JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

Article

pubs.acs.org/JAFC

Chemical and Bioactive Quality Traits During Fruit Ripening in Eggplant (*S. melongena* L.) and Allied Species

Giuseppe Mennella,*[,†] Roberto Lo Scalzo,[‡] Marta Fibiani,[‡] Antonietta D'Alessandro,[†] Gianluca Francese,[†] Laura Toppino,[§] Nazzareno Acciarri,[∥] Adelia Emilia de Almeida,[⊥] and Giuseppe Leonardo Rotino[§]

[†]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, CRA-ORT Centro di Ricerca per l'Orticoltura, via Cavalleggeri 25, 84098 Pontecagnano-Faiano (Salerno), Italy

[‡]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, CRA-IAA Unità di Ricerca per i Processi dell'Industria Agroalimentare, via Venezian 26, 20133 Milan, Italy

[§]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, CRA-ORL Unità di Ricerca per l'Orticoltura, via Paullese 28, 26836 Montanaso Lombardo (Lodi), Italy

[∥]Consiglio per la Ricerca e la Sperimentazione in Agricoltura, CRA-ORA Unità di Ricerca per l'Orticoltura, via Salaria 1, 63030 Monsampolo del Tronto (Ascoli Piceno), Italy

[⊥]Universidade Estadual Paulista Júlio de Mesquita Filho, Departamento de Fármacos e Medicamentos, Faculdade de Ciências Farmacêuticas - Rodovia Araraquara - Jaú, km 1, Araraquara SP, Brazil

Supporting Information

ABSTRACT: A chemical and bioactive quality evaluation of phytochemicals content of 10 eggplant lines and three allied species (*S. sodomaeum, S. aethiopicum* and *S. integrifolium*) was performed. The eggplant lines were divided into the two subgroups of delphinidin-3-rutinoside (D3R) and nasunin (NAS) typologies, on the basis of the anthocyanin detected in their fruit skin. The allied species had higher glycoalkaloids content, lower soluble solids and PPO activity and absence of anthocyanins compared to the eggplant lines; *S. sodomaeum* stood out for high phenols content. Orthogonal contrast revealed a higher sugar content and low PPO activity in NAS- compared to D3R-typologies, whereas higher chlorogenic acid and anthocyanin contents were present in D3R-typologies. The main effect of the ripening was a decrease in phenols and in the PPO activity, not evidenced in *S. sodomaeum,* and an increase of glycoalkaloids in overripe fruits.

A good relationship was found between superoxide anion scavenging capacity and chlorogenic acid. This study highlighted the pattern of accumulation, also evidencing variations, of several phytochemicals during the eggplant fruit development and ripening.

KEYWORDS: *glycoalkaloids, phenols, antioxidants, nasunin, delphinidin 3-rutinoside, S. aethiopicum, S. integrifolium, S. sodomaeum*

INTRODUCTION

The improvement of quality traits in fruits/vegetables, among other purposes, is aimed at producing fruits/vegetables rich in compounds important for human health by the evaluation and manipulation of particular phytochemical components and, consequently, of their biological activity.[1,2] These molecules, such as the phenolics considered "nutraceuticals", may have an important role in the resistance/tolerance to biotic[3] and/or abiotic stresses.[4] Eggplant (*S. melongena* L.) is an interesting fruit/vegetable in this respect because it is well-known to contain phytochemicals, mainly phenolics and steroids, which are considered to have, respectively, beneficial effects and toxic/medicamentous properties according to their dosage.[5−8] Eggplant berries have a high antioxidant capacity mainly due to chlorogenic acid[7,9,10] and the anthocyanin pigments delphinidin-3-rutinoside (D3R) and/or nasunin.[11,12]

Eggplant lines and relatives have shown interesting variations in their chemical composition with regard to phenolic and steroid compounds.[9,13,14] Employment of exotic eggplant germplasm in the breeding programs may represent a valid option to manipulate the content of such compounds. Moreover, the use of allied species allowed successful incorporation of disease resistance/tolerance traits into the eggplant gene pool[15] and the introgression lines developed showed a wide range of variation in their phytochemical content.[14]

The knowledge of the phytochemical variations during eggplant fruit development could greatly help the improvement of the commercial and nutritional value of the fruit. In fact, the physiological ripeness of the eggplant fruit is not coincident with the commercial ripeness and, furthermore, overripe fruits are unmarketable because of the presence of mature seed, and also of modifications in taste and firmness. During the eggplant fruit development and ripening, relevant changes occur in the color of the skin and flesh, in the fruit firmness and texture, and in seed maturation. Very little information is available about the correspondent modification in the biochemical composition and molecular aspects of the transition of eggplant fruit from commercial to physiological ripeness.

Received: August 29, 2012
Revised: November 6, 2012
Accepted: November 8, 2012
Published: November 8, 2012

This work is aimed at the evaluation of the quality traits regarding the presence of the phytochemicals and their antioxidant capacity in 10 eggplant lines from two different fruits typologies and in three allied genotypes (*S. linnaeanum* = *S. sodomaeum*, *S. aethiopicum* gr. *gilo* and *S. aethiopicum* gr. *aculeatum* = *S. integrifolium*). These allied species have been employed in an introgression breeding program mainly devoted to development of fungal wilt resistant eggplant lines.[16] The 10 eggplant genotypes were divided into two groups according to their anthocyanin form, one containing delphinidin-3-rutinoside (D3R) and the other containing nasunin, delphinidin-3-(*p*-coumaroylrutinoside)-5-glucoside (NAS), which correspond to a previous subdivision made by Azuma et al.[11] in Japanese (NAS) and non-Japanese (D3R) eggplant types. The NAS- and D3R-containing eggplant breeding lines have been employed in our introgression breeding programs as recurrent genotypes and, among other traits, according to local Southern Italy lore they differ each other by a sweeter taste and a lower tendency to brown after cutting in NAS-type eggplants with respect to D3R-types. The fruits of all the genotypes were collected during three phenological stages (unripe, commercially ripe and physiologically ripe fruit), to monitor the evolution of phytochemicals of interest for their human-health properties. Information about a different pattern of accumulation of health-related compounds might be efficiently exploited to breed eggplants with superior nutritional value.

MATERIALS AND METHODS

Plant Material. Fruits from eggplant (*Solanum melongena* L., *S. mel*) and from three allied species *S. sodomaeum* (*S. sod*), *S. aethiopicum* gr. *gilo* (*S. aeth*) and *S. integrifolium* (*S. int*) were collected from 18 plants for each genotype, grown during 2006 season in an experimental field located in Montanaso Lombardo (Lodi, Italy) at the Consiglio per la Ricerca e la Sperimentazione in Agricoltura, Unità di Ricerca per l'Orticoltura. Three D3R-containing eggplant lines (*S. mel* 1−3) exhibiting fruits with a long shape and a deep purple color and seven NAS-containing lines (*S. mel* 4−10) with spherical pale purple (violet) fruits color belonging to "Tunisina" typology were evaluated. The fruit peels of the allied parents did not exhibit detectable levels of these anthocyanin pigments.

The samplings were carried out at three different fruit ripening stages, unripe [A, approximately 21 days after flowering (DAF)], commercial (B, approximately 38 DAF) and physiological ripening (C, approximately 60 DAF). The fruits at the stage A were actively growing, presumably because of cellular enlargement, close to half of the final size, the skin color was glossy, the calyx and peduncle were quite tender and flexible, the flesh appeared still soft and greenish in the D3R-types and white in the NAS-types, the seeds had not reached final size and they displayed a white tegument. The fruit at the stage B had reached their final size, the skin color became less brilliant and in some lines was a little dull; the fruit, calyx, and peduncle had the typical commercial firmness, the flesh became less greenish in the DR3-types, which showed the characteristic green ring next to the skin, and the seeds had almost reached their final size but were still immature. The fruits harvested at the stage C had an increased firmness, the calyx and peduncle were quite lignified, the peel color turned brownish, the flesh became spongy and thready with a white-yellowish color containing visible hard mature seeds showing a brown tegument. Fruits of the ripening stages A and B were simultaneously harvested.

The experimental sample was constituted by portions obtained from 5−8 fruits, sampled in duplicate. Flesh cubes from peeled fruits and peel slices of about 1.5−2 cm, made within 2 h after harvest, were immediately frozen in liquid N_2 and lyophilized. The weight of the fresh flesh and peel samples and the correspondent weight at the end of drying process was detected for the conversion to fresh weight of the compounds analyzed (Table 1). The freeze-dried tissue was powdered and held at −80 °C. All results were referred to as dry weight (dw).

Table 1. Average Moisture Content (%) of the Assayed *Solanum* spp. Fruits before Freeze-Dried Process

genotype	moisture content (%)		
	stage A	stage B	stage C
Allied			
S. integrifolium	86.2	85.0	83.0
S. aethiopicum	88.3	85.8	87.3
S. sodomaeum	82.1	79.6	79.0
D3R-Containing Typology			
S. melongena 1	87.7	91.0	91.8
S. melongena 2	92.1	91.8	91.9
S. melongena 3	92.1	92.5	89.3
NAS-Containing Typology			
S. melongena 4	92.2	92.1	91.8
S. melongena 5	92.8	90.8	90.0
S. melongena 6	93.8	93.4	92.6
S. melongena 7	92.1	91.9	91.7
S. melongena 8	92.3	90.5	91.3
S. melongena 9	93.3	91.4	91.4
S. melongena 10	92.6	92.6	91.3

Chemical Quality Traits. The extraction and the analysis of anthocyanins was carried out on 200 mg of lyophilized and powdered peel, diluted in 10 mL of methanol containing 3% trifluoroacetic acid (TFA), as previously reported.[8] RP-HPLC analysis was performed through a Waters E-Alliance HPLC system constituted by a 2695 separations module with quaternary pump, autosampler, and a 2996 photodiode array detector; data were acquired and analyzed with Waters Empower software on a PC. The chromatographic separations were performed at a flow rate of 0.8 mL/min and at 0.1 AUFS. Purified D3R (Polyphenols Laboratories AS, Sandnes, Norway) was used as external standard in RP-HPLC analyses, with a different retention time (23.9 min) compared to nasunin, that was eluted at a longer retention time (25.8 min for *cis*-nasunin and 26.1 min for *trans*-nasunin, respectively; see Supporting Information). As for nasunin quantification, a partially purified standard was used according to Lo Scalzo et al.[17] The results were expressed as μmol/100 g of peel dw; the limit of detection was 2.00 μmol/100 g of peel dw.

Soluble refractometric residue (SRR) was measured on the vortexed and centrifuged extract of 30 mg of powdered flesh with 1 mL of 1 mM HCl (1 + 5 min at room temperature), and it was expressed as percent substance on dw by refractometry (°Bx).

Glycoalkaloids, solamargine and solasonine, were extracted from 0.5 g samples of lyophilized and powdered flesh tissue by 95% ethanol as described by Birner[18] with some modifications. The analyses were performed by means of RP-HPLC according to Kuronen et al.,[6] using partially purified solasonine and solamargine as the external standard. The data were expressed as mg/100 g dw; the limit of detection was 0.03 mg/100 g of dw.

Phenolic acids were extracted and analyzed according to Whitaker and Stommel[7] with minor modifications. A binary mobile phase gradient of methanol in 0.01% aqueous phosphoric acid was used according to this procedure: 0−15 min, linear increase from 5 to 25% methanol; 15−28 min, linear increase from 25 to 50% methanol; 28−30 min, linear increase from 50 to 100% methanol; 30−32 min, 100% methanol; 32−36 min, linear decrease from 100 to 5% methanol; 36−43 min, 5% methanol. The flow rate was 0.8 mL/min. Quantification of chlorogenic acid (CA), carried out after a RP-HPLC separation, was based on absorbance at 325 nm relative to the sesamol internal standard and an external standard of authentic CA (Sigma-Aldrich, St. Louis, MO). The results were expressed as mmol/100 g of dw.

Total phenols index (TP) was assayed through a spectrophotometric method. TP was evaluated by a modified Folin-Ciocalteu

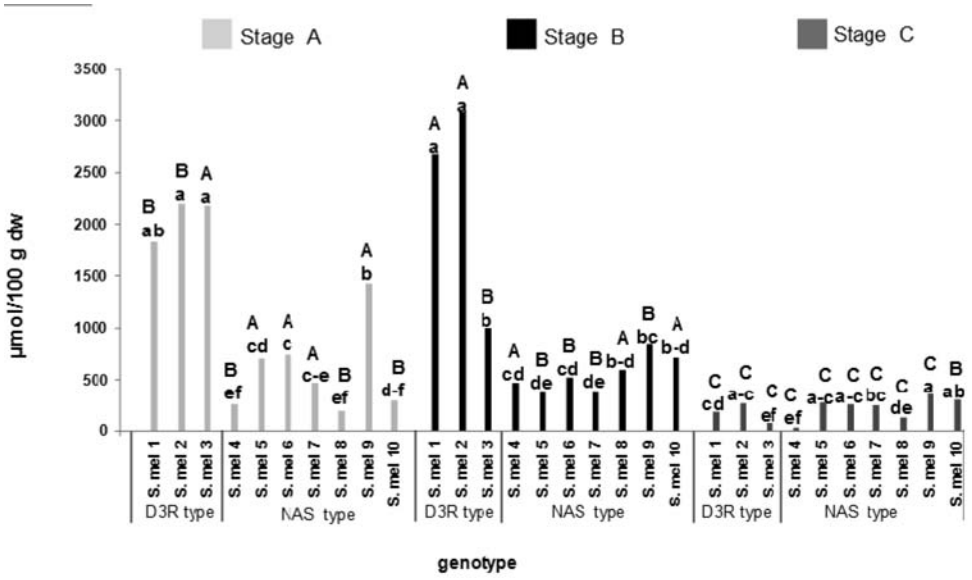

Figure 1. Level of anthocyanins in single D3R-containing and NAS-containing eggplant lines during three ripening stages (A, B, and C). The values of allied species have not been shown because of their absence. The respective significance was obtained by orthogonal contrasts between values of D3R- vs NAS-typologies in each ripening stage. Stage A, $F = 734.06$**; stage B, $F = 944.52$**; stage C, $F = 14.98$**. (** significant at the 0.01 level of probability). For each ripening stage, different lower-case letters indicate significantly different means among different lines at $p \leq 0.05$ (Tukey HSD test). For each line, different capital letters indicate significantly different means among different ripening stages at $p \leq 0.05$ (Tukey HSD test).

method[19] on a McIlvaine buffer (pH 3.0, 1 mL) extract of 30 mg of lyophilized and powdered flesh tissue. Results were expressed as mmoles CA per 100 g of dw. CA was used because it is the main phenolic compound in the eggplant fruit.

Biological Quality Traits. The PPO activity was assayed following the Fujita and Tono[20] method, using 30 mg of lyophilized flesh, extracted with 1 mL of McIlvaine buffer (pH 5.0). Results were expressed as U/100 mg of dw, with 1U = 0.01 absorbance unit variation/min, using CA as substrate at 420 nm.

The assays of antiradical activity were performed on superoxide anion and hydroxyl radical by electron spin resonance spectrometry at 25 °C, following the method used by Privat et al.[21] and by Valavanidis et al.,[22] with some modifications. The free radical generation (2.8 mM KO_2-crown-ether-18−6 1:1 in dimethylsulfoxide for superoxide anion; 2 mM Fenton system in 0.1 M phosphate buffer pH 7.4 for hydroxyl radical) was followed by spin trapping with 5,5-dimethyl-1-pyrrolin-*N*-oxide 25 mM and 10 mM dissolved in 0.1 M phosphate buffer pH 7.4 for superoxide anion and hydroxyl radical, respectively. The reaction was elapsed for exactly 1 min, after this time the spectra were recorded in presence and absence of eggplant extract (supernatant of 30 mg of flesh powder with 1 mL HCl 1 mM), respectively. In order to calculate the scavenging index, the main band amplitude measure was used applying the equation $I = 100\text{-}(I_x/I_0 \times 100)$, where I_x is the spectrum amplitude in presence of eggplant extract and I_0 is the spectrum amplitude in its absence. The results were expressed as mmol CA equivalents per 100 g of dw, by interpolating the data from flesh extracts with the scavenging index of chlorogenic acid solutions at known concentrations.

Data Analysis. All the determinations were carried out at least three times. Data were subjected to ANOVA performed by JMP (SAS Institute, Cary, NC) according to a completely randomized design. Means were compared by using Tukey HSD test ($p \leq 0.05$). For each parameter considered, orthogonal contrasts were used to determine significant differences between the two subgroups of *S. melongena* L. (i.e., D3R- vs NAS-type). For each genotype and parameter studied, the three stages of ripening were analyzed by ANOVA and means compared through Tukey HSD test ($p \leq 0.05$). The correlation indexes (rxy) were calculated by simple linear regression analysis.

RESULTS AND DISCUSSION

Genotypic Difference. The first approach for the eggplant quality analysis was the pigment evaluation in the fruit peel. The allied species do not have the typical anthocyanins from *S. melongena*, represented by D3R and/or nasunin,[11] therefore eggplant lines were defined as D3R-types due to the presence of delphinidin-3-rutinoside or NAS-type, containing nasunin. However, as eggplants have a huge morphological diversity in shape and size of fruit further investigations, using other eggplant types belonging to both D3R- and NAS-type, are needed in order to verify if the differences observed are maintained.

For each cultivar (except *S. mel* 10), the anthocyanin content was significantly higher in the stage A and B with respect to the stage C. The fruits of D3R typologies had significantly higher anthocyanin levels than NAS-containing fruits in stage A and B as confirmed by the orthogonal contrast, the averages being 2069 vs 587 and 2252 vs 563 µmol/100 g of peel dw, respectively. On the contrary, the NAS-type showed a mean value significantly higher than D3R one (233 vs 181 µmol/100 g of peel dw) in the stage C (Figure 1).

The highest amount of anthocyanin was detected in *S. mel* 2 line (D3R-type), both in the stage A and B, 2198 and 3080 µmol/100 g of peel dw, respectively. Among the NAS-type, a noticeable level of the pigment in the three ripening stages for *S. mel* 9 (1425, 849, and 256 µmol/100 g of peel dw, respectively) was found. The lowest amounts of the pigment, for the three ripening stages, were detected in the lines belonging to NAS-type [stage A, *S. mel* 8 (194 µmol/100 g); stage B, *S. mel* 7 (387 µmol/100 g); stage C, *S. mel* 4 (37 µmol/100 g of peel dw)] (Figure 1).

The allied species (except *S. aethiopicum* in the stages A and C) showed a significantly lower SRR amount than eggplant lines (Table 2). The two eggplant subgroups had a significant difference in the SRR content, on the average the NAS-types were significantly higher than D3R ones in all ripening stages

Table 2. Soluble Refractometric Residue (SRR) in Fruits of the Three *Solanum* spp., of the Three D3R- and of the Seven NAS-Containing Eggplant Typologies Analyzed[a] and the Estimated Orthogonal Contrasts

	SRR (% on dw)		
genotype/contrast	stage A	stage B	stage C
Allied			
S. integrifolium	36.32 f A	32.40 g B	30.08 g C
S. aethiopicum	44.17 e B	37.65 f C	51.34 cd A
S. sodomaeum	33.41 f A	28.60 h C	31.62 g B
D3R-Containing Typology			
S. melongena 1	44.59 e C	50.70 c A	48.40 de B
S. melongena 2	54.26 a-c A	50.35 c A	46.10 e B
S. melongena 3	50.83 cd A	43.70 e B	39.54 f C
mean	49.89	48.25	44.68
NAS-Containing Typology			
S. melongena 4	45.80 e B	54.25 b A	57.50 a A
S. melongena 5	50.05 d A	46.95 d B	39.25 f C
S. melongena 6	52.65 b-d B	56.66 a A	56.10 ab A
S. melongena 7	55.15 ab A	53.60 b AB	51.05 cd B
S. melongena 8	55.85 ab A	47.70 d B	45.85 e C
S. melongena 9	54.65 ab A	51.30 c A	52.45 c A
S. melongena 10	57.40 a A	57.75 a A	53.20 bc B
mean	53.08	52.60	50.77
D3R- vs NAS-Containing Typologies	$F = 39.60^b$	$F = 234.50^b$	$F = 205.05^b$

[a]For each ripening stage, different lower-case letters indicate significantly different means among different genotypes at $p \leq 0.05$ (Tukey HSD test). For each genotype, different capital letters indicate significantly different means among different ripening stages at $p \leq 0.05$ (Tukey HSD test). [b]Significant at the 0.01 level of probability.

and particularly in the B and C stages as evidenced by orthogonal contrast (53.08, 52.60, and 50.77 vs 49.89, 48.25, and 44.68% on dw, respectively).

The level of steroidal glycoalkaloids has to be kept in great account, due to both their toxicity for humans and the high amount in the fruits of allied species.[14] A recommended safety limit value of 200 mg/100 g of dw, as total glycoalkaloids, has been set up for potatoes.[23] Several authors consider such value as a safe limit also for eggplant,[24−26] however it should be probably adjusted for *S. melongena* due to the presence of a different aglycone (solasodine instead of solanidine) with hypothetical different activity and synergistic effects. The present study evaluated the glycoalkaloid content across fruit ripening both in allied species and in *S. melongena* L. lines, some of which were also used as recurrent parents (i.e., for backcrosses) in the course of introgression breeding programs.

The content of solamargine and solasonine, characteristic glycoalkaloids of *S. melongena* L. and of the allied species studied, is reported in Table 3. The above-mentioned limit is abundantly exceeded (4−7 fold), by the sum of the two glycoalkaloids, in all the ripening stages of *S. sodomaeum*. The safety value is exceeded, at various extent, in the stages A and C of *S. aethiopicum* and in stage C of *S. integrifolium*, while it is almost reached in the stage B of *S. integrifolium* (Table 3). In the *S. melongena* lines, the total glycoalkaloids amount was well below the safety values at the stage A and B and these data are in accordance with a previous work;[27] however, it was clearly evidenced a progressive and noticeable increase at the physiological stage of fruit ripening. In fact, in the stage C, the levels sometimes reached and exceeded the reference level (lines *S. mel* 9, 6, 7, and *1*).

Interestingly, in some lines such as *S. mel* 4 and *S. mel* 10, the solasonine level resulted <0.03 mg/100 g of dw for all the ripening stages, while it showed very low values in *S. mel* 5. As for stage A, *S. mel* 3, 6, and 9 resulted under the limit of detection too, and *S. mel* 1 exhibited a very low solasonine value, whereas *S. mel* 2 and 8 had the highest amounts (14.8 and 14.3 mg/100 g of dw, respectively), but with no significant difference compared to the other eggplant lines. As for stage B, all lines showed very low values, except for *S. mel* 8 and 7 (37.5 and 19.0 mg/100 g of dw, respectively). On the other hand, in the stage C high contents occurred in some lines, with remarkable values in *S. mel* 6, 9, 7, and *1*, reaching the safety value (Table 3). Orthogonal contrast between D3R- and NAS-containing lines was nonsignificant for this character in the stage A and significant ($p \leq 0.05$) in the stages B and C (Table 3).

The solamargine levels were lower than solasonine ones in *S. sod*, while this trend was more variable in *S. aeth* and *S. int*. Specifically, solamargine amount in the stage A is much higher than solasonine one in *S. aeth*, whereas in the stage C it is much lower than solasonine amount in both *S. aeth* and *S. int* (Table 3). Further investigation, also using other accessions, may better clarify the glycoalkaloids composition during fruit ripening in these allied species.

It has to be noted that the structural difference, at a molecular level, between the two steroidal glycoalkaloids is located in their glycosidic "tail", with a higher presence of rhamnose in solamargine (the glycoside being chacotriose containing rhamnose-rhamnose-glucose) with respect to solasonine (the glycoside being solatriose containing rhamnose-glucose-galactose), the former resulting with higher biological activity with respect to the latter.[28,29] The ratio solasonine vs solamargine showed a noteworthy variation, from values of about zero to value of 4.33 (Table 3). In eggplant lines, most ratio values were less than 1 in the stages A and B, with average values of 0.61 and 0.47. In the stage C the ratio often exceeded the value of 1, especially for D3R-types. Orthogonal contrast between D3R- and NAS-containing lines was nonsignificant for solasonine/solamargine ratio in the stage A only (Table 3).

The phenolic pattern of the eggplant fruit flesh has been evaluated by the dosage of total phenols index and by the differential RP-HPLC quantification of CA, the major phenolic compound present in the fruit.

The TP content displayed the highest value in *S. sod* (range 11.86−14.70 mmol of CA eq/100 g of dw) and the lowest one in both *S. int* and *S. aeth* (range 1.90−4.15 mmol of CA eq/100 g of dw); the TP content for the eggplant lines resulted intermediate (Table 4). The difference between the two subgroups were mainly shown in the stage A, as orthogonal contrast revealed significant difference between the average values of 12.46 vs 8.61 mmol CA eq/100 g of dw in D3R- and NAS-containing eggplant lines, respectively (Figure 2). This difference was confirmed in the stage B (6.43 vs 5.60 mmol CA eq/100 g of dw), with a lower F value, and it was inverted in the stage C (4.86 vs 6.41 mmol CA eq/100 g of dw; Figure 2). Orthogonal contrast between D3R- and NAS-containing lines was also highly significant for total phenols in the three ripening stages (Table 4).

The CA content was significantly correlated to that of TP (rxy =0.80). *S. sod* showed the highest values followed by the

Table 3. Solamargine, Solasonine, Total Glycoalkaloids and Solasonine/Solamargine Ratio in Fruits of the Three *Solanum* spp., of the Three D3R- and of the Seven NAS-Containing Eggplant Typologies Analyzed[a] and the Estimated Orthogonal Contrasts

	solamargine (mg/100 g of dw)			solasonine (mg/100 g of dw)			total glycoalkaloids (mg/100 g of dw)			solasonine/solamargine ratio		
genotype/contrast	stage A	stage B	stage C	stage A	stage B	stage C	stage A	stage B	stage C	stage A	stage B	stage C
Allied												
S. integrifolium	45.08 c B	102.12 b A	86.00 ef A	36.88 c B	96.18 b B	335.71 b A	81.96 c C	198.31 b B	421.72 bc A	0.82 c B	0.94 d B	3.93 a A
S. aethiopicum	387.00 b A	44.77 c C	75.02	62.99 b C	91.34 b B	199.64 de A	449.99 b A	136.11 c C	274.66 d B	0.16 d C	2.04 c B	2.66 cd A
S. sodomaeum	482.10 a A	185.04 a C	394.53 a B	764.77 a B	690.49 a B	1104.94 a A	1246.87 a B	875.53 a C	1499.47 a A	1.59 a C	3.74 a A	2.80 c B
D3R-Containing Typology												
S. melongena 1	10.98 de C	23.44 de B	41.60 hi A	2.06 d B	10.72 de B	179.95 e A	13.04 d-g C	34.16 e B	221.55 d A	0.20 d B	0.46 e B	4.33 a A
S. melongena 2	10.28 de C	18.38 ef B	98.73 de A	14.77 d A	<0 03 e B	<0.03 g B	25.05 d B	18.38 e-g C	98.73 ef A	1.44 ab A	0 f B	0 f B
S. melongena 3	18.82 d B	<0.03 h C	61.82 gh A	<0.03 d B	<0 03 e B	71.04 f A	18.82 d-f B	<0.03 h C	132.86 e A	0 d B	0 f B	1.15 e A
mean	13.36	13.94	67.38	5.61	3.57	83.66	18.97	17.51	151.04	0.55	0.15	1.83
NAS-Containing Typology												
S. melongena 4	9.34 de C	33.43 d B	106.11 de A	<0.03 d A	<0.03 e A	<0.03 g A	9.34 e-g C	33.43 e B	106.11 e A	0 d A	0 f A	0 f A
S. melongena 5	2.90 e C	10.83 fg B	25.02 i A	2.24 d A	0.68 e B	<0.03 g B	5.14 fg C	11.51 f-h B	25.02 f A	0.84 c A	0.06 f B	0 f B
S. melongena 6	0.92 e C	2.75 gh B	109.49 d A	<0.03 d B	<0.03 e B	259.74 c A	0.92 g C	2.75 gh B	369.23 c A	0 d B	0 f B	2.37 d A
S. melongena 7	9.01 de B	5.56 gh C	154.62 c A	10.51 d C	19.03 d B	198.63 de A	19.52 de B	24.59 ef B	353.25 c A	1.17 bc B	3.42 b A	1.28 e B
S. melongena 8	11.18 de C	51.51 c B	74.04 fg A	14.32 d B	37.46 c A	<0.03 g C	25.50 d B	88.96 d A	74.04 ef A	1.28 ab A	0.72 de B	0 f C
S. melongena 9	4.28 e B	5.78 gh B	227.57 b A	<0.03 d B	<0.03 e B	241.19 cd A	4.28 g B	5.78 gh B	468.76 b A	0 d B	0 f B	1.06 e A
S. melongena 10	2.86 e C	10.31 f-h B	87.26 ef A	<0.03 d A	<0.03 e A	<0.03 g A	2.86 g C	10.31 f-h B	87.26 ef A	0 d A	0 f A	0 f A
mean	5.78	17.17	112.02	3.87	8.17	99.94	9.65	25.34	211.96	0.47	0.6	0.67
D3R- vs NAS-Containing Typologies	$F = 15.93^{d}$	$F = 5.44^{c}$	$F = 244.65^{d}$	$F = 0.51$ ns[b]	$F = 5.52^{c}$	$F = 4.44^{c}$	$F = 25.41^{d}$	$F = 11.51^{d}$	$F = 37.99^{d}$	$F = 2.28$ ns[b]	$F = 117.19^{d}$	$F = 609.50^{d}$

[a]For each ripening stage, different lower-case letters indicate significantly different means among different genotypes at $p \leq 0.05$ (Tukey HSD test). For each genotype, different capital letters indicate significantly different means among different ripening stages at $p \leq 0.05$ (Tukey HSD test). ns. [b]ns, nonsignificant. [c]Significant at the 0.05 level of probability. [d]Significant at the 0.01 level of probability, < 0.03, limit of detection.

dx.doi.org/10.1021/jf3037424 | J. Agric. Food Chem. 2012, 60, 11821−11831

Table 4. Chlorogenic Acid (CA), Total Phenols (TP) and CA/TP Ratio in Fruits of the Three *Solanum* spp., of the Three D3R- and of the Seven NAS-Containing Eggplant Typologies Analyzed[a] and the Estimated Orthogonal Contrasts

	CA (mmol/100 g of dw)			TP (mmol of CA eq/100 g of dw)			CA/TP ratio		
genotype/contrast	stage A	stage B	stage C	stage A	stage B	stage C	stage A	stage B	stage C
Allied									
S. integrifolium	1.20 j A	0.22 j B	0.24 k B	2.46 f A	2.11 g AB	2.07 g B	0.49 de A	0.10 h B	0.12 f B
S. aethiopicum	3.14 i A	0.45 i C	0.72 j B	4.15 ef A	1.90 g B	4.03 f A	0.78 bc A	0.24 g B	0.18 ef B
S. sodomaeum	7.82 c C	9.22 a B	9.81 a A	14.36 a A	11.86 a B	14.70 a A	0.54 de B	0.78 b A	0.67 a B
D3R-Containing Typology									
S. melongena 1	6.10 e A	5.92 c B	1.86 e C	12.91 ab A	7.90 b B	4.12 f C	0.47 e B	0.75 bc A	0.45 c C
S. melongena 2	9.01 a A	3.95 d B	3.20 c C	11.34 bc A	5.56 de B	5.65 de B	0.79 b A	0.71 b-d B	0.57 b C
S. melongena 3	8.05 b A	3.87 d B	3.36 b C	13.14 ab A	5.84 de B	4.80 ef C	0.61 c-e A	0.66 cd A	0.71 a A
mean	7.72	4.58	2.81	12.46	6.43	4.86	0.62	0.71	0.58
NAS-Containing Typology									
S. melongena 4	4.68 g A	1.71 h B	1.74 f B	8.31 d A	3.99 f C	6.77 c B	0.56 de A	0.43 e B	0.26 d C
S. melongena 5	5.02 f A	3.52 e B	2.30 d C	10.06 cd A	5.22 e B	5.54 de B	0.50 de B	0.68 b-d A	0.41 c C
S. melongena 6	3.55 h A	2.04 g B	1.12 h C	5.48 e B	4.89 ef C	8.97 b A	0.65 b-d A	0.42 e B	0.12 f C
S. melongena 7	3.26 i B	7.37 b A	0.85 i C	11.33 bc A	7.11 bc B	4.81 ef C	0.29 f B	1.04 a A	0.18 ef B
S. melongena 8	6.15 e A	1.98 g B	1.54 g C	9.74 cd A	6.58 cd B	7.11 c AB	0.65 b-d A	0.30 fg B	0.22 de B
S. melongena 9	7.77 c A	2.26 f B	1.61 g C	9.58 cd A	5.53 de B	6.03 cd B	0.82 b A	0.41 ef B	0.27 d C
S.melongena 10	7.35 d A	3.64 e B	2.37 d C	5.79 e A	5.89 de A	5.64 de A	1.27 a A	0.63 d B	0.42 c C
mean	5.40	3.22	1.65	8.61	5.60	6.41	0.68	0.56	0.27
D3R- vs NAS-Containing Typologies	$F = 11775.22^{c}$	$F = 6898.59^{c}$	$F = 6536.22^{c}$	$F = 238.61^{c}$	$F = 29.85^{c}$	$F = 111.10^{c}$	$F = 4.88^{b}$	$F = 100.07^{c}$	$F = 892.29^{c}$

[a]For each ripening stage, different lower-case letters indicate significantly different means among different genotypes at $p \leq 0.05$ (Tukey HSD test). For each genotype, different capital letters indicate significantly different means among different ripening stages at $p \leq 0.05$ (Tukey HSD test). [b]Significant at the 0.05 level of probability. [c]Significant at the 0.01 level of probability.

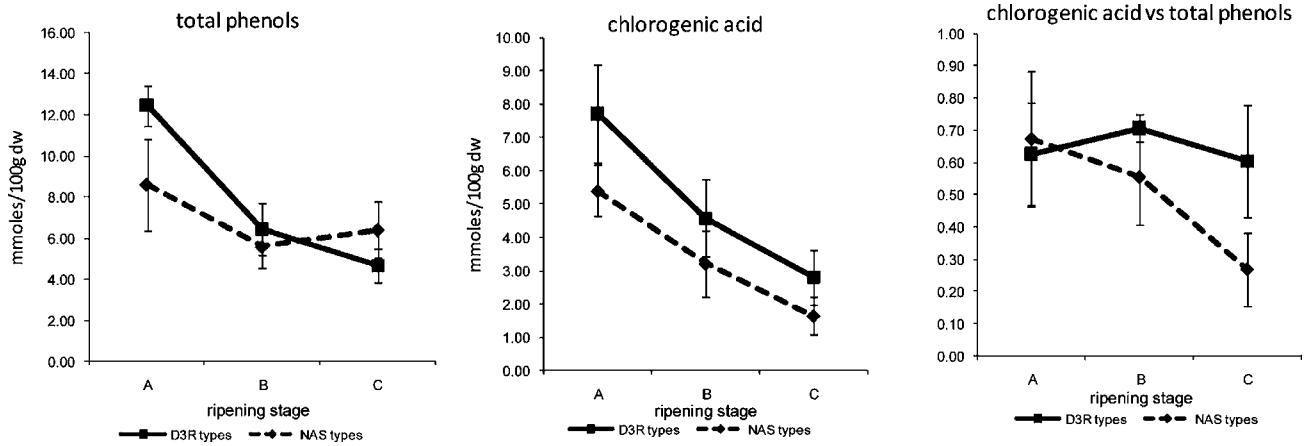

Figure 2. Trend with ripening in the stages A, B, and C (average ± sd) of total phenols (mmol CA equivalents/100 g of dw), chlorogenic acid (mmol/100 g of dw), and their ratio in D3R- and NAS-containing eggplant lines.

eggplant lines which showed significantly higher values than *S. aeth* and *S. int*. Relatively to the stages B and C, the allied species *S. sod* showed significantly higher values with respect to the other genotypes analyzed whereas the eggplant lines showed significantly higher values than *S. aeth* and *S. int* (Table 4). The elution profiles of the allied species analyzed in the present study did not show the typical pattern of CA derivatives as in eggplant chromatograms, in fact some minor peaks (not characterized) were detected at a longer retention times that could correspond to new CA derivatives identified by some authors in different wild eggplant relatives.[30,31] Generally, D3R-containing typologies had a higher average CA content than NAS-types as evidenced by highly significant F values (Figure 2, Table 4).

Strong variations have been found in the content of CA with respect to the TP amount, as measured by their ratio. Its value ranged from 0.10 (i.e., 10% of CA with respect to TP) in *S. int* (stage B), to an unique presence of CA as polyphenols in the eggplant fruit (*S. mel* 10, stage A; *S. mel* 7, stage B) (Table 4). Considering the B and C stages, significantly higher average ratio values have been found in D3R-subgroup with respect to NAS-types eggplant (0.71 vs 0.56 and 0.58 vs 0.27, respectively), confirmed by a highly significant orthogonal contrast (Table 4, Figure 2). An opposite trend characterized the stage A (Table 4, Figure 2). However, a remarkable

Table 5. Polyphenoloxidase (PPO) Activity, Superoxide Anion and Hydroxyl Radical Scavenging Capacity in Fruits of the Three *Solanum* spp., of the Three D3R- and of the Seven NAS-Containing Eggplant Typologies Analyzed[a] and the Estimated Orthogonal Contrasts

	PPO activity (U/100 mg of dw)			superoxide anion scavenging capacity (mmol of CAeq/100 g of dw)			hydroxyl radical scavenging capacity (mmol of CA eq/100 g of dw)		
genotype/contrast	stage A	stage B	stage C	stage A	stage B	stage C	stage A	stage B	stage C
Allied									
S. integrifolium	7.46 g A	5.15 fg AB	3.99 e-g B	1.60 g A	1.23 h B	0.68 g C	36.67 b-d B	37.58 cd B	42.03 b A
S. aethiopicum	16.52 f A	14.56 de AB	11.55 b B	1.33 g B	1.09 h C	1.71 ef A	43.13 a A	35.27 de B	45.81 a A
S.sodomaeum	2.13 h A	0.52 g B	0.71 h B	2.94 bc A	2.87 ab A	2.74 ab B	26.93 g A	23.21 g B	28.43 f A
D3R-Containing Typology									
S. melongena 1	36.84 c B	61.85 a A	12.83 b C	2.50 d-f A	2.51 c-e A	2.12 c-e A	33.25 d-f C	37.79 cd B	47.66 a A
S.melongena 2	52.18 b B	63.26 a AB	74.06 a A	2.48 ef A	2.23 ef A	2.29 cd A	33.48 d-f B	36.41 de A	32.97 e B
S. melongena 3	60.47 a A	13.63 de B	5.87 c-e C	3.01 bc A	2.76 a-c AB	2.24 cd B	33.14 d-f B	39.78 bc A	37.94 d A
mean	49.83	46.25	30.92	2.66	2.50	2.22	33.29	37.99	39.52
NAS-Containing Typology									
S. melongena 4	9.04 g A	8.84 ef A	4.17 e-g B	2.79 c-e A	1.77 g C	2.46 bc B	33.00 ef B	40.70 a-c A	41.70 bc A
S. melongena 5	32.06 d A	13.49 de B	3.25 fg C	3.00 bc A	2.14 f B	1.50 f C	37.30 bc B	27.20 f C	46.90 a A
S. melongena 6	27.24 e A	21.06 cd B	2.42 gh C	2.22 f B	1.73 g C	2.98 a A	33.65 d-f C	41.25 ab A	37.10 d B
S. melongena 7	26.90 e B	44.58 b A	5.99 c-e C	2.80 cd A	3.01 a A	2.21 cd B	37.75 bc B	43.45 a A	38.30 cd B
S. melongena 8	10.86 g B	38.02 b A	6.66 cd C	3.13 ab A	2.32 d-f C	2.76 ab B	35.00 c-e B	34.10 e B	41.70 bc A
S. melongena 9	34.11 cd A	15.74 de B	5.15 d-f C	3.40 a A	2.65 bc B	1.97 de C	39.55 b A	35.20 de B	39.00 b-d AB
S. melongena 10	10.67 g B	28.51 c A	7.96 c C	2.23 f C	2.54 cd B	3.09 a A	31.40 f C	35.55 de B	40.40 b-d A
mean	21.55	24.32	5.09	2.80	2.31	2.42	35.38	36.78	40.73
D3R- vs NAS-Containing Typologies	$F = 2477.87^{c}$	$F = 394.72^{c}$	$F = 7779.82^{c}$	$F = 9.63^{c}$	$F = 22.71^{c}$	$F = 14.12^{c}$	$F = 19.34^{c}$	$F = 7.36^{b}$	$F = 6.81^{b}$

[a]For each ripening stage, different lower-case letters indicate significantly different means among different genotypes at $p \leq 0.05$ (Tukey HSD test). For each genotype, different capital letters indicate significantly different means among different ripening stages at $p \leq 0.05$ (Tukey HSD test). [b]Significant at the 0.05 level of probability. [c]Significant at the 0.01 level of probability.

difference in the relationship between CA and TP can be observed in the two eggplant types, with D3R typologies having a high correlation index (rxy = 0.89) and the NAS typologies showing a weak correlation (rxy = 0.41).

The measurement of PPO activity (Table 5) revealed low values in all the allied species; *S. sod* showed the lowest activity in all the ripening stages, the minimum value being 0.52 U/100 mg of dw in the stage B; this character makes such allied species particularly suitable for breeding programs aiming to obtain lines showing reduced flesh browning.[32] On the contrary, *S. melongena* lines generally showed higher PPO activity values in stages A and B with respect to the allied species and, however, were characterized by a huge variability (Table 5). Interesting lines for the low PPO activity in all the ripening stages are *S. mel* 4 with 9.04, 8.84, and 4.17 U/100 mg of dw, for stages A, B, and C, respectively (Table 5).

It has to be pointed out that the PPO activity mean value of the analyzed NAS-type eggplants is always significantly lower than that of D3R-types, thus confirming previous empirical observations about the minor tendency to browning of NAS-type eggplant fruits after cutting. Such finding is validated by the highly significant orthogonal contrast for PPO activity in the three ripening stages (Table 5).

The two antioxidant assays showed a very different behavior of the genetic materials studied, meaning a differentiated role of the fruit phytochemicals in the action against two different free radicals species. In fact, the simple regression analysis of the main antioxidant compounds, such as TP, CA, and anthocyanins, showed different correlation indexes with the two assays. In accordance with previous data,[13] the superoxide anion scavenging capacity significantly correlated with TP (rxy = 0.74), and a significant correlation with CA amount was found (rxy = 0.61), whereas the anthocyanins pattern, in the eggplant lines, gave no significant correlation values with the antioxidant data (rxy = 0.33).

The hydroxyl radical scavenging was negatively and weakly correlated with the measured phytochemicals (rxy = −0.53, −0.57, and −0.08, for TP, CA, and anthocyanins, respectively), this result is in accordance with previous considerations about the role of other unusually considered antioxidant molecules.[33]

The correlation data further confirmed the difference in the phenolic activity against superoxide anion of the two eggplant subgroups. In fact, for chlorogenic acid amount a significant index of 0.64 has been found in D3R-type, whereas for NAS-type it resulted lower and not significant (rxy = 0.48) underlining the non exclusive role of chlorogenic acid in the antioxidant action in NAS-type.

Relevant values have been found in the eggplant lines *S. mel* 9, *S. mel* 7, and *S. mel* 10, for the ripening stages A, B, and C, respectively (Table 5). Significant differences were evidenced between the two subgroups in the three ripening stages: F-values of orthogonal contrast between D3R- and NAS-containing lines being 9.63, 22.71, and 14.12 for the stages A, B and C, respectively.

As regards the hydroxyl radical scavenging capacity, the lowest values were showed, in all the ripening stages, by *S. sod*, with an average of 26.19 mmol of CA eq/100 g of dw; when considering the stage B and C, the other two allied species and the eggplant lines were not significantly different (Table 5).

Biochemical Changes during Ripening. High average values of anthocyanins were found in the stages A and B (1032 and 1070 μmol/100 g of peel dw, respectively) of the eggplant

lines, whereas a strong depletion was observed in the stage C (218 μmol/100 g of peel dw).

As for D3R-types, the levels peaked in the stage B for *S. mel* 1 and *S. mel* 2, and successively significantly dropped from stage B to stage C (Figure 1). The *S. mel* 3 showed a different trend, with a continuous and significant decrease from stage A to stage C. In general, the NAS-containing lines had about the same average content in the stages A and B (587 and 563 μmol/100 g of peel dw) with a less marked decrease in the ripening stage C (233 μmol/100 g of peel dw) with respect to the D3R-types. It is worth noting that among the 7 NAS-type analyzed, 4 (*S. mel* 5, 6, 7, and 9) showed their higher anthocyanin content in the stage A, whereas the remaining three genotypes had their maximum content in the stage B. All the NAS-lines had their significantly lower content at the stage C except *S. mel* 10 that in the stage C had an amount of NAS similar to that of stage A. A different trend has been observed for the line *S. mel* 9, showing a continuous marked and significant decrease from stage A to C. These data suggest that a possible developmental genetic variation about the metabolic pathway of anthocyanin exists among different eggplant genotypes which could differently react to the light intensity and quality as confirmed by Honda et al. in eggplant[34] and Lowry et al. on *Mimulus guttatus*.[35] Further studies are necessary to better clarify and dissect the genetic regulation of the anthocyanin pathway in *S. melongena* which would allow to breed more easily improved genotypes with higher pigment content coupled with a longer lasting "commercial" skin coloration.

The SRR level in the allied species (Table 2) showed a significant decrease with ripening from stage A to stage B, while the trend to the stage C was more variable according to the species. *S. int* had a slight but significant decrement also in the stage C. On the contrary, *S. sod* and *S. aeth* showed a significant increment from B to C with the latter species having its maximum value at stage C of ripening. On the average, the D3R- and NAS-containing eggplant lines showed a slight decrease along ripening from stage A to C. Esteban et al.[36] reported the maximum in the sugars accumulation 6 weeks after fruit set, which would correspond to the late B stage of this investigation; most of our lines (7 out of the 10 studied) agreed to this trend, peaking in B stage or maintaining a similar higher content as the stage A. The remainders (*S. mel* 4, 6, and 9), belonging to the NAS-type, seemed to follow a different developmental ripening process, as they had the maximum at the stage C. These data evidenced that variability exists with regard to the trend of SSR accumulation in the fruits that may imply a possible different genetic regulation.

As reported by some authors for other *Solanum* species, it is a general concept that the level of glycoalkaloids tends to a strong decrease with ripening,[37] but other authors revealed that in *Solanum melongena* there is a retention with ripening, often exceeding the level of the immature fruit.[38,39] The trend detected in the eggplant lines in the present study is in good agreement with these findings. In fact, the glycoalkaloids amount shows a tendency to an increase in the physiologically ripe fruits (stage C) in most of the lines both for solasonine and solamargine. On this subject, it has been observed that several *Solanum* species retained potentially lethal levels of secondary compounds (such as steroidal glycoalkaloids) in their physiologically ripe fruits and some hypotheses were proposed to explain the adaptive significance of secondary metabolites in ripe fleshy fruits and their implications for seed dispersal,[40] often linked to phenomena of seed germination inhibition and attraction/repulsion toward vertebrate seed dispersers. Such phenomenon also involves humans since the glycoalkaloids increase, determining a marked flesh bitterness,[41] makes unmarketable the physiologically ripen eggplant fruits (also due to the other changes previously reported in MM).

In the allied species, the level of solasonine, solamargine and their sum tended to significantly increase during the three ripening stages in *S. int* (Table 3). On the contrary, in *S. aeth* only the less represented solasonine had a significant continuous increment from stage A to C; whereas the most abundant solamargine had its higher content in stage A, dramatically dropped in stage B and significantly increased in stage C, therefore the total glycoalkaloids content had a trend similar to solamargine (Table 3). In *S. sod*, the most represented glycoalkaloid was solasonine whose content was unchanged in stage A and B, whereas it significantly increased in stage C, solamargine had a trend of accumulation similar to *S. aeth* being its significant higher and lower values, respectively, in stage A and B. The trend of total glycoalkaloids content displayed by *S. sod* had its maximum in stage C, followed, with significant lower amount, by stage A and B (Table 3). By considering the relative and total content of the two glycoalkaloids analyzed, the three allied species displayed a different pattern of accumulation along with the ripening stages of the fruit. This fact is surely to be taken into account in the introgression breeding programs based on the utilization of these or other allied species.[14] In fact, these evidence, from one hand, underline the importance to monitor the accumulation pattern of these toxic compounds and, from the other hand, may open up the possibility to genetically manipulate this pattern in the cultivated *S. melongena*.

The eggplant lines had the significant highest level of total glycoalkaloids in the overripe fruits (stage C). In the case of the most abundant solamargine, the general trend to reach the maximum content was a continuous significant increment from stage A to C except for *S. mel* 3 and 7 which had the minimum level at stage B and *S. mel* 9 which maintains a similar amount in stage A and B. For the generally less represented solasonine a sharp increment was often displayed from the stage A/B (which had almost undetectable amounts) to C where, in the *S. mel* 1, 6, 7, and 9 the amount overtook or was close to the safety value of 200 mg/100 g dw. It is worth to note that these four latter mentioned lines are the ones with the higher total glycoalkaloid content due to the remarkable and concurrent increment of solasonine and solamargine in the stage C, as confirmed by the higher solasonine/solamargine ratios; whereas, in the case of almost all the remaining lines (i.e., *S. mel* 2, 4, 5, 8, and 10) only the solamargine significantly increased in the stage C. This behavior might suggest an independent genetic control of the solamargine and solasonine accumulation during the eggplant fruit ripening.

The trend of TP in allied species showed a slight but significant decrease in *S. int* from the stage A to stage C, whereas the other two species showed a significant reduction in the stage B, being not different the A and C stages. Except *S. mel* 10, exhibiting unchanged values in all three stages, the TP content in all the other eggplant lines showed clearly a significant decrease from stages A to B which in the case of *S. mel* 4 and 6 reached their minimum level. A great variability was found in the TP pattern from B to C stages: *S. mel* 1, 3, and 7 showed a significant decrease, whereas all the other lines showed a stability with the exception of *S. mel* 6 that reached a content significantly higher than those of stage B and A (Table

4, Figure 2). Esteban et al.[36] reported the peak in phenols and soluble sugars content after 42 days of fruit set, and this is not in accordance with our data. In fact, the trend in sugars content is different from that of polyphenols in the present study. A possible cause could be the difference of the assayed genotypes and the very different methods of extraction of phenols, with boiling methanol by Esteban et al.[36] and by homogenizing at room temperature with an aqueous buffer in the present study.

In the allied species, the level of CA during fruit development was very different (Table 4). *S. sod* had higher amounts and showed a significant increasing along the 3 ripening stages analyzed. On the contrary, the other two allied species had a significant decrease from stage A to B; in the overripe fruit with respect to stage B, the CA content was unchanged in *S. int* while had slight significant increment in *S. aeth* (Table 4). In eggplant lines there was a general continuous significant decrease from A to C, with the only exceptions of *S. mel* 7 that showed a strong increase from A to B followed by a sharp decrement in the overripe fruits (Table 4, Figure 2).

The trend of the ratio CA/TP in the allied species *S. int* and *S. aeth* showed a significant decrement from A to B and remained unchanged in the stage C; *S. sod*, instead, had a different behavior with a significant increase from stage A to B followed by a slight, non significant, decrease in the stage C. This indicates the CA replacement by other unidentified substances that, because detected by Folin reaction, could not belong to the phenol classes.

In six eggplant lines the ratio CA/TP showed an average trend to a decrease with ripening (*S. mel* 2, 4, 6, 8, 9, and 10), a significant higher ratio was found at the commercial stage B in three lines (*S. mel* 1, 5, and 7) and, finally, *S. mel* 3 maintained a similar ratio of about 0.66 in the three analyzed fruit ripe stages (Table 4). Literature data generally report a decrease of total phenols with ripening in fruit of various species, although exceptions for some single class exist, such as anthocyanins. Nevertheless, our data can be considered in general good accordance with these findings, although most of the available research has been carried out on different species than eggplant.[42−44] However, further studies are needed to better understand and, possibly, exploit the variation in CA and TP accumulation here evidenced both among the eggplant lines and in the accessions of the allied species analyzed.

As regards PPO changes, a significant reduction was detected with ripening, especially considering the enzyme activity variation from the unripe to the overripe fruit; this was a general behavior of the allied and eggplant genotypes with the exception of *S. mel* 2 that had a significant increment from A to C stage (Table 5). High variation was evidenced in the transition between the stage A to B, with lines showing significant decrement (*S. mel* 3, 5, 6, and 9), significant increment (*S. mel* 1, 7, 8, and 10) or unchanged (*S. mel* 2, 4) PPO activity. No other data are available, to our knowledge, on eggplant PPO variations with ripening, however the present findings are in good accordance with those found on pear fruit,[45] especially in relation to the trend in the decrease with ripening as well as with regard to the genotype effect. However, no accordance was found with the PPO activity in olive and grape fruits[46,47] that showed an increase with ripening.

Finally, the antioxidant parameters gave different trend in the eggplant lines. The superoxide anion scavenging capacity showed a very large variability across the three stages of ripening.

The hydroxyl radical scavenging capacity, in the allied and eggplant genotypes, showed a certain tendency to increase along with the fruit ripening (Table 5). The eggplant lines reached their maximum hydroxyl scavenging capacity in the stage C, except *S. mel* 2, 6, and 7 for which the maximum was in the stage B.

The biochemical analyses confirmed that a high variability exists for the analyzed traits both within the eggplant lines and with respect to the three allied species studied. By performing analyses along three stages of fruit development, variation was evidenced in the trend of accumulation of the compounds and biochemical activities studied, as well. The three allied species which have been employed to introgress into the cultivated eggplant (*S. melongena* L.) useful disease resistance and tolerance traits, here also proved to be a potential, useful source for the improvement of fruit qualitative traits (e.g., the low PPO activity in the 3 allied species, the high content of total phenols and chlorogenic acid in *S. sod*). A wide variation was evidenced in the content and pattern of accumulation of the anthocyanins, SSR, PPO activity and glycoalkaloids. In the case of the steroidal glycoalkaloids, a clear different trend of accumulation was evidenced by the solasonine in the stage C of fruit ripening (with a range from almost undetectable value to above the safety content), which could be exploited to select higher- or lower-producing glycoalkaloids genotypes.

A consideration can be pointed out on the difference in quality traits between the two eggplant typologies. A higher SRR content and a low PPO activity can be found in NAS-type with respect to the D3R ones.

The antioxidant capacity seems to be mainly influenced by TP and CA and to a low extent by the amount of the pigments, but antioxidant substances other than CA seem to influence the antioxidant capacity in NAS-typologies. Further studies are needed to better understand the chemical composition of NAS-type eggplants, that can justify this difference. These substances could be a less suitable substrates for PPO enzymes than CA, so explaining the lower tendency to browning of NAS-type eggplants.

These first insights revealed that biochemical compositional changes occurred across eggplant fruit development and ripening; the trend and the extent of such changes seem to be genetically regulated. Additional biochemical and metabolomic studies connected with the exploitation of the new molecular tools, like the dense intraspecific genetic maps[48,49] and the availability of the genome sequences of the solanaceous species tomato and potato[50,51] may help to localize the genomic regions (QTLs) and, hopefully, to clone the structural and regulatory genes, which modulate some ripening traits (e.g., color, firmness, SRR, TP, and glycoalkaloids content) linked to several qualitative properties of the eggplant fruit.

■ ASSOCIATED CONTENT

Supporting Information

RP-HPLC elution profiles at 520 nm of D3R- and Nasunin-containing eggplant peel extracts. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone +39-089-386233; fax +39-089-384170; e-mail giuseppe.mennella@entecra.it.

Funding

This research was partially supported by the Italian Ministry of Agricultural, Alimentary, and Forest Politics, with funds released by CIPE (resolution 17/2003), in the framework of the PROM project

Notes

The authors declare no competing financial interest.

REFERENCES

(1) Scheerens, J. C. Phytochemicals and the consumer: factors affecting fruit and vegetable consumption and the potential for increasing small fruit in the diet. *HortTechnology* **2001**, *11*, 547−556.

(2) Keller, I.; Tukuitonga, C. The WHO/FAO fruit and vegetable promotion initiative. In *Proceedings of the 1st International Symposium on Human Health Effects of Fruits and Vegetables*, FAVHEALTH 2005, Quebec City, Canada, 17−20 August 2005; Acta Hort. (ISHS), **2007**; 744, 27−37.

(3) Montero, C.; Cristescu, S. M.; Jiménez, J. B.; Orea, J. M.; Lintel Hekkert, S.; Harren, F. J. M.; Gonzàlez Ureña, A. *Trans*-resveratrol and grape disease resistance. A dynamical study by high-resolution laser-based techniques. *Plant Physiol* **2003**, *131*, 129−138.

(4) Rivero, R. M.; Ruiz, J. M.; Garcìa, P. C.; Lòpez-Lefebre, L. R.; Sànchez, E.; Romero, L. Resistance to cold and heat stress: accumulation of phenolic compounds in tomato and watermelon plants. *Plant Sci.* **2001**, *160*, 315−321.

(5) Jones, P. G.; Fenwick, G. R. The glycoalkaloid content of some edible solanaceous fruits and potato products. *J. Sci. Food Agric.* **2006**, *32*, 419−421.

(6) Kuronen, P.; Väänänen, T.; Pehu, E. Reversed-phase liquid chromatographic separation and simultaneous profiling of steroidal glycoalkaloids and their aglycones. *J. Chromatogr., A* **1999**, *863*, 25−35.

(7) Whitaker, B. D.; Stommel, J. R. Distribution of hydroxycinnamic acid conjugates in fruit of commercial eggplant (*Solanum melongena* L.) cultivars. *J. Agric. Food Chem.* **2003**, *51*, 3448−3454.

(8) Ichiyanagi, T.; Kashiwada, Y.; Shida, Y.; Ikeshiro, Y.; Kaneyuki, T.; Konishi, T. Nasunin from eggplant consists of *cis-trans* isomers of delphinidin 3-[4-(*p*-coumaroyl)-L-rhamnosyl (1→6) glucopyranoside]-5-glucopyranoside. *J. Agric. Food Chem.* **2005**, *53*, 9472−9477.

(9) Prohens, J.; Rodriguez-Burruezo, A.; Raigon, M. D.; Nuez, F. Total phenolic concentration and browning susceptibility in a collection of different varietal types and hybrids of eggplant: implications for breeding for higher nutritional quality and reduced browning. *J. Am. Soc. Hortic. Sci* **2007**, *132*, 1−9.

(10) Luthria, D. L.; Mukhopadhyay, S. Influence of sample preparation on assay of phenolic acids from eggplant. *J. Agric. Food Chem.* **2006**, *54*, 41−47.

(11) Azuma, K.; Ohyama, A.; Ippoushi, K.; Ichiyanagy, T.; Takeuchi, A.; Saito, T.; Fukuoka, H. Structures and antioxidant activity of anthocyanins in many accessions of eggplant and its related species. *J. Agric. Food Chem.* **2008**, *56*, 10154−10159.

(12) Matsubara, K.; Kaneyuki, T.; Miyake, T.; Mori, M. Antiangiogenic activity of nasunin, an antioxidant anthocyanin, in eggplant peels. *J. Agric. Food Chem.* **2005**, *53*, 6272−6275.

(13) Hanson, P. M.; Yang, R.-Y.; Tsou, S. C. S.; Ledesma, D.; Engle, L.; Lee, T. C. Diversity in eggplant (*Solanum melongena*) for superoxide scavenging activity, total phenolics and ascorbic acid. *J. Food Comp. Anal.* **2006**, *19*, 594−600.

(14) Mennella, G.; Rotino, G. L.; Fibiani, M.; D'Alessandro, A.; Francese, G.; Toppino, L.; Cavallanti, F.; Acciarri, N.; Lo Scalzo, R. Characterization of health-related compounds in eggplant (*Solanum melongena* L.,) lines derived from introgression of allied species. *J. Agric. Food Chem.* **2010**, *58*, 7597−7603.

(15) Toppino, L.; Vale', G.; Rotino, G. L. Inheritance of *Fusarium* wilt resistance introgressed from *Solanum aethiopicum* Gilo and Aculeatum groups into cultivated eggplant (*S. melongena*) and development of associated PCR-based markers. *Mol. Breed.* **2008**, *22*, 237−250.

(16) Acciarri, N.; Sabatini, E.; Voltattorni, S.; Ciriaci, T.; Tamietti, G.; Valentino, D.; Mennella, G.; Cavallanti, F.; Tacconi, M. G.; Toppino, L.; Grazioli, G.; Pedretti, R.; Alberti, P.; Rotino, G. L. First eggplant pure lines of different typologies derived from sexual and somatic hybridization with *S. sodomaeum* and *S. aethiopicum* gr. *aculeatum* and gr. *gilo*. In *Proceedings of the XIIIth EUCARPIA Meeting on Genetics and Breeding of Capsicum and Eggplant, Warsaw, Poland, 5−7 September, 2007*; Niemirowicz-Szezytt, K., Ed.; University of Life Science Press: Warsaw, Poland, 2007; pp 327−338.

(17) Lo Scalzo, R.; Fibiani, M.; Mennella, G.; Rotino, G. L.; Dal Sasso, M.; Culici, M.; Spallino, A.; Braga, P. C. Thermal treatments of eggplant (*Solanum melongena* L.) increases the antioxidant content and the inhibitory effect on human neutrophil burst. *J. Agric. Food Chem.* **2010**, *58*, 3371−3379.

(18) Birner, J. A method for the determination of total steroid bases. *J. Pharm. Sci.* **1969**, *58*, 258−259.

(19) Singleton, V. L.; Orthofer, R.; Lamuela-Raventos, R. M. Analysis of total phenols and other oxidation substrates and antioxidant by means of Folin-Ciocalteu reagent. *Methods Enzymol.* **1974**, *299*, 152−178.

(20) Fujita, S.; Tono, T. Purification and some properties of polyphenoloxidase in eggplant (*Solanum melongena*). *J. Sci. Agric.* **1988**, *46*, 115−123.

(21) Privat, C.; Telo, J. P.; Bernardes-Genisson, V.; Vieira, A.; Souchard, J. P.; Nepveu, F. Antioxidant properties of *trans-ε*-viniferin as compared to stilbene derivatives in aqueous and non-aqueous media. *J. Agric. Food Chem.* **2002**, *50*, 1213−1217.

(22) Valavanidis, A.; Nisiotou, C; Papageorgiou, Y; Kremli, I.; Satravelas, N.; Zinieris, N.; Zygalaki, H. Comparison of the radical scavenging potential of polar and lipidic fractions of olive oil and other vegetable oils under normal conditions and after thermal treatment. *J. Agric. Food Chem.* **2004**, *52*, 2358−2365.

(23) Jones, P. G.; Fenwick, G. R. The glycoalkaloid content of some edible solanaceous fruits and potato products. *J. Sci. Food Agric.* **2006**, *32*, 419−421.

(24) Roddick, J. G.; Weissenberg, M.; Leonard, A. L. Membrane disruption and enzyme inhibition by naturally-occurring and modified chacotriose-containing *Solanum* steroidal glycoalkaloids. *Phytochemistry* **2001**, *56*, 603−610.

(25) Shiu, L. Y.; Chang, L. C.; Liang, C. H.; Huang, Y. S.; Sheu, H. M.; Kuo, K. W. Solamargine induces apoptosis and sensitizes breast cancer cells to cisplastin. *Food Chem. Toxicol.* **2007**, *45*, 2155−2164.

(26) Hall, C. A.; Hobby, T.; Cipollini, M. Efficacy and mechanisms of solasonine- and solamargine-induced cytolysis on two strains of *Trypanosoma cruzii*. *J. Chem. Ecol.* **2006**, *32*, 2405−2416.

(27) Sanchez-Mata, M.-C.; Yokoyama, W. E.; Hong, Y.-J.; Prohens, J. *α*-Solasonine and *α*-solamargine contents of gboma (*Solanum macrocarpon* L.) and scarlet (*Solanum aethiopicum* L.) eggplants. *J. Agric. Food Chem.* **2010**, *58*, 5502−5508.

(28) Roddick, J. G.; Rijnenberg, A. L.; Weissenberg, M. Membrane-disrupting properties of the steroidal glycoalkaloids solasonine and solamargine. *Phytochemistry* **1990**, *29*, 1513−1518.

(29) Roddick, J. G.; Rijnenberg, A. L.; Weissenberg, M. Alterations to the permeability of liposome membranes by the solasodine-based glycoalkaloids solasonine and solamargine. *Phytochemistry* **1992**, *31*, 1951−1954.

(30) Ma, C.; Whitaker, B. D.; Kennelly, E. J. New 5-*O*-caffeoylquinic acid derivatives in fruit of the wild eggplant relative *Solanum viarum*. *J. Agric. Food Chem.* **2010**, *58*, 11036−11042.

(31) Ma, C.; Dastmalchi, K.; Whitaker, B. D.; Kennelly, E. J. Two new antioxidant malonated caffeoylquinic acid isomers in fruits of wild eggplant relatives. *J. Agric. Food Chem.* **2011**, *59*, 9645−9651.

(32) Todaro, A.; Cavallaro, R.; Argento, S.; Branca, F.; Spagna, G. Study and characterization of polyphenol oxidase from eggplant (*Solanum melongena* L.). *J. Agric. Food Chem.* **2011**, *59*, 11244−11248.

(33) Halliwell, B.; Aeschbach, R.; Löliger, J.; Aruoma, O. I. The characterization of antioxidants. *Food Chem. Toxicol.* **1995**, *33*, 601−617.

(34) Honda, T.; Zushi, K.; Matsuzoe, N. Inheritance of anthocyanin pigment and photosensitivity in eggplant (*Solanum melongena* L.) fruit. *Environ. Control Biol* **2012**, *50*, 75−80.
(35) Lowry, D. B.; Sheng, C. C.; Lasky, J. R.; Willis, J. H. Five anthocyanin polymorphisms are associated with an *R2R3-MYB* cluster in *Mimulus guttatus* (Phrymaceae). *Am. J. Bot* **2012**, *99*, 82−91.
(36) Esteban, R. M.; Mollà, E. M.; Robredo, L. M.; Lòpez-Andrèu, F. J. Changes in the chemical composition of eggplant fruit during development and ripening. *J. Agric. Food Chem.* **1992**, *40*, 998−1000.
(37) Friedman, M. Tomato glycoalkaloids: role in the plant and in the diet. *J. Agric. Food Chem.* **2002**, *50*, 5751−5780.
(38) Aubert, S.; Daunay, M. C.; Pochard, E. Saponosides steroidiques de l'aubergine (*Solanum melongena* L.). 1. Interet alimentaire, methodologie d'analyse, localisation dans le fruit. *Agronomie* **1989**, *9*, 641−651.
(39) Aubert, S.; Daunay, M. C.; Pochard, E. Saponosides steroidiques de l'aubergine *(Solanum melongena* L.. 2. Variations des teneurs lieus aux conditions de raccolte, aux genotypes et in la quantité de graines des fruits. *Agronomie* **1989**, *9*, 751−758.
(40) Cipollini, M. L.; Levey, D. J. Secondary metabolites of fleshy vertebrate-dispersed fruits: adaptive hypotheses and implications for seed dispersal. *Amer. Nat.* **1997**, *150*, 346−372.
(41) Chating, B.; Chami, L.; Mendez, R.; O'Callaghan, J.; Usubillaga, A.; LaCruz, L. Toxicological effects of α-solamargine in experimental animals. *Phytother. Res.* **2003**, *17*, 254−258.
(42) Lima, V. L. A. G.; Mèlo, E. A.; Maciel, M. I. S.; Prazeres, F. G.; Musser, R. S.; Lima, D. E. S. Total phenolics and carotenoid content in acerola genotypes harvested at three ripening stages. *Food Chem.* **2005**, *90*, 565−568.
(43) Castrejon, A. D. R.; Eichholz, I.; Rohn, S.; Kroh, L. W.; Huyskens-Keil, S. Phenolic profile and antioxidant activity of highbush blueberry (*Vaccinium corimbosum* L.) during fruit maturation and ripening. *Food Chem.* **2008**, *109*, 564−572.
(44) Delgado, R.; Martìn, P.; del Alamo, M.; Gonzàlez, M.-R. Changes in the phenolic composition of grape berries during ripening in relation to vineyard nitrogen and potassium fertilization rates. *J. Sci. Food Agr* **2004**, *84*, 623−630.
(45) Amiot, M. J.; Tacchini, M.; Aubert, S.; Nicolas, J. Influence of cultivar, maturity stage, and storage condition on phenolic composition and enzymatic browning of pear fruits. *J. Agric. Food Chem.* **1995**, *43*, 1132−1137.
(46) Ortega-Garcia, F.; Peragon, J. Phenylalanine ammonia-lyase, polyphenol oxidase, and phenol concentration in fruits of *Olea europaea* L. cv. Picual, Verdial, Arbequina, and Frantoio during ripening. *J. Agric. Food Chem.* **2009**, *57*, 10331−10340.
(47) López-Miranda, S.; Hernández-Sánchez, P.; Serrano-Martínez, A.; Hellín, P.; Fenoll, J.; Núñez-Delicado, E. Effect of ripening on protein content and enzymatic activity of Crimson Seedless table grape. *Food Chem.* **2011**, *127*, 481−486.
(48) Fukuoka, H.; Miyatake, K.; Nunome, T.; Negoro, S.; Shirasawa, K.; Isobe, S.; Asamizu, E.; Yamaguchi, H.; Ohyama, A. Development of gene-based markers and construction of an integrated linkage map in eggplant by using *Solanum* orthologous (SOL) gene sets. *Theor. Appl. Genet.* **2012**, *125*, 47−56.
(49) Barchi, L.; Lanteri, S.; Portis, E.; Valè, G.; Volante, A.; Pulcini, L.; Ciriaci, T.; Acciarri, N.; Barbierato, V.; Toppino, L.; Rotino, G. L. A RAD tag derived marker based eggplant linkage map and the location of QTLs determining anthocyanin pigmentation. *PLoS ONE* **2012**, *7*, e43740 DOI: 10.1371/journal.pone.0043740.
(50) The Tomato Genome Consortium. The tomato genome sequence provides insights into fleshy fruit evolution. *Nature* **2012**, *485*, 635−641. DOI: 10.1038/nature11119.
(51) The Potato Genome Sequencing Consortium. Genome sequence and analysis of the tuber crop potato. *Nature* **2011**, *475*, 189−195. DOI: 10.1038/nature10158.