar. The ratio

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Table 3. Isoprenoid Contents of Purified Plastids Isolated from the Mesocarp of Traditional (Donald) and High-Pigment (HLY-18) Tomato Cultivars during Ripening[a]

	content (μg/mg of plastid proteins) stage of ripening							
	green		green-orange		orange-red		red-ripe	
isoprenoid	Donald	HLY-18	Donald	HLY-18	Donald	HLY-18	Donald	HLY-18
phytoene			2.9	42.9	4.7	40.9	26.8	52.0
lycopene			10.9	23.2	36.6	284.1	165.2	315.3
total acy Car			**13.8**	**66.1**	**41.3**	**325.0**	**192.0**	**367.3**
δ-carotene			0.7	6.4	1.3	5.9	5.4	7.8
lutein	3.2	8.3	9.6	4.3	2.7	11.5	4.5	13.7
γ-carotene			0.8	3.2	1.1	5.4	4.8	6.9
β-carotene	0.4	2.1	11.5	7.9	5.1	15.7	12.5	15.3
violaxanthin	0.7	1.2	1.8	5.3	0.3	0.6	0.9	1.6
total cy Car	**4.3**	**11.6**	**24.4**	**27.1**	**10.5**	**39.1**	**28.1**	**45.3**
Car acy:cyc			*0.57*	*2.44*	*3.93*	*8.31*	*6.83*	*8.11*
chlorophyll *a*	0.7	1.2	3.7	8.9				
chlorophyll *b*	3.9	5.1	3.4	5.0				
Chl:Car	*1.07*	*0.54*	*0.19*	*0.15*				
α-tocopherol		0.0	0.7	8.9	0.6	2.8	12.5	4.3
total isoprenoids	**8.9**	**17.9**	**46.0**	**116.0**	**52.4**	**366.9**	**232.6**	**416.9**
	content (μg/mg of plastid f-dm) stage of ripening							
	green		green-orange		orange-red		red-ripe	
isoprenoid	Donald	HLY-18	Donald	HLY-18	Donald	HLY-18	Donald	HLY-18
phytoene			0.3	2.4	0.6	2.2	1.5	2.7
lycopene			1.2	1.3	4.6	15.3	9.3	16.1
total acy Car			**1.5**	**3.7**	**5.2**	**17.5**	**10.8**	**18.8**
δ-carotene			0.1	0.4	0.2	0.3	0.3	0.4
lutein	0.9	1.8	1.0	0.2	0.3	0.6	0.3	0.7
γ-carotene			0.1	0.2	0.1	0.3	0.3	0.4
β-carotene	0.1	0.4	1.2	0.4	0.6	0.9	0.7	0.8
violaxanthin	0.2	0.3	0.2	0.3	0.1	0.1	0.1	0.1
total cy Car	**1.2**	**2.5**	**2.6**	**1.5**	**1.3**	**2.2**	**1.7**	**2.4**
Car acy:cy			*0.58*	*2.47*	*4.00*	*7.95*	*6.35*	*7.83*
chlorophyll *a*	0.2	0.3	0.4	0.5				
chlorophyll *b*	1.1	1.1	0.4	0.3				
Chl:Car	*1.08*	*0.56*	*0.20*	*0.15*				
α-tocopherol			0.1	0.5	0.1	0.2	0.7	0.2
total isoprenoids	**2.5**	**3.9**	**5.0**	**6.5**	**6.6**	**19.9**	**13.2**	**21.4**

[a]Data, expressed as μg/mg of plastid proteins and μg/mg of plastid f.-d.m, are the means of two replicates, with standard deviations generally below 10% of means. Car, carotenoid; acy, acyclic; cy, cyclic; Chl, chlorophyll. Italic formatting identifies dimensionless ratios.

chlorophylls to carotenoids decreased in both cultivars during fruit ripening, remaining always lower in HLY-18 cultivar than in Donald cultivar.

α-Tocopherol was detectable starting from the green-orange stage, and its amount, referred to milligrams of plastid f-dm, remained generally low in both cultivars (Table 3). When the content of α-tocopherol was expressed as micrograms per milligram of plastid proteins, the highest amount was recorded at the red-ripe stage (12.5) for Donald cultivar and at the green-orange stage in HLY-18 cultivar (8.9).

As a whole, total isoprenoids, referred to either milligrams of plastid f-dm or plastid proteins, markedly increased during tomato fruit ripening; in HLY-18 cultivar each value was higher than in Donald cultivar at all stages of tomato fruit ripening.

Table 4 reports the total amount of phospholipids in purified plastids isolated during tomato fruit ripening in traditional (Donald and Incas) and high-pigment (Kalvert and HLY-18) cultivars. The phospholipid content increased markedly from the green to the red-ripe stages in all cultivars under investigation. This increase was highly significant ($p < 0.001$) in all of the analyzed cultivars. At the green, green-orange, and orange-red stages of maturation, the amounts of phospholipids significantly differed among the four investigated cultivars, showing the highest content in the high-pigment cultivars; however, at the red-ripe stage, the differences in the mean values of phospholipid content among the analyzed cultivars were not statistically significant ($p = 0.809$).

The neutral lipids were analyzed by TLC separation (Figure 1) and quantified referring to milligrams of plastid proteins (Table 5). TLC separation pattern showed that the most representative spots corresponded to the authentic markers for triacylglycerols (TG), diacylglycerols (DG), and free fatty acids (FA). Some unidentified spots were also present. At the green stage, the amounts of TG and DG did not vary significantly among tomato cultivars ($p = 0.153$, $p = 0.828$, respectively), whereas significant differences were detected in the amounts of

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Table 4. Phospholipid Content in Purified Plastids Isolated from the Mesocarp of Traditional (Donald, Incas) and High-Pigment (Kalvert, HLY-18) Tomato Cultivars at Four Different Ripening Stages[a]

	content (μg/mg of plastid proteins) stage of ripening			
cultivar	green	green-orange	orange-red	red-ripe
Donald	27.4 ± 1.2aA	62.7 ± 12.0bA	87.6 ± 7.4cA	296.0 ± 13.4dA
Incas	39.3 ± 5.0aB	89.5 ± 9.1bB	220.8 ± 1.2cB	296.5 ± 18.0dA
Kalvert	62.7 ± 2.2aC	208.4 ± 12.4bD	261.1 ± 23.7cD	294.6 ± 34.0dA
HLY-18	80.1 ± 3.3aD	184.8 ± 2.0bC	240.1 ± 10.2cC	286.0 ± 12.0dA

[a]Data are the mean ± standard deviation of six independent replicates. Lower case letters indicate mean separation among stages of ripening within cultivar by the Holm−Sidak test, $p < 0.05$. Upper case letters indicate mean separation among cultivars within stage of ripening by the Holm−Sidak test, $p < 0.05$.

FA ($p = 0.006$) (Table 4). During fruit ripening, a general decrease in the amount of TG was observed in all cultivars. This decrease, at the red-ripe stage, was more pronounced in the traditional (∼62−64%) than in the high-pigment (∼33−45%) cultivars. DG was the predominant neutral lipid detected in all cultivars, and its content showed only slight variations at the different ripening stages. The amounts of FA, approximately 180 μg/mg of plastid proteins (Donald and HLY-18) and 170 μg/mg of plastid proteins (Kalvert and Incas) at the green stage, decreased during ripening in the high-pigment (from 23 to 21%) more than in the traditional (from 19 to 12%) cultivars.

The amount of sterols increased approximately 5-fold from the green to the red-ripe stage in all cultivars, even if the kinetics of this increase was different (Table 6). This increase was statistically highly significant ($p < 0.001$) in all of the assayed cultivars. At red-ripe stage, the highest amount of sterols, referred to milligrams of plastid proteins, was recorded in high-pigment HLY-18 (212.0 μg) followed by Kalvert (174.8 μg), Incas (154.0 μg), and Donald (122.4 μg) cultivars.

Figure 2 shows the qualitative and quantitative patterns of free fatty acids in purified plastids isolated from tomato fruits during ripening in the four cultivars under investigation. At all stages of ripening and in all cultivars, palmitic acid (16:0) was the major fatty acid (ranging from 35 to 52%), followed by stearic (18:0) (from 20 to 38%), oleic (18:1) (from 4 to 12%), linoleic (18:2) (from 3 to 12%), linolenic (18:3) (from 3 to 12%), and myristic (14:0) (from 1 to 2.5) acids. Changes in the fatty acid relative percentage were observed among cultivars and in the same cultivar at the different stages of ripening. In general, the levels of saturated fatty acids (palmitic and stearic) increased during tomato ripening in all investigated cultivars, whereas the levels of unsaturated fatty acids (oleic, linoleic, and linolenic) generally decreased. In all cultivars and at all stages of ripening myristic acid was present in the lowest amount (<2.5%).

SDS-PAGE of plastid proteins isolated from tomato fruits of the traditional Donald and high-pigment HLY-18 cultivars at different ripening stages showed some qualitative and quantitative differences between the two and during ripening (Figure 3). In both cultivars a decrease in band intensity and number occurred during ripening, generally starting from the green-orange stage. Only in the HLY-18 cultivar did the 41.6 kDa band appear at the orange-red and red-ripe stages. Furthermore, the polypeptides of 44.5 and 16.0 kDa were characteristic of the green and green-orange stages of HLY-18 cultivar, whereas the bands of 58.2 (all stage of ripening) and 39 kDa (green-orange and orange-red stages) were exclusive to the Donald cultivar.

DISCUSSION

This study reports changes in the amounts of isoprenoids, phospholipids, neutral lipids, and sterols and protein distribution patterns in purified plastids from tomato mesocarp cells of fresh fruits in different ripening stages, sampled from traditional (Donald and Incas) and commercial high-pigment (Kalvert and HLY-18) tomato cultivars.

At the green stage, acyclic carotenoids (phytoene, phytofluoene, ζ-carotene, neurosporene, and lycopene) were undetectable in chloroplasts isolated from mesocarp tomato cells of all investigated cultivars, whereas cyclic carotenoids, such as lutein, β-carotene, and violaxanthin, and obviously chlorophylls (*a* and *b*) were present in quantifiable amounts, reflecting their association with thylakoid membranes and photosynthetic function. Thus, although the nuclear genes involved in carotenoid biosynthesis are likely expressed throughout tomato fruit development and ripening,[5] fine and highly regulated controls must be present to allow the exclusive formation of cyclic carotenoids in tomato fruit chloroplasts.

Starting from the green-orange stage, only phytoene and lycopene were detectable among the acyclic carotenoids, and their levels drastically increased in all investigated cultivars as ripening proceeded. In the traditional cultivar Donald, the amount of phytoene significantly rose during chromoplast differentiation when referred to milligrams of either plastid proteins or f-dm. On the contrary, phytoene level remained almost stable in chromoplasts isolated from mesocarp cells of HLY-18 cultivar, although it was higher than that measured in cultivar Donald chromoplasts at the green-orange, orange-red, and red-ripe stages of fruit ripening. It is well-known that during tomato ripening, the mRNA levels for the lycopene-producing enzymes phytoene synthase (PSY) and phytoene desaturase (PDS) increase, determining a rise in phytoene and a 500-fold boost in lycopene concentrations.[15] The stability in phytoene concentration observed in the chromoplasts of HLY-18 cultivar may be possibly due to a differential transcription of the PSY and PDS mRNAs, in terms of time and level, which cause a steady state in phytoene concentration within the differentiating chromoplasts.

As expected, lycopene was the major carotenoid accumulated in chromoplasts of the red-ripe tomato fruits analyzed regardless of cultivar and detection method used (Tables 2 and 3). Chromoplasts of red-ripe tomato fruits of high-pigment cultivars accumulated approximately twice as much as lycopene as ordinary cultivars (Tables 2 and 3). In particular, the amount of lycopene measured in fully differentiated chromoplasts isolated from red-ripe tomatoes of the HLY-18 cultivar (16.1 μg/mg f-dm or 315.3 μg/mg of protein) was approximately slightly less than

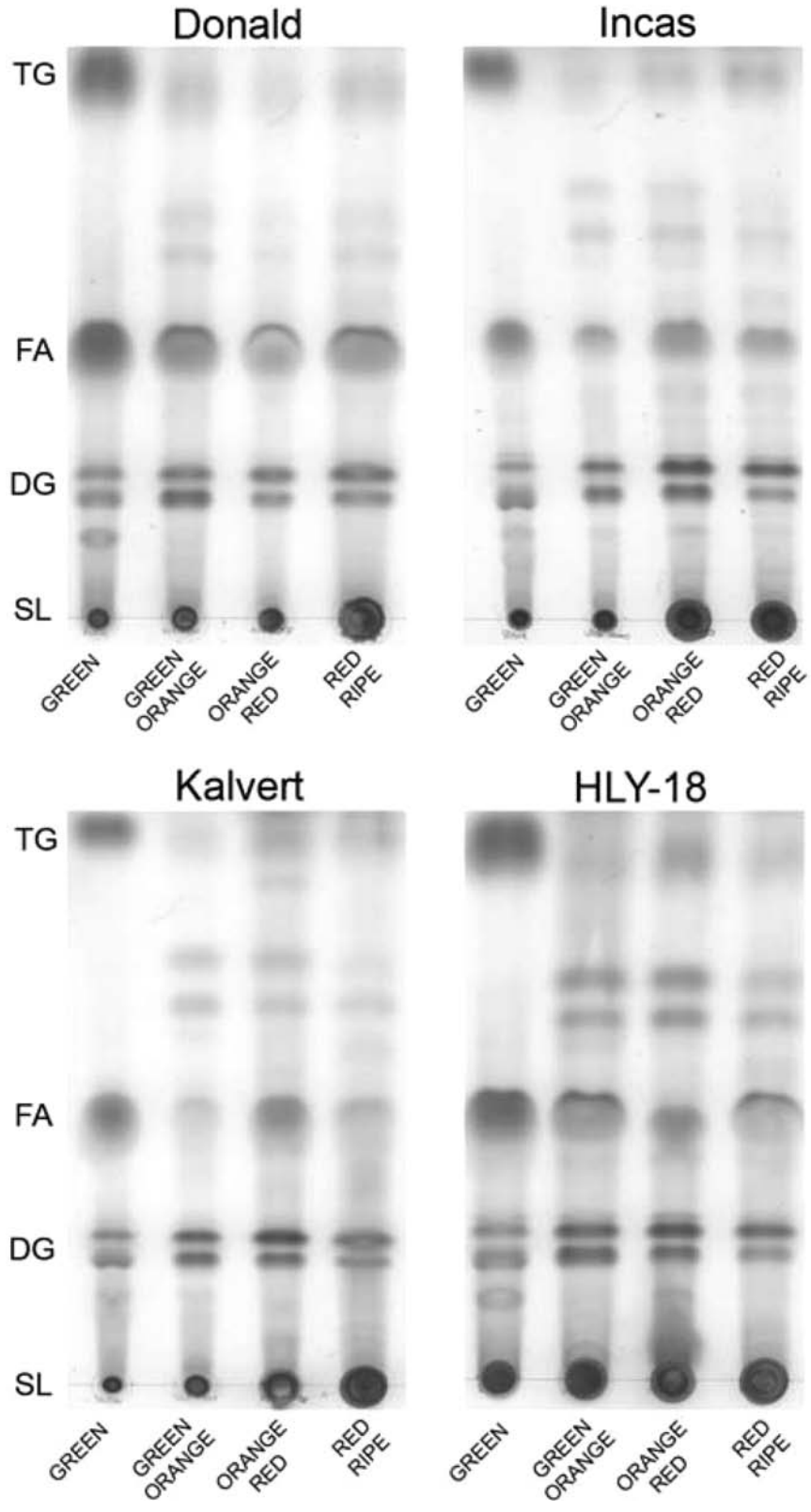

Figure 1. TLC separation of neutral lipids extracted from purified plastids isolated from the mesocarp of traditional (Donald, Incas) and high-pigment (Kalvert, HLY-18) tomato cultivars at four different ripening stages. TG, triacylglycerols; DG, diacylglycerols, FA, free fatty acids; SL, starting line.

2-fold higher than in cultivar Donald (9.3 μg/mg f-dm or 165.2 μg/mg of protein) (Table 3).

The high-pigment cultivars used in this study have been developed through conventional plant-breeding techniques taking into account the careful selection of the high-lycopene trait. This important commercial trait is commonly due to the presence of light-responsive high-pigment (hp) mutations such as hp-1, hp-1^{w}, hp-2, hp-2^{j}, hp-2^{dg}, and hp-3, which lead to an

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Table 5. Content of Neutral Lipids in Purified Plastids Isolated from the Mesocarp of Traditional (Donald, Incas) and High-Pigment (Kalvert, HLY-18) Tomato Cultivars at Four Different Ripening Stages[a]

	content (μg/mg of plastid proteins) stage of ripening			
cultivar/neutral lipid	green	green-orange	orange-red	red-ripe
Donald				
TG	179.6 ± 15.3cA	84.2 ± 3.1bB	61.2 ± 4.0aA	64.1 ± 15.3aA
DG	314.2 ± 29.7aA	338.5 ± 19.7aAB	307.7 ± 20.2aA	314.5 ± 15.3aA
FA	185.4 ± 20.1cA	159.5 ± 10.3bC	132.6 ± 8.2aA	149.3 ± 15.3abB
Incas				
TG	170.9 ± 12.1cA	50.4 ± 5.4aA	66.2 ± 5.1bA	65.1 ± 6.2bA
DG	313.7 ± 17.5aA	312.7 ± 20.2aA	335.4 ± 17.7aAB	323.8 ± 26.5aA
FA	163.1 ± 9.8bB	132.9 ± 10.1aB	163.1 ± 11.1bB	143.5 ± 10.7aAB
Kalvert				
TG	168.5 ± 8.8cA	93.8 ± 7.8aC	110.2 ± 9.1bC	105.1 ± 4.0aB
DG	324.0 ± 13.4aA	349.2 ± 25.3abB	377.9 ± 23.0bC	335.6 ± 21.2aA
FA	162.4 ± 6.3cB	106.7 ± 11.5aA	163.9 ± 13.5cB	125.0 ± 10.9bA
HLY-18				
TG	182.6 ± 10.2bA	92.1 ± 7.1aBC	90.0 ± 6.4aB	100.1 ± 10.7aB
DG	318.1 ± 21.7aA	342.5 ± 18.2aAB	353.8 ± 21.1aBC	331.9 ± 25.7aA
FA	181.6 ± 9.9cAB	160.0 ± 7.4bC	156.2 ± 12.5abB	143.3 ± 9.9aAB

[a]Data are the mean ± standard deviation of six independent replicates. Lower case letters indicate mean separation among stages of ripening within cultivar by the Holm−Sidak test, $p < 0.05$. Upper case letters indicate mean separation among cultivars within stage of ripening and class of compound by the Holm−Sidak test, $p < 0.05$. TG, triacylglycerols; DG, diacylglycerols; FA, free fatty acids.

Table 6. Sterol Content in Purified Plastids Isolated from the Mesocarp of Traditional (Donald, Incas) and High-Pigment (Kalvert, HLY-18) Tomato Cultivars at Four Different Ripening Stages[a]

	content (μg/mg of plastid proteins) stage of ripening			
cultivar	green	green-orange	orange-red	red-ripe
Donald	24.1 ± 4.8aA	55.2 ± 5.5bA	61.4 ± 6.4bA	122.4 ± 9.2cA
Incas	31.5 ± 2.9aB	69.7 ± 9.7bB	74.1 ± 7.6bB	154.0 ± 8.8cB
Kalvert	36.4 ± 1.9aC	47.8 ± 6.2bA	90.7 ± 6.8cC	174.8 ± 7.2dC
HLY-18	43.4 ± 2.4aD	173.7 ± 5.3bC	171.7 ± 1.9bD	212.0 ± 15.6cD

[a]Data are the mean ± standard deviation of six independent replicates. Lower case letters indicate mean separation among stages of ripening within cultivar by the Holm−Sidak test, $p < 0.05$. Upper case letters indicate mean separation among cultivars within stage of ripening by the Holm−Sidak test, $p < 0.05$.

increase of carotenoid and flavonoid biosynthesis.[19,21] However, the accumulation of carotenoids within the chromoplasts is also influenced by the control of other metabolic events such as storage/sequestration of carotenoid biosynthetic pathway end-products[42−44] and from the progressive reduction of lycopene β-cyclase (CYC-B) and ε-cyclase (LCY-E) enzymes converting lycopene to β-carotene and α-carotene, respectively.[14,45] This aspect is strengthened by the recent observations evidencing the important role of chromoplast plastoglobules in carotenoid biosynthesis, storage, and sequestration.[46,47] In addition, the ripening process is under endogenous hormonal control. The transcriptional level of the carotenoid biosynthetic genes *Psy-1* and *Pds* is ethylene dependent, whereas the transcription of genes involved in lycopene cyclization (LCY-B, CYC-B) is independent from ethylene.[48,49] Hence, the enhanced capability of commercial high-pigment tomato cultivars in accumulating lycopene, and more generally isoprenoids, in fruit chromoplasts, is likely due to a complex combination of factors comprising a superior biosynthetic and accumulation/sequestration potential of the metabolites in these cultivars than in the traditional ones. As a consequence of lycopene accumulation, the ratio between the total amount of acyclic/cyclic carotenoids markedly increased in both HLY-18 and Donald cultivars during the conversion of chloroplasts to fully differentiated chromoplasts but with a differential behavior likely due to differences in the regulation of the genes involved in carotenoid biosynthesis between the studied traditional and high-pigment tomato cultivars.

Recently, the highest amount of lycopene found in red-ripe fruits of the transgenic DET1 down-regulated tomato varieties (2A11, TFM7, and P119) and hp2 mutant was approximately 1575 μg/g dw (P119), 5-fold higher than in the reference wild type (T56) variety (322 μg/g dw)[29] but considerably lower than those we found in freeze-dried chromoplasts isolated from Donald (9300 μg/g f-dm) and HLY-18 (16100 μg/g f-dm) cultivar red-ripe tomato fruits and those we previously reported for red-ripe tomato fruits of the same cultivars,[28] which were approximately 50% lower than the amount in differentiated chromoplasts. The comparison among these data indicates that plastid preparations are more appropriate than the whole tomato fruits to obtain a high concentration of lycopene or, more generally, isoprenoids. In fact, the elimination of all the other cellular structures and particularly of the cell walls, which considerably contribute to f-dm weight, determines a reliable concentration of lycopene in the lyophilized chromoplasts.

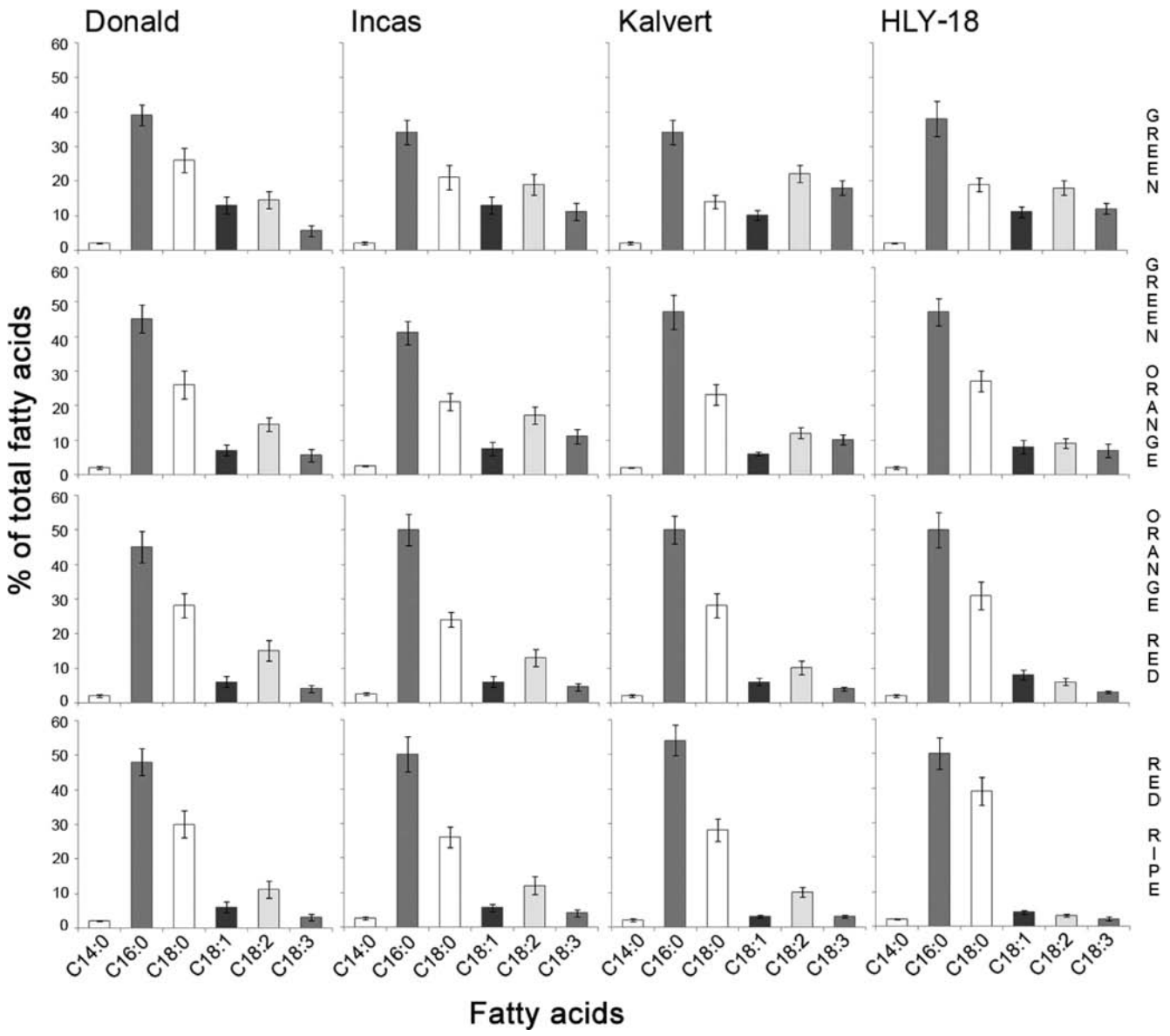

Figure 2. Free fatty acid composition in purified plastids isolated from the mesocarp of traditional (Donald, Incas) and high-pigment (Kalvert, HLY-18) tomato cultivars at four different ripening stages. Data are the mean ± standard deviation of three independent replicates.

Thus, isolated tomato plastids could represent excellent quality ingredients in the formulation of lycopene-containing functional foods, cosmetic products, nutraceuticals, and pharmaceuticals. In addition, plastids, being similar to bilamellar liposomes, could protect lycopene from oxidation/degradation in the commercial products and facilitate biosorption and tissue delivery. Our data also indicate that the compartmentalization of bioactive molecules in plant organelles (plastids) represents an important target for future green biotechnological applications that can be achieved by a non-genetic-modification approach.

It is well documented that plastoglobules are present in all plastid types and that their shape, size, and number change during plastid development, and differentiation and under stress conditions.[46,47] In chloroplasts, these lipoprotein particles are physically connected to a thylakoid membrane via a half-lipid bilayer and mainly composed of α-tocopherol, plastoquinone, triacylglycerols, sterol esters, and mono- and digalactosyl diacylglycerols. They are virtually devoid of carotenoids and chlorophylls.[50] In chromoplasts, plastoglobules play a specific role in carotenoid biosynthesis in addition to the well-known carotenoid storage/sequestering functions.[42,51] In the traditional and high-pigment tomato cultivars investigated in this study, the amount of phospholipids, referred to milligrams of plastid proteins, markedly increased during the chloroplast-to-chromoplast transition. This increase can be explained with the de novo synthesis of new membranes required for the sequestration and growth of lycopene crystals. Thus, our data support the idea that, during tomato ripening, the progressive disassembling of thylakoid membranes associated with chlorophyll degradation is related with the de novo synthesis of membranes required for lycopene sequestration and growth of crystals in chromoplast tubules[52] as well as for stromule formation.[53] The presence of lycopene crystals has been reported in different types of chromoplasts.[54,55] In *Palisota* fruits, carotenoid-accumulating plastoglobules progressively transform into tubules with a simultaneous de novo synthesis of a 30 kDa polypeptide located at the surface of the tubules.[56] This polypeptide appeared to be the major protein of all tubule fractions. Similar observations

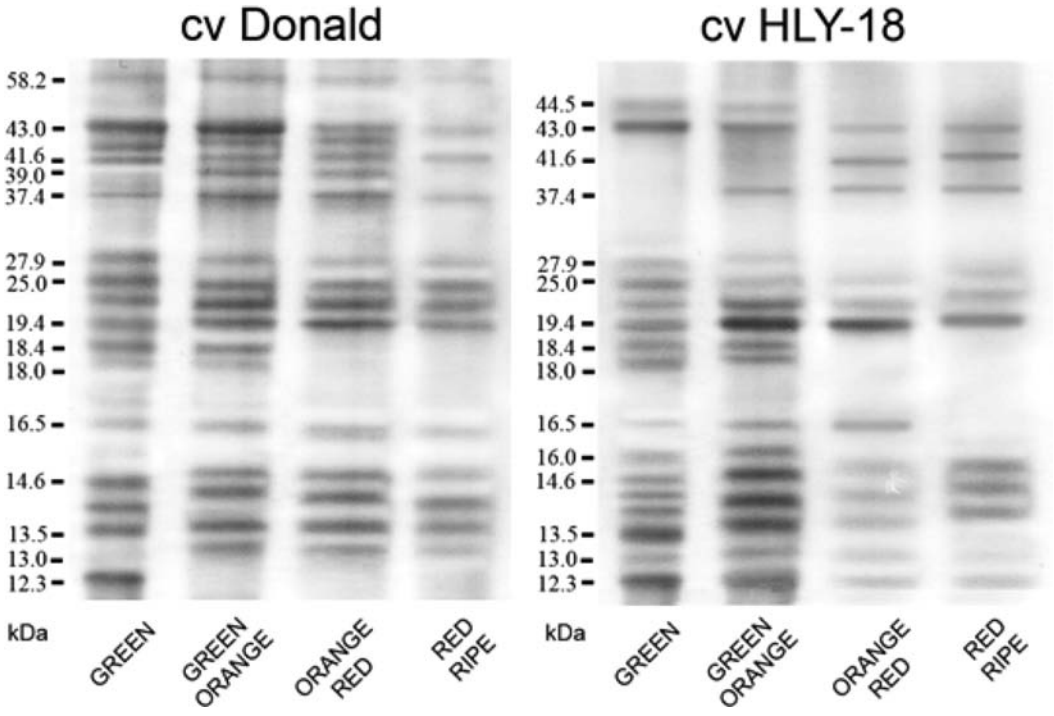

Figure 3. SDS-PAGE of plastid protein isolated from the mesocarp of the traditional Donald and high-pigment HLY-18 tomato cultivars at four different ripening stages.

were made on chromoplast tubules of *Tropaeolum* petals[57] and *Rosa* hips.[58] It has been recently reported that in fibrillin-overexpressing tomato plants, carotenoid crystals grow from stellate nucleation centers instead of developing in the long axis as in the wild type chromoplasts.[52] Due to the fact that when expressed on a plastid protein basis the amount of phospholipids in fully differentiated chromoplasts remained almost unchanged in all of the analyzed cultivars, it seems interesting to consider that the accumulation of phospholipids precedes or occurs simultaneously with that of lycopene during chloroplast-to-chromoplast transition. The role of newly synthesized phospholipids, likely associated with newly synthesized membranes enclosing carotenoid crystal, remains to be established.

The total amount of DG remained almost unchanged during plastid transition from chloroplasts to chromoplasts in almost all of the cultivars under investigation with the exception of Kalvert cultivar, for which statistically significant variations were evidenced ($p < 0.05$). Free fatty acid contents significantly ($p < 0.05$) changed during chloroplast-to-chromplast transition without showing a particular trend. Furthermore, the free fatty acid composition did not show significant changes among the different cultivars. Instead, TG content markedly decreased from plastids of the green stage to those of the green-orange stage and remained almost similar in the other two stages (orange-red and red-ripe). This decrease in TG content was more pronounced in plastids of traditional cultivars than in those of high-pigment cultivars. Interestingly, the decrease of TG content was accompanied by the appearance of two neutral lipid compounds not yet characterized (Figure 1). High levels of TG were found in plastoglobules isolated from *Viola tricolor* chromoplasts.[59] Furthermore, an enhanced synthesis of TG and an increase in the number and size of plastoglobules have been reported in senescing rosette leaves of *Arabidopsis*.[60]

During the transition from chloroplast to chromoplast, in traditional (Donald, Incas) and high-pigment (HLY-18, Kalvert) tomato cultivars, the phytosterol content markedly increased with different patterns. The highest amount of phytosterols was recorded in the chromoplasts isolated from red-ripe tomatoes. It was approximately 5 times higher than that of green tomato chloroplasts. The identification of sterol composition would be of interest to understand their role during plastid interconversion as well as considering that dietary phytosterols reduce the intestinal absorption of cholesterol and hence have positive effects on plasma lipid profiles.[9,61]

The analyses of monodimensional SDS-PAGEs of tomato plastid proteins (Figure 3) showed that most of the proteins were constantly present in all stages of ripening, whereas some others disappeared during chloroplast-to-chromoplast transition. An exception is represented by the 41.6 kDa protein expressed in the last two stages of ripening in HLY-18 cultivar. Therefore, our data clearly indicate a sharp qualitative and quantitative variation of the protein pattern during ripening. With regard to this, it has been reported that most, but not all, plastid genes are translationally down-regulated during chloroplast-to-chromoplast transition and that a low level of plastid gene expression is required in chromoplasts.[62] Furthermore, a progressive reduction of gene expression during tomato fruit ripening has been also recently reported in transgenic DET1 down-regulated cultivars.[29]

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■ ABBREVIATIONS USED

DG, diacylglycerols; DMAPP, dimethylallyl diphosphate; DPA, days post anthesis; FA, fatty acids; FAMEs, fatty acid methyl esters; f-dm, freeze-dried matter; hp, high-pigment; IPP, isopentenyl diphosphate; MEP, 2-*C*-methyl-D-erythritol 4-phosphate; MVA, mevalonic acid; PDS, phytoene desaturase; TG, triacylglycerols; PSY, phytoene synthase.

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