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Disposition of Selected Flavonoids in Fruit Tissues of Various Tomato (*Lycopersicon esculentum* Mill.) Genotypes

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Flavonoids have been studied extensively because they offer great potential health benefits. In this study, enzymatic hydrolysis of glycosylated quercetin, kaempferol, and naringin was used to obtain their sugar-free aglycones. The investigation also employed a validated HPLC method to obtain the chiral disposition of the aglycone naringenin enantiomers. These analyses were conducted on exocarp, mesocarp, and seed cavity tissues of field-grown tomato (*Lycopersicon esculentum* Mill.) mutants (*anthocyanin absent*, *atroviolacea*, and *high pigment-1*) and their nearly isogenic parent (cv. Ailsa Craig) at immature green, "breaker", and red ripe maturity stages. Concentrations of all flavonoids using enzymatic hydrolysis were significantly higher than previously reported concentrations using acid hydrolysis. Presumably, this occurred due to a more specific and rapid hydrolysis of the glycoside moiety by the β-glucosidase enzyme. The glycoside *S*-naringin was the predominant enantiomer in all fruit tissues, although the aglycones free *R*- and *S*-naringenin were detected in both exocarp and mesocarp. Whereas there was significantly more quercetin than kaempferol in exocarp tissue, they were present in about equal concentrations in the mesocarp. Quercetin concentrations were higher in the exocarp and mesocarp of immature green and breaker fruit of the *high pigment-1* mutant than in the other genotypes, supporting the observed photoprotection and potential health benefits of the *high pigment-1* tomato genotype.

KEYWORDS: Quercetin; kaempferol; naringenin; naringin; enantiomer; maturity stage; HPLC; enzymatic hydrolysis

INTRODUCTION

Flavonoids are an important group of secondary plant metabolites derived from the phenylpropanoid biochemical pathway. Their basic structure consists of two aromatic benzene rings separated by an oxygenated heterocyclic ring. Although they are present in many plant tissues, they are usually located in the cell vacuoles. The flavonoids have important functions in plants, including defense against pathogens and protection against ultraviolet B (UV-B) radiation (*1*−*3*). In mammals, they have been shown to have multiple health benefits as antioxidants and anti-inflammatory, anti-cancer, and antiviral compounds (*4*−*7*). Flavanones are a class of flavonoids that have been determined to influence the sensory characteristics of fruits. For instance, they have been proposed to be responsible for the bitter flavor of citrus fruits (*8*).

Flavonols and flavanones are the major subclasses of flavonoids in fruits. These flavonoids are usually present as *O*-glycosides having a conjugated sugar moiety such as glucose, rhamnose, rutinose, galactose, or arabinose. The presence of these moieties increases the polarity of flavonoids so that they can be stored in vacuoles. However, flavonols and flavanones can also be present without the attached sugar moiety, called aglycones. The two major flavonols in fruits are quercetin and kaempferol, although their glycoside forms are primarily found in tomatoes (*9*, *10*). Le Gall et al. (*11*) identified quercetin-3-*O*-rutinoside (rutin), a type of kaempferol glycoside, and naringenin-7-*O*-glucoside and naringenin chalcone as the major flavonoid components of whole ripe tomato fruits. Naringenin is a flavanone synthesized upstream from quercetin and kaempferol. Different ratios of the chiral flavanone 7-*O*-glycoside naringin [(±)-4′,5,7-trihydroxyflavanone 7-rhamnoglucoside] to its aglycone, naringenin [(±)-4′,5,7-trihydroxyflavanone], were detected in tomato and citrus fruits (*12*−*14*). Compared to citrus fruits, tomatoes have relatively higher naringenin concentrations than the glycosylated naringin. Moreover, several studies have been unable to determine either naringin or naringenin-7-*O*-glucoside in tomato paste (*15*−*18*).

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Although flavonoids are abundant in tomato fruit, the purported antioxidant properties of tomato consumption have been mostly attributed to the fruit's major red carotenoid pigment, lycopene (*7*). However, when lycopene was administered along with various polyphenols, including flavonoids, lycopene's antioxidant properties were determined to be enhanced (*19*).

In the present study we investigate naringenin and naringin, together with quercetin and kaempferol, profiles in tomato fruit tissues (exocarp, mesocarp, and seed cavity) at different fruit maturity stages of four tomato genotypes. Because naringenin and naringin are chiral compounds, either or both of its enantiomers may be the active component(s) with regard to potential health benefits. This study also presents, for the first time, the quantitative analysis of naringenin and naringin enantiomers in tomatoes using a validated stereoselective, isocratic, reversed-phase high-performance liquid chromatography (HPLC) method. The quantification of all flavonoids involved the use of enzymatic hydrolysis for the removal of the sugar moiety instead of the typical acid hydrolysis approach used in similar studies of fruits and vegetables.

MATERIALS AND METHODS

Plant Material. Fruit samples from four tomato (*Lycopersicon esculentum* Mill.) genotypes, the seeds of which were obtained from the C. M. Rick Tomato Genetics Resource Center (University of California, Davis, CA), were used. The genotypes were (1) *anthocyanin absent (aa)* (LA 3617), nearly isogenic in cv. Ailsa Craig with mutation on chromosome 2 and anthocyanin completely absent in all plant parts (*33, 39*); (2) *atroviolacea* (atv) (LA 3736), nearly isogenic in Ailsa Craig with enhanced photoresponsiveness (*20*) elevated anthocyanin levels in all plant parts, the main anthocyanin present in leaves and stems being peonidin-3-(*p*-coumaryl rutinoside)-5-glucoside (*33, 39*); (3) *high pigment-1 (hp-1)* (LA 2838A), a highly studied genotype nearly isogenic in Ailsa Craig with recessive nonallelic mutation in locus 12 (monogenic), first identified in 1917, corresponding to a negative regulator of the phytochrome signal translator resulting in an exaggerated photoresponse (*20*) [chlorophyll, carotenoids (lycopene and β-carotene), and ascorbic acid contents of fruit intensified (*35*−*37*), including anthocyanins (*20*), a characteristic found in breeding lines using *hp-1* (*36*)]; and (4) the nearly isogenic parent (Ailsa Craig) of these mutants.

Seeds were germinated and seedlings grown in cell packs in a greenhouse until they had several true leaves, at which time they were transplanted into replicated plots in the field in a randomized complete-block design consisting of four blocks with six plants per plot. Plants were spaced 1.2 m apart in the rows, with rows 2.4 m apart.

Field Site. The research site was located in Lewiston, ID (46° 23′ N; 116° 59′ W) at an elevation of 430 m above sea level. The site was level of a uniform Nez Perce silty, clay-loam textured soil (fine, montmorillonitic, mesic Xeric Argialbolls). This region would be classified as desert steppe with summer (June−September) mean maximum temperatures of 30 °C on generally cloudless days, with most precipitation occurring only in the winter months.

All plants were irrigated with buried drip tape, 1 h per day during the first 30 days after transplanting and 2 h per day from 30 days after planting until the end of the growing season. The output of the emitters was 1.9 L h^{-1}. Plants were surface fertilized with calcium nitrate (30 g per plant) only once during the season, 2 weeks after transplanting.

Fruit Sampling. Five, healthy, equal-sized fruits were harvested from each block and genotype at three maturity stages: (1) green (immature), (2) "breaker" (intermediate), and (3) red (fully ripe).

From each fruit, the exocarp (peel, ~2 mm thick), mesocarp (flesh), and jelly-like seed cavity (including columella, placenta tissue, and seeds) tissues were separately collected, immediately frozen using liquid N_2, transported to the laboratory on dry ice, and stored at −80 °C. The frozen tissue was later ground to a fine powder using a mortar and pestle and liquid N_2 and stored at −80 °C until analysis. Fully expanded leaves were also collected for analysis.

Chemicals and Reagents. HPLC grade methanol, hexane, acetonitrile, and water were purchased from J. T. Baker (Phillipsburg, NJ). Phosphoric acid was purchased from Aldrich Chemical Co. Inc. (Milwaukee, WI). Quercetin, kaempferol, naringenin, 7-ethoxycoumarin, daidzein, and *Helix pomatia* type HP-2 were purchased from Sigma-Aldrich (St. Louis, MO).

Flavonoid Extraction. Flavonoids were extracted from fresh tissue using a ratio of tissue/100% methanol/hexane of 0.1:1:0.5 (w/v/v). First, powdered tissue was ground with methanol using a homogenizer (Talboys Engineering Corp., Montrose, PA) for 2 min. The slurry was then transferred to Eppendorf tubes, and hexane was added. After samples had been centrifuged at 13000*g* for 10 min, the hexane layer was discarded and the methanol fraction was dried completely under a stream of purified N_2 gas. Samples were maintained at 2 °C throughout the extraction procedure.

Dried samples were then reconstituted in mobile phase (400 μL) for measurement of naringenin by HPLC or enzymatically hydrolyzed to measure the total individual flavanones, quercetin, kaempferol, and naringenin.

Enzymatic Hydrolysis. The enzymatic hydrolysis of flavonoids was performed according to the method described by Yañez and Davies (*21*) with some modifications. Dried samples from the flavonoid extraction were resuspended in 0.78 M acetate buffer (pH 4.8), 0.1 M ascorbic acid, and *H. pomatia* type HP-2 β-glucosidase (100 μL). The mixture was incubated for 17−24 h at 37 °C, after which samples were centrifuged at 7000*g* for 10 min at room temperature (25 °C). Each sample was divided into two equal aliquots, one for naringenin, to which 25 μL of daidzein (0.1 mg mL^{-1}) was added as internal standard, and the other one for quercetin and kaempferol detections, to which 25 μL of 7-ethoxycoumarin (0.1 mg mL^{-1}) was added as internal standard. In both aliquots, 1 mL of 100% cold acetonitrile was added and vortexed for 1 min. Samples were then centrifuged at 7000*g* for 5 min at room temperature. Supernatants were transferred to new Eppendorf tubes and dried completely under a stream of purified N_2 gas. Dried samples were then reconstituted in 400 μL of mobile phase for naringenin or quercetin and kaempferol assays, vortexed for 1 min, and centrifuged at 7000*g* for 5 min. Supernatants were transferred to HPLC vials. In all cases the injection volume was 150 μL.

Standards Solutions. All standards were made at 1 mg mL^{-1} stock solutions in methanol. They were protected from light and stored at −20 °C for up to 3 months.

Chromatographic Conditions. Extracts and standards were injected into a Shimadzu HPLC system (Kyoto, Japan), consisting of an LC-10AT VP pump, an SIL-10AF autoinjector, an SPD-M10A VP spectrophotometric diode array detector, and an SCL-10A system controller. Integration and collection of data were carried out using the Shimadzu EZ Start 7.1.1. SP1 software (Kyoto, Japan).

Naringenin enantiomers were separated using a Chiralcel OD-RH column (150 mm × 4.5 mm i.d., 5-μm particle size; Chiral Technologies Inc., Exton, PA) under isocratic conditions at 25 °C. Separation was carried out using a mobile phase of acetonitrile/water/phosphoric acid (30:70:0.04, v/v/v) and a flow rate of 0.4 mL min^{-1}. Naringenin enantiomers were detected at 292 nm. This stereoselective, reverse-phase HPLC method has been previously validated and described in detail by Yañez and Davies (*21*).

Quercetin and kaempferol were also separated isocratically by a Chiralcel AD-RH column (150 mm × 4.5 mm i.d., 5-μm particle size; Chiral Technologies Inc.). The mobile phase was acetonitrile/water/phosphoric acid (42:58:0.01, v/v/v) at a flow rate of 0.6 mL min^{-1}. Both flavonoids were detected at 370 nm. Mobile phase solvents were filtered and degassed before use.

Statistical Analysis. Flavonoid contents were quantified on the basis of standard curves constructed using peak area ratio (PAR) against standard concentrations. PAR was obtained by dividing the peak area of each flavonoid by the peak area of the respective internal standard. Least-squares linear regression was used for this purpose.

The experimental design was a split-block with two factors: (A) genotypes and (B) maturity stage. The analysis was carried out separately for three different fruit tissues: (1) exocarp, (2) mesocarp,

Table 1. Total *R*- and *S*-Naringenin and Total Naringenin in Fruit Exocarp, Mesocarp, and Seed Cavity in Different Genotypes (A) and Fruit Maturity Stages (B), and Their Interaction (A × B)

	total *R*-naringenin (μg g^{-1} of FW)			total *S*-naringenin (μg g^{-1} of FW)			total naringenin (*R* + *S*) (μg g^{-1} of FW)		
factor	exocarp	mesocarp	seed cavity	exocarp	mesocarp	seed cavity	exocarp	mesocarp	seed cavity
genotype (A)									
Ailsa Craig	141	24.0	22.0	910	35.4	27.8	1051	59.4	49.8
aa	191	23.8	22.7	1233	35.4	33.1	1424	59.2	55.8
atv	182	24.5	23.2	985	37.0	32.8	1167	61.4	56.0
hp-1	210	24.3	21.9	1160	42.1	28.4	1370	66.5	50.3
P value	0.002	0.872	0.342	0.002	0.525	0.263	0.001	0.494	0.1957
maturity stage (B)									
green immature	29.6	20.2 a[a]	20.8 a	65.0	23.2 a	26.2 a	94.6	43.4 a	47.0 a
breaker	268	22.8 b	22.9 b	2295	47.3 b	34.2 b	2569	70.1 b	57.1 b
red	248	29.5 c	23.6 b	862	41.9 b	31.3 b	1110	71.3 b	54.9 b
P value	<0.0001	<0.0001	0.001	<0.0001	<0.0001	0.010	<0.0001	<0.0001	0.0058
A × B *P* value	0.007	0.938	0.790	<0.0001	0.763	0.936	<0.0001	0.872	0.9102

[a] Different letters indicate statistical differences. Mean separation by protected LSD (<0.05).

and (3) seed cavity. Analysis of variance and mean separation were performed only after data met the assumption of normality, which in some cases was achieved by transforming data using the ladder of powers ($x = y^p$). When statistical differences were determined, a protected LSD test ($P < 0.05$) was used for mean separation. The analysis was performed using the statistical package SAS (SAS Institute Inc., Cary, NC).

RESULTS

All compounds, quercetin, kaempferol, naringenin enantiomers, and internal standards, were separated successfully, without interfering peaks coeluting with them (**Figure 1**). Interestingly, enzymatic hydrolysis to obtain free plus conjugated flavonoid compounds resulted in significantly greater concentrations of all compounds compared to those reported for the commonly used acid hydrolysis method (*9*, *15*, *22*−*26*).

Naringenin. *R*- and *S*-naringenin and their internal standard (IS) eluted at approximately 46, 50, and 25 min, respectively (**Figure 1A**). In all tomato fruit tissues (exocarp, mesocarp, and seed cavity), total concentrations of *S*-naringenin were greater than those of *R*-naringenin (**Table 1**). In agreement with another study (*9*) that reported that tomato skin has the highest concentration of these flavonoids, the exocarp of tomato fruit contained ~92% of total naringenin (*R* + *S* configurations). Fruit mesocarp (flesh) and seed cavity contained only 4.5 and 3.5%, respectively, of the total concentration of naringenin in the whole tomato. When enantiomers were analyzed separately, the percentage of the *R*-enantiomer in the exocarp dropped to 80%, whereas that in flesh and seed cavity increased to 10% in each tissue type.

Genotype and maturity stage influenced the change in flavonoid concentrations in the different tissues studied. There was a significant interaction between both factors (genotype × maturity stages) in fruit exocarp (**Table 1**). Total naringenin, as well as the *R* and *S* configurations, was consistently higher in the exocarp of *hp-1* at the immature green stage (**Figure 2**). Although not always statistically significant, all tomato mutants generally demonstrated higher total naringenin than their parent Ailsa Craig (**Figure 2**). In both mesocarp and seed cavity tissues, immature green fruit had the lowest total naringenin and *R*- and *S*-naringenin concentrations (**Table 1**). In general, there was a prominent increase in both naringenin enantiomers at the breaker (intermediate) stage, which later decreased at the red-ripe stage.

Free naringenin (before enzymatic hydrolysis) accounted for ~30% of the total naringenin concentration (after enzymatic hydrolysis) of the fruit exocarp tissue, with the majority present as the free *S*-enantiomer. Therefore, the glycoside naringin accounted for ~70% of the total naringenin concentration in exocarp tissue.

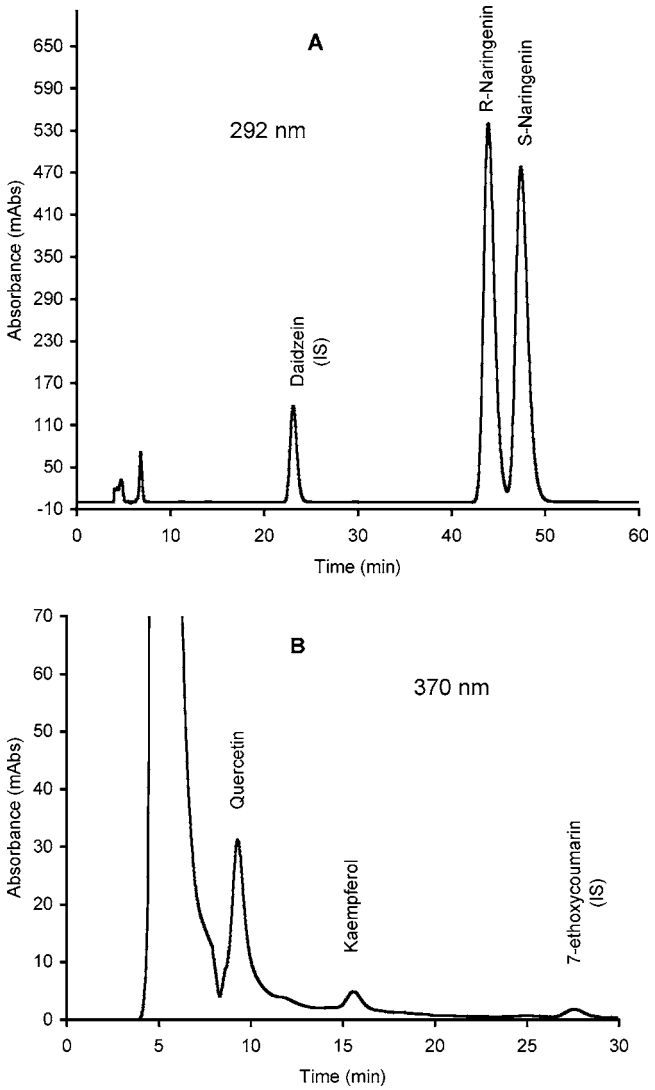

Figure 1. Typical chromatograms of a fruit tissue extract after enzymatic hydrolysis using β-glucuronidase from *H. pomatia* type H-2: (**A**) peaks for *R*-naringenin, *S*-naringenin, and diadzein (IS); (**B**) peaks quercetin, kaempferol, and 7-ethoxycoumarin peaks.

Table 2. Free *R*- and *S*-Naringenin and Total Free Naringenin in Fruit Exocarp, Mesocarp, and Seed Cavity in Different Genotypes (A) and Fruit Maturity Stages (B), and Their Interaction (A × B)

	total free *R*-naringenin (μg g^{-1} of FW)			fotal free *S*-naringenin (μg g^{-1} of FW)			total free naringenin (*R* + *S*) (μg g^{-1} of FW)		
factor	exocarp	mesocarp	seed cavity	exocarp	mesocarp	seed cavity	exocarp	mesocarp	seed cavity
genotypes (A)									
Ailsa Craig	100	11.5	10.2	360	11.2	10.3	460.0	22.8	20.5
aa	207	10.4	9.5	212	10.0	9.3	419	20.4	18.8
atv	106	9.7	9.7	107	9.6	9.6	213	19.3	19.3
hp-1	164	14.2	9.7	175	13.9	9.6	339	28.1	19.4
P value	0.237	0.003	0.466	0.248	0.006	0.201	0.243	0.004	0.3155
maturity stage (B)									
green immature	13.1	10.0	9.9	12.8	9.8	9.8	25.8	19.7	19.7
breaker	411	14.2	9.8	429	13.8	9.8	839	28.1	19.6
red	196	10.2	9.7	199	9.5	9.5	395	20.2	19.2
P value	<0.0001	<0.0001	0.826	<0.0001	<0.0001	0.212	<0.0001	<0.0001	0.5415
A × B *P* value	0.025	0.007	0.020	0.028	0.007	0.007	0.027	0.006	0.0106

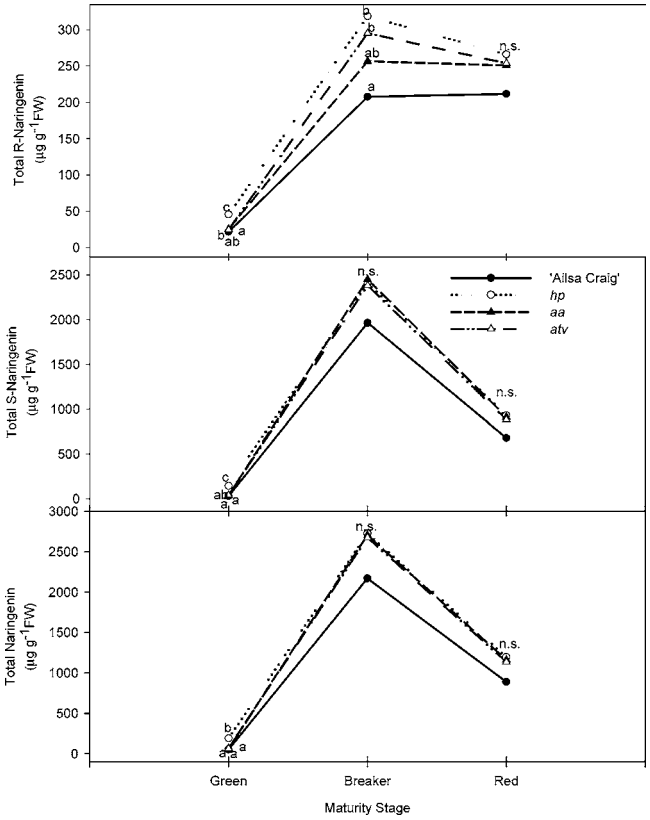

Figure 2. Genotype × maturity stage interaction for total *R*- and *S*-naringenin and total naringenin concentrations in fruit exocarp. Mean separation by protected LSD ($P < 0.05$). Different letters between genotypes at each maturity stage indicate statistical differences. n.s. = nonstatistically different.

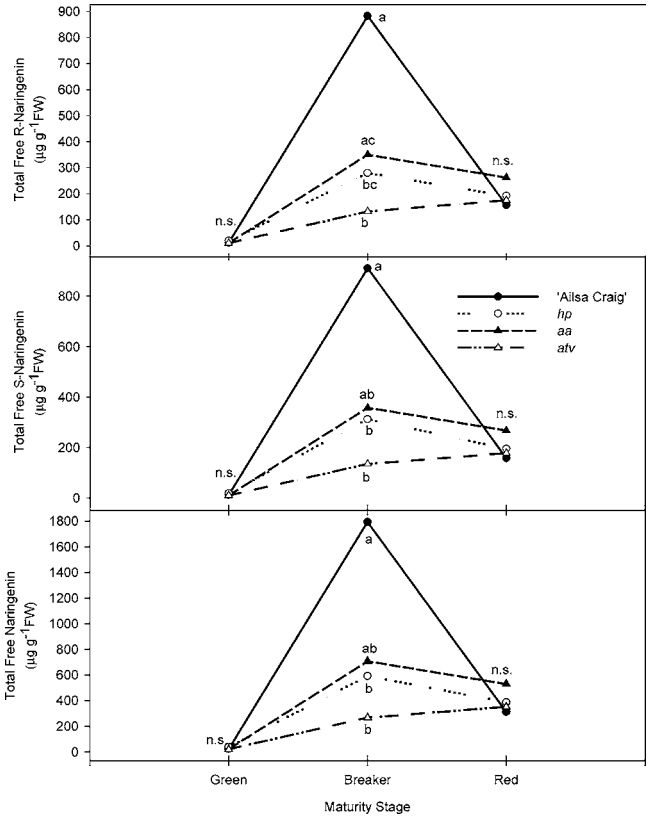

Figure 3. Genotype × maturity stage interaction for free (nonconjugated aglycone) *R*- and *S*-naringenin and total naringenin concentrations in fruit exocarp. Mean separation by protected LSD ($P < 0.05$). Different letters between genotypes at each maturity stage indicate statistical differences. n.s. = nonstatistically different.

In all tissue types, concentrations of both *R*- and *S*-enantiomers of free naringenin showed significant interactions between genotypes and maturity stages (**Table 2**). In fruit exocarp and mesocarp, genotypes were statistically different only at the breaker maturity stage (**Figures 3** and **4**). In the exocarp, Ailsa Craig showed significantly higher concentrations of *R*- and *S*-naringenin than *hp-1* and *atv* fruit (**Figure 3**), whereas in the mesocarp *hp-1* fruit exhibited the highest concentrations of naringenin enantiomers (**Figure 4**). In contrast, seed cavity tissue from green fruit of Ailsa Craig showed the highest levels of both *R*- and *S*-naringenin (**Figure 5**).

Quercetin and Kaempferol. Retention times for quercetin, kaempferol, and their internal standard (IS) were approximately 10, 16, and 27 min, respectively (**Figure 1B**).

In fruit exocarp there was an interaction between genotype and maturity stages for both quercetin and kaempferol concentrations (**Table 3**). For both flavonoids, *hp-1* fruit yielded the highest concentrations among genotypes and the highest total quercetin concentration at the breaker stage (**Figure 6**). Only in mesocarp tissue was there an interaction evident between genotype and maturity stage for quercetin concentration (**Table 3**). Immature green and breaker fruit from *hp-1* demonstrated a significantly higher concentration of quercetin than the other genotypes examined (**Figure 7**).

Overall, the concentration of kaempferol was only about 10% that of quercetin in fruit exocarp tissue and 75 and 110% of quercetin in fruit mesocarp and seed cavity tissues, respectively. These differences were also reflected in the quercetin/kaempferol

Table 3. Total Quercetin and Kaempferol and Their Ratio in Fruit Exocarp, Mesocarp, and Seed Cavity in Different Genotypes (A) and Fruit Maturity Stages (B) and Their Interaction (A × B)

	total quercetin (μg g^{-1} of FW)			total kaempferol (μg g^{-1} of FW)			quercetin/kaempferol		
factor	exocarp	mesocarp	seed cavity	exocarp	mesocarp	seed cavity	exocarp	mesocarp	seed cavity
genotype (A)									
Ailsa Craig	1118	65.4	61.7	144	61.4	67.5 ab[a]	7.7 a	1.1 a	0.91
aa	1137	81.8	57.8	142	64.2	66.8 a	7.6 a	1.3 a	0.87
atv	1110	63.4	56.6	131	61.7	70.6 ab	8.1 a	1.0 a	0.80
hp-1	2254	116	70.2	156	66.7	78.1 b	14.2 b	1.7 b	0.90
P value	0.001	0.001	0.153	0.419	0.341	0.035	<0.0001	0.003	0.1317
maturity stage (B)									
green immature	1311	88.1	65.2	142	67.0	64.7 a	8.3 a	1.3 b	1.00 b
breaker	1337	89.8	59.5	131	61.8	74.1 b	9.9 b	1.5 b	0.80 a
red	1597	67.4	60.0	159	61.7	73.4 b	10.1 b	1.1 a	0.81 a
P value	0.163	0.001	0.870	0.006	0.104	0.004	0.043	0.001	0.0150
A × B *P* value	0.007	0.027	0.866	0.014	0.318	0.302	0.113	0.280	0.6176

[a] Different letters indicate statistical differences. Mean separation by protected LSD (<0.05).

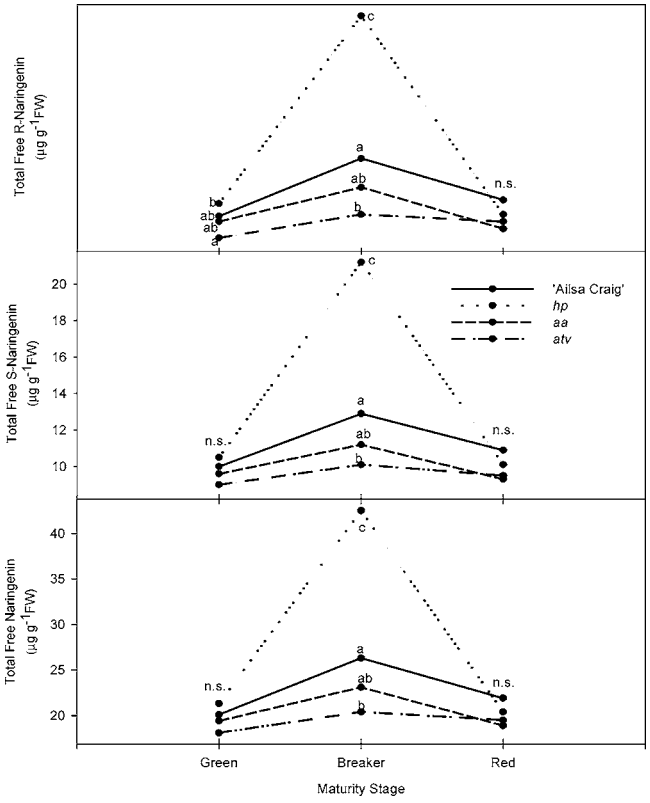

Figure 4. Genotype × maturity stage interaction for free (nonconjugated aglycone) *R*- and *S*-naringenin and total naringenin concentrations in fruit mesocarp. Mean separation by protected LSD ($P < 0.05$). Different letters between genotypes at each maturity stage indicate statistical differences. n.s. = nonstatistically different.

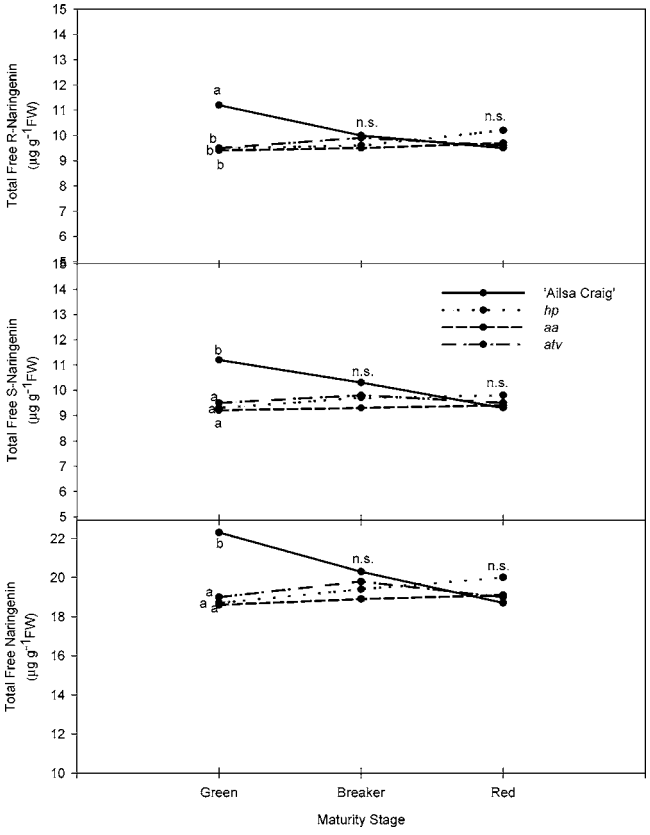

Figure 5. Genotype × maturity stage interaction for free (nonconjugated aglycone) *R*- and *S*-naringenin and total naringenin concentrations in fruit seed cavity. Mean separation by protected LSD ($P < 0.05$). Different letters between genotypes at each maturity stage indicate statistical differences. n.s. = nonstatistically different.

(Q/K) ratio, which was over 7.5 in fruit exocarp but ~1.0 in mesocarp and seed cavity tissues (**Table 3**). The Q/K ratio also differed among genotypes and maturity stages. For instance, in fruit exocarp of *hp-1*, the Q/K ratio was almost twice those in the other genotypes, due to the elevated quercetin concentration in *hp-1* exocarp (**Figure 6**). A high Q/K ratio was also apparent but less pronounced in fruit mesocarp. Only in the exocarp was there an increase in the Q/K ratio as fruit maturity progressed (**Table 3**).

DISCUSSION

In the present work we report for the first time the naringenin enantiomer concentrations in different tomato fruit tissues during development, using a stereospecific HPLC method validated for biological fluids by Yañez and Davies (*21*). The rapid, sensitive detection of the selected flavonoids obtained by these HPLC methods was enhanced by enzymatic hydrolysis to detect total aglycones. Furthermore, enzymatic hydrolysis resulted in >90% improved efficiency in flavonoid detection than acid hydrolysis on the basis of our preliminary analysis (data not shown). Acid hydrolysis is the most commonly used technique for flavonoid determination in fruits and vegetables (*9*, *22*, *24*, *26*−*29*), and many of these reported concentrations are significantly lower than those presented in this study (**Table 4**). One explanation for this discrepancy is that β-glucuronidase is more specific and

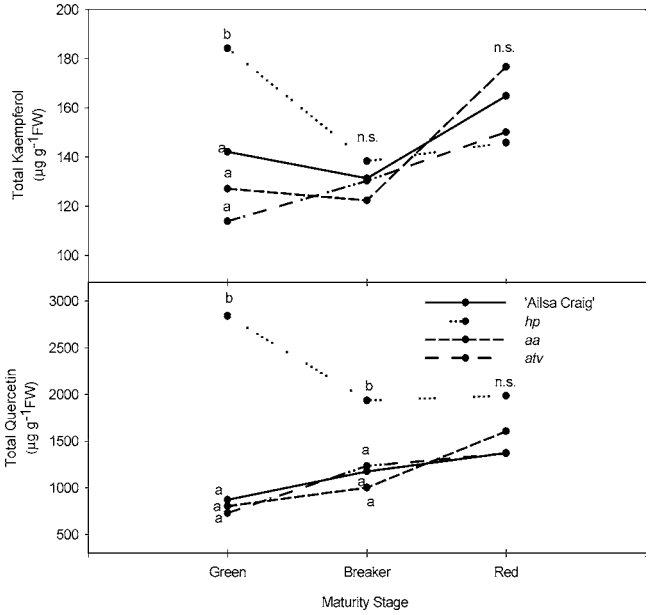

Figure 6. Genotype × maturity stage interaction for total kaempferol and quercetin concentrations in fruit exocarp. Mean separation by protected LSD ($P < 0.05$). Different letters between genotypes at each maturity stage indicate statistical differences. n.s. = nonstatistically different.

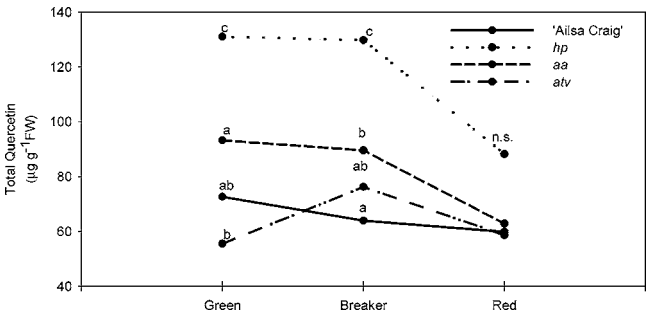

Figure 7. Genotype × maturity stage interaction for total quercetin concentration in fruit mesocarp. Mean separation by protected LSD ($P < 0.05$). Different letters between genotypes at each maturity stage indicate statistical differences. n.s. = nonstatistically different.

efficient in deglycosylation, and this allows it to free a larger number of aglycones (flavonoids) from their sugar moieties than acid hydrolysis and/or elevated temperatures. This critical hydrolysis step is key to determining the total amount of flavonoids, both glycosylated and aglycones, to effectively quantify specific flavonoid concentrations in fruits and vegetables. Although we consistently detected higher concentrations of flavonoids than reported in other studies, differences among genotypes, sampling procedures, and storage conditions could also account for some of the variation in flavonoid concentrations among studies.

In contrast to Muir et al. (*29*), who did not detect any naringenin aglycones in tomato skin, we found that 30% of total naringenin was present as aglycone in fruit exocarp. Moreover, we were also able to detect naringenin aglycones in fruit flesh. Furthermore, Paganga et al. (*15*) and Wardale (*18*) reported that only the aglycone naringenin was present in tomato fruit.

As in citrus juice (*21*), *S*-naringin was found to be the predominant glycosylated enantiomer in all tissue types. *S*-Naringenin accounted for 85% of total naringenin in fruit exocarp and for ~60% of total naringenin in fruit mesocarp and seed cavity tissues. This information could be important for bioavailability studies, because chirality may have a significant influence on physiology and pharmacological action and disposition (*30*). In the case of naringenin, our preliminary studies have shown that *S*-naringenin has a longer biological half-life than *R*-naringenin (data not shown).

Table 4. Concentration Ranges or Averages for Total Quercetin (Q), Kaempferol (K), or Naringenin (N) in Tomato (*L. esculentum* Mill.) Fruit Tissue after Acid Hydrolysis Reported by Different Authors

fruit material red-ripe tomato	concn range or av (μg g^{-1} of FW)	authors
skin[a]	Q = 136–138 K = 4.5–4.7	Stewart at al. (*9*)
flesh[a]	Q = 0–0.9 K = 0.3–0.4	
seed[a]	Q = 1.1–1.2 K = 0.4	
whole[b]	Q = 7.2–43.6 K = 0–2.1 N = 4.5–12.6	Martinez-Valverde et al. (*26*)
whole[b]	Q = 15 N = 15	Justesen et al. (*24*)
whole[c]	Q = 5–9 K = 2–3 N = 25–35	Bovy et al. (*27*)

[a] Cultivar Paloma. [b] From local supermarket(s). [c] Line FM6.

Quercetin and naringenin have been reported to be the major flavonoids in tomato fruit (*11*, *14*, *15*, *24*). In our study we found that this applied only for fruit exocarp, which had approximately 50% naringenin, 45% quercetin, and 5% kaempferol. However, in mesocarp and seed cavity tissues, naringenin, quercetin, and kaempferol were all present in approximately equal amounts.

Kaempferol is often not detected in samples of whole tomato fruit (*15, 26*), yet we were able to detect it in both mesocarp and seed cavity tissues. Just as for naringenin and quercetin, exocarp tissue contained the highest concentration of kaempferol, twice that detected in mesocarp and seed cavity tissues. The concentration of kaempferol in the exocarp was at least 7 times higher than the concentration in red-ripe tomato skin reported by Steward et al. (*9*).

Interestingly, the kaempferol concentration difference between the fruit exocarp and mesocarp did not appear to decline as significantly as did the quercetin concentrations between these two tissues. Whereas kaempferol concentrations decreased by 53% between the exocarp and mesocarp, quercetin concentrations decreased by 93%. Because the accumulation of quercetin glycosides in fruit exocarp plays an important role in UV-B protection (*32*), plants may avoid expenditure of additional energy by not synthesizing excess quercetin in unexposed mesocarp tissue.

In our study there was not a common trend for quercetin, kaempferol, and naringenin concentrations with fruit maturity among genotypes. In agreement with Muir et al. (*29*), quercetin levels in fruit exocarp (peel) increased with maturity, although this was not true for the *hp-1* mutant. In contrast, naringenin enantiomer concentrations were highest at the breaker stage, declining in red-ripe fruit, a pattern similar to that reported by Muir et al. (*29*) and Le Gall et al. (*11*) for naringenin chalcone, a precursor of naringenin in the biosynthetic pathway. These results indicate that the *hp-1* mutant has higher concentrations of flavonoids during the red-ripe stage compared to the immature green stage. Tomatoes are generally consumed when they are red-ripe, and thus, this finding might have a nutritional impact for human health. Even though flavonoids are important health promoters and contribute to the nutritional value of foods, there

are other phytochemical components that are as important as flavonoids, such as vitamins and carotenoids (lycopene). Thus, more comprehensive examinations of other phytochemicals in the *hp-1* mutant are warranted.

Differences in flavonoid concentrations among tomato genotypes have been reported in the literature (*9*, *14*, *26*). Therefore, we also expected to find genotype differences in flavonoid concentrations. All mutants had either similar or higher concentrations of total quercetin, kaempferol, and naringenin than their parent, Ailsa Craig. Because *aa* apparently does not synthesize anthocyanin in any plant part (*33*), its mutation is likely located at the last step of anthocyanin biosynthesis, that is, downstream from naringenin and dihydrokaempferol precursors. In contrast, the *atv* mutant, described as having enhanced anthocyanins in all plant parts (*33*), did not have higher flavonoid concentrations than the other genotypes tested. On the other hand, the *hp-1* mutant has been widely studied due to its increased photoresponsiveness and its higher concentrations of chlorophyll, carotenoids, phenolics, anthocyanins (*14*, *34*−*37*), and flavonoids, especially quercetin as determined in this study. Furthermore, the higher flavonoid concentrations of *hp-1* would partially explain its increased tolerance to photo-oxidative stress under natural environmental conditions (*38*). Moreover, Stewart et al. (*9*) analyzed a number of genotypes of different fruit colors (red, purple, yellow) and concluded that flavonol content (quercetin and kaempferol) did not necessarily correlate with accumulation of anthocyanins in these genotypes.

Another interesting observation was the lack of statistical differences among leaf samples of the tomato genotypes for quercetin, kaempferol, or naringenin concentrations. The concentrations of these flavonoids were on average 1360 μg g^{-1}of fresh weight (FW) (±231.2) for quercetin, 97.4 μg g^{-1} of FW (±10.1) for *R*-naringenin, 95.3 μg g^{-1} of FW (±13.1) for *S*-naringenin, and 330 μg g^{-1} of FW (±19.1) for kaempferol.

In conclusion, enzymatic hydrolysis dramatically increased the apparent total flavonoid quantification of tomato fruit tissues. This is the first reported stereospecific detection of naringenin and naringin enantiomers in tomato tissues. In the future, additional research needs to be undertaken to elucidate the physiological and pharmacological significance of naringenin enantiomers and the pharmacokinetic disposition and stereospecificity of their actions. Finally, the *hp-1* gene was once again determined to be an interesting molecular insertion, which could be incorporated into breeding programs for enhancing tomato fruit flavonoid concentrations.

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