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Copigmentation Triggers the Development of Skin Burning Disorder on Peach and Nectarine Fruit [*Prunus persica* (L.) Batsch]

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ABSTRACT: Skin burning is a new type of skin damage related to exposure to high pH values during the brushing−waxing postharvest operations that has been observed recently on some newly released peach and nectarine [*Prunus persica* (L.) Batsch] cultivars. In this work, we described this skin disorder for the first time and studied its triggers and biological basis. Different skin burning susceptibility was observed after screening 21 peach and nectarine cultivars. The stability of the skin phenolic extracts to pH in the range 7−10 was studied by UV−visible spectroscopy. This study demonstrated that fruit skin phenolics are not stable at high pH and that the transformations occurring at high pH are reversible and time-dependent. The changes on the UV−visible absorption spectra at different pH values pointed out the copigmentation of anthocyanins as the mechanism beyond the skin burning disorder. Finally, some recommendations to minimize this postharvest damage are also discussed.

KEYWORDS: Absorption spectra, antioxidant potential, HPLC, pH, total phenolics

INTRODUCTION

Recently, a high level of a new type of skin damage has been observed on new releases of well-colored peaches and nectarines. This is the first report on this darkening of the fruit skin affecting peaches and nectarines, and we have named it "skin burning". It is a cosmetic disorder since it affects the appearance of the fruit, whereas the tissue underneath remains undamaged. One unique characteristic of this type of skin damage, contrary to other previously described skin disorders such as field inking,[1,2] is that the incidence increases after fruit packing and handling. Our findings indicate that exposure to high pH water during washing−brushing operations could induce the development of this cosmetic disorder on the fruit when combined with handling physical damage or abrasion.

Skin color disorders in the fruit can emanate from the transformation of the molecular structure of anthocyanins, abundant in the peach skin cells.[3,4] The absorption spectra and extinction coefficients of phenols are influenced by the nature of the solvent, electron-withdrawing and electron-donating substituents in the benzene ring(s), intra- and intermolecular hydrogen bonding, steric effects, and the pH-dependent formation of resonance forms with altered conjugation as compared to the parent compounds.[3,5] Moreover, anthocyanins can interact with other colorless organic compounds to form molecular or complex associations (copigmentation), generating an increment in the color intensity.[6] This anthocyanin−copigment interaction produces a hyperchromic (intensity) and a bathochromic (position) shift in the absorption spectra (UV−visible), which can be easily seen by absorption spectroscopy.[7,8] This copigmentation has already been reported as the main mechanism of stabilization of colors in flowers[9,10] and is responsible for the color changes in wine aging.[8,11]

The aim of this work was to understand the triggers and the biological basis of the skin burning disorder. As a first step, we characterized the skin burning disorder that affects the appearance of peach and nectarine fruit. We screened 21 different cultivars of peach and nectarine for susceptibility to skin burning and determined the phenolic composition and content of skin extracts. We also studied the stability of the phenolic skin extracts from those peach and nectarine cultivars in contact to buffers in the pH range from 7 to 10. The effect of contact time with high pH and the reversibility of the damage were also investigated. Finally, we tested the hypothesis of phenolic copigmentation as the biological mechanism that leads to this skin disorder. The results of this study will enable us to develop practical strategies to control the incidence of this fruit skin disorder. The remarkable economical impact of this cosmetic problem for the fruit industry worldwide encourages further detailed research to fully understand its causes and its biological basis.

MATERIALS AND METHODS

Plant Material. Peaches and nectarines from 21 different cultivars were collected from different sources to eliminate source as a variable to influence skin burning susceptibility. Peach and nectarine cultivars, white and yellow fleshed, and low and high acidity were used in this test. Cultivars were selected based on our previous preliminary work[2,12−14] and feedback from the Californian peach and nectarine industry. Three repetitions of approximately 100 g of skin tissue of each cultivar−source combination were frozen immediately in liquid nitrogen, freeze-dried, and stored at −80 °C until the phenolics biochemical determinations were carried out.

Effect of Contact with Different pH Solutions on the Fruit. To study the effect of physical damage or abrasion that occurred on the fruit during harvesting and postharvest operations on the development of skin burning disorder, pH treatments were applied to abraded and nonabraded fruit. Solutions of 0.1 M Tris were used to make buffers at pH 7, 7.5, 8, 8.5, 9, 9.5, and 10. They were adjusted to the desired pH by adding HCl. All solutions were prepared daily.

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Fruit was abraded with a rotary automatic toothbrush connected to a fixed axis. During abrasion, the fruit was hand-rotated forward and backward, while the automatic toothbrush head abraded the skin.[12] This abrasion only damaged epidermal cells, as observed under light and scanning electron microscopy.[14] The hand-rotation speed was kept as consistent as possible for all fruit samples. The effect of abrasion intensity on the skin burning development was also tested in one of the experiments, abrading the fruit at different intensities (from 0 to 5 times) as described by Cheng and Crisosto.[12]

After abrasion, fruit were incubated for 15 min in the different pH solutions. Then, the fruit were removed from the solutions and allowed to air dry for about 15 min. The effect of contact time with the solution on the development of skin burning was tested in one of the experiments, leaving the fruit immersed in the different pH solutions for 1, 15, 30, and 45 min. The control treatment was dry fruit, in contact with no solution (air). Ten fruits were used for each treatment.

For each subtreatment (abraded and nonabraded fruit), six skin disks of 15 mm diameter were randomly marked with a silver marker on the skin surface of each fruit to measure the color change after the contact with the solution (Figure 1). Each disk was identified by the layout order in the following experiments. Before and after treatment, color on the skin disks was measured with a Minolta Colorimeter CR 200 in the $L^*a^*b^*$ color notation system (C illuminant, calibrated with standard white plate, and 0° viewing angle). The change on the skin color was expressed by the relative change in the color value L^* (lightness), which reflects the darkening of the fruit skin. The change in L^* was calculated as the difference between L^* before treatment and L^* after incubation. The higher L^* difference revealed a darker color change.

On the basis of the results of the skin burning disorder developed on the fruit skin on the previous experiments, cultivars were assigned to different categories of skin burning disorder susceptibility: 1, very low/non-susceptible; 2, susceptible; and 3, very susceptible.

Phytochemical Analysis. The finely ground frozen skin tissue (0.5 g) was extracted with 80% methanol (v/v). The mixture was incubated overnight at 4 °C and then centrifuged for 10 min at 4 °C and 17000*g*. The supernatant was recovered, and the volume was measured. This hydroalcoholic skin extract was used for HPLC analyses, total phenolics content (TPC), antioxidant capacity (AOC), and absorption spectrum assays.

Samples of 20 μL of extracts were analyzed using a HPLC system coupled with a photodiode array detector (DAD) and an autosampler (Agilent 1200 Series), operated by Agilent Chemstation Software. The mobile phases used for gradient elution were 0.1% formic acid (A) and acetonitrile (B). The following gradient elution program was carried out: 0−5 min, 95% A; 5−25 min, 95−85% A; 25−32 min, 85−75% A; and 32−35 min, 75−70% A. The flow rate was kept at 1 mL min^{-1}. Data were collected at 254, 280, 340, 360, and 510 nm. The phenolic compounds in the skin fruit extracts were identified by their UV spectra, by chromatographic comparisons with authentic markers. Quantification was made by comparisons with external standards at their maximum absorbance wavelengths: 510 (anthocyanins), 340 (flavonols and hydroxycinnamic acid derivatives), and 280 nm (flavan 3-ols). The data of each measurement are the average of three replicates.

The content of total phenolic compounds in methanol extracts was determined based on the Folin−Ciocalteu method.[15] The method consisted of mixing 100 μL of the extract with 750 μL of Folin−Ciocalteu's reagent diluted in water (1:14 v/v). After 3 min of reaction, 150 μL of 1 N sodium carbonate (Na_2CO_3) was added. The tubes were mixed for 15 s and then allowed to stand for 120 min at room temperature. The absorbance was measured at 765 nm using a spectrophotometer (Beckman Coulter DU 800). The standard calibration curves were daily prepared using gallic acid (3,4,5-trihydroxy-benzoic acid). For each sample, three separate determinations were carried out. The phenolic content was expressed in micrograms of gallic acid equivalents (GAE) per gram of fresh weight (FW).

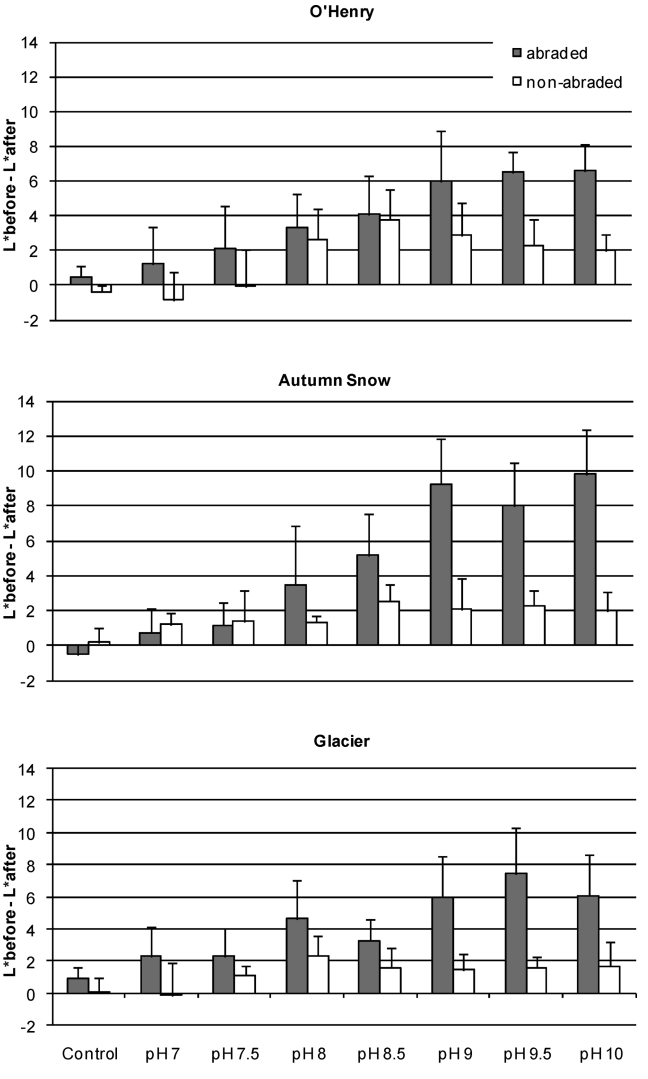

Figure 1. Increase in darkness (decrease of lightness, L^*) on the prelabeled fruit skin surface of O'Henry, Autumn Snow, and Glacier cultivars, after exposing the fruit to different pH solutions (0.1 M) for 15 min. Air was used as a control in this experiment. Data are means of 10 replications. Vertical bars represent SDs.

The AOC was measured using the 1,1-diphenyl-2-picrylhydrazyl (DPPH) method adapted from Brand-Williams et al.[16] Briefly, 50 μL of the methanolic extract was added to 950 μL of fresh DPPH radical solution (0.1 mM in methanol), mixed in the dark by vortex at room temperature, and incubated overnight in the dark. The absorbance of the samples was measured at 515 nm and subtracted from the absorbance of the DPPH radical solution. For each sample, three separate determinations were carried out. The standard calibration curves were prepared daily using Trolox (6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid). Results were expressed in micrograms of Trolox equivalent antioxidant capacity (TEAC) per g FW.

Stability of Phenolic Skin Extracts at Different pH Values. Tris base was used to prepare 0.5 M buffers at pH values 7, 8, 9, and 10. They were adjusted to the desired pH by adding HCl (0.25 and 2 N) or NaOH (0.25 and 1 N). The phenolic skin extracts were then diluted (1:9 v/v) with H_2O and one of the buffers to a final concentration of 0.1 M.

The UV and visible spectra were recorded on a Beckman Coulter TM DU 640 UV/vis scanning spectrophotometer (Brea, CA) with a wavelength accuracy of 0.1 nm. UV spectra were measured at wavelengths

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ranging from 200 to 700 nm in 1 nm increments immediately after final dilutions were made. Readings of the skin extracts at pH values 7, 8, 9, and 10 were made against buffers. By comparing variations of spectrum shapes, absorption peaks, and maximum absorbances, the effects of different pH solutions on skin extract phenolics of different peach and nectarine cultivars were determined. To study the effect of different pH values throughout time, measurements were made immediately after the dilution with the pH 9 solution and after 1, 12, and 24 h. The solutions and the buffers were stored in the refrigerator (4 °C) between determinations.

To establish whether the spectral changes observed after mixing with high pH solutions were reversible or irreversible, the skin extracts incubated in pH 10 for 2 h were neutralized to neutral pH (pH 7) by using 0.25 and 1 N HCl. The UV−visible absorbance was measured immediately after the dilution of the skin extracts with pH 10, after 2 h of incubation, and immediately after the neutralization to pH 7. In each experiment, two replicates per cultivar were used for the UV spectra measurements.

Statistical Analysis. All statistical analyses were performed using SPSS 17.0 for Windows (SPSS, Chicago, IL). To obtain basic statistics for the entire plant materials studied, the number of observed samples, maximum, minimum, and mean values, and standard deviation for each trait were recorded. Duncan's multiple-range test ($P \leq 0.01$) was used to estimate cultivar means and to find differences in the phytochemical profile among them. A t test ($P \leq 0.01$) was run to compare pairs of fruit types (peach or nectarine, yellow or white flesh). The difference in the skin phenolics profile among different categories of skin burning susceptibility cultivars was analyzed by drawing boxplots. Finally, correlations between traits to reveal possible relationships were calculated from raw data using the Pearson correlation coefficient at $P \leq 0.01$.

RESULTS AND DISCUSSION

Skin Burning Disorder Development on the Fruit Due to High pH Solutions. After the washing−waxing operations during the packing process, some abraded fruit skin areas turn dark, possibly as a consequence of temporary increases in the tissue pH. As conditions of high pH often exist in the sanitation water during postharvest handling, this may be an important factor in skin burning. Usually, water pH during washing and hydrocooling varies from 7.5 to 8.0. However, the postharvest use of sodium hypochlorite and fruit coatings has the potential to increase the pH, reaching values around pH 9.0. After testing this hypothesis in the lab by submerging the abraded and nonabraded fruit into different pH solutions, our results showed that contact of fruit skin with high pH solutions (over 8.0−8.5) induced skin darkening (skin burning) on different cultivars (Figures 1 and 2). Extremely dark skin color change was observed after the fruit was exposed to pH 9.0, 9.5, and 10.0 solutions in susceptible cultivars as O'Henry, Autumn Snow, and Glacier (Figure 1).

It is noteworthy that the skin burning incidence was high on the abraded fruit, whereas low or no significant damage was observed on the non-abraded fruit, even after exposure to the highest pH solutions. This result confirms our hypothesis that the skin burning disorder is triggered by the combination of abrasion and subsequent exposure to high pH. Previous anatomical studies using light microscopy and scanning electron microscopy have demonstrated that low physical damage (abrasion) was the primary physical injury associated with skin inking development.[14] Phenolic compounds are secondary plant metabolites found in all fruits and vegetables,[17,18] mainly concentrated in the epidermal and subepidermal layers of the fruit.[19,20] Abrasion damage releases anthocyanin/phenolic pigments from the epidermal and subepidermal cells, allowing the reaction of these pigments with different external contaminants causing skin color change on the fruit.[2,13]

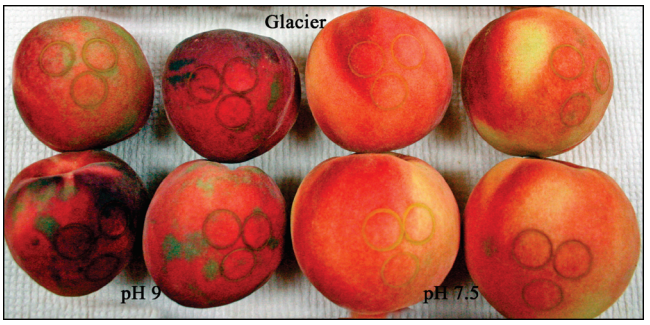

Figure 2. Glacier peach (subjected to abrasion) after 15 min in contact with pH 9, 0.1 M (left), and pH 7.5, 0.1 M (right), solutions. Skin disks of 15 mm diameter were randomly marked on the skin surface of each fruit to measure the color change after the contact with the different pH solutions.

Our results also indicated different levels of susceptibility to skin burning development among the 21 different peach and nectarine cultivars studied. While some cultivars did not have any damage after exposure to very high pH values (8.5 or 9.0), some others started to show some skin darkening at pH 7.5. According to those results, we classified the 21 tested cultivars depending on their susceptibility to develop skin disorder due to high pH (Table 1). August Pearl, Honey Blaze, and Honey Fire were within the group of very susceptible cultivars, whereas Summer Bright, Grand Sweet, and Sweet Dream, among others, showed very low or no susceptibility to skin burning. As can be observed in Table 1, this susceptibility does not seem to be related to the breeding program source.

Once we demonstrated that abrasion was a critical factor on the incidence of skin burning, we studied the importance of abrasion intensity by abrading the fruit from 0 to 5 times. Fruit skin darkening developed after the combination of skin abrasion with exposure to high pH (9.0) in susceptible cultivars. Non-abraded fruit showed none or much less skin burning than abraded fruit for all of the treatments and cultivars tested, as previously described. However, no consistent differences were found among different intensities of abrasion (data not shown). Once the fruit skin is damaged by abrasion, the collapse of the skin cells leads to the release of phenolic pigments that will trigger dark discoloration when exposed to high pH. Therefore, once the skin cells were already damaged, phenolic pigments were ready to react with even the lowest abrasion intensities.

The effect of contact time with a high pH solution on the development of skin burning was also assayed (Figure 3). The skin burning intensity increased with time of exposure with a high pH solution (pH 9.0) for the different cultivars tested. Skin darkening increased (decrease in L^*) progressively with contact time with pH 9 solutions for Snow King and Snow Princess white-fleshed peach cultivars (Figure 3). Darkening of the skin was already observed in Snow King and Snow Princess after 15 min of contact with the pH 9 solution. However, no skin darkening was observed for the Sweet Dream cultivar, even after 45 min of incubation with a pH 9 solution. These results confirm the differences on skin burning susceptibility among cultivars and show the importance of contact time with the high pH solution during the postharvest washing−brushing operation on the skin burning intensity on susceptible cultivars.

Table 1. Classification of 21 Different Peach and Nectarine Cultivars According to Their Susceptibility To Develop Skin Burning Disorder in Response to High pH Solutions[a]

fruit	flesh color	skin burning susceptibility	plant breeding program source
peach	yellow	susceptible	Peters
nectarine	white	very susceptible	Bradford
peach	white	very susceptible	Zaiger
nectarine	white	susceptible	Bradford
nectarine	yellow	susceptible	Bradford
peach	yellow	susceptible	Lewis
nectarine	white	susceptible	Bradford
nectarine	yellow	very susceptible	Bradford
peach	white	susceptible	Zaiger
nectarine	yellow	low/not susceptible	Bradford
nectarine	yellow	very susceptible	Zaiger
nectarine	yellow	very susceptible	Zaiger
peach	yellow	low/not susceptible	Zaiger
peach	yellow	susceptible	The Burchell Nursery
peach	yellow	susceptible	Zaiger

[a] The plant breeding program from where the cultivar was obtained is shown.

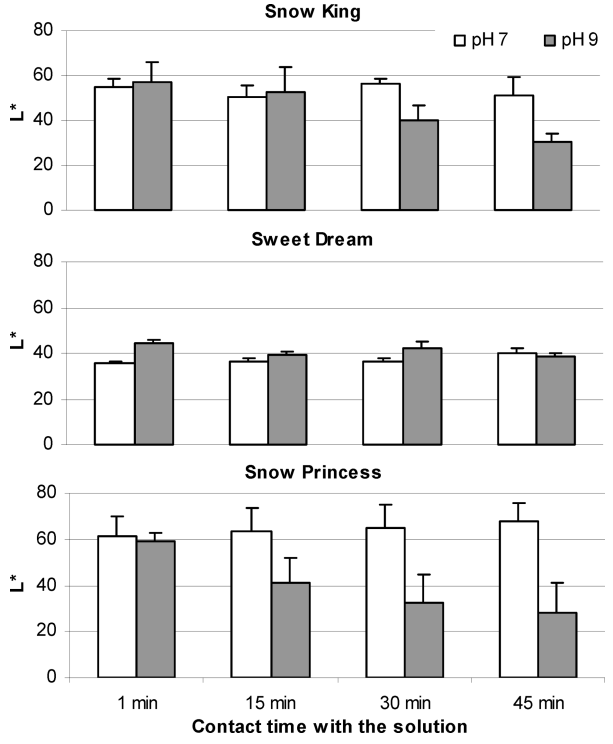

Figure 3. Effect of contact time with pH 7 and pH 9 solutions (0.1 M) on fruit skin darkening of Snow King (skin burning very susceptible), Sweet Dream (skin burning low/non-susceptible), and Snow Princess (skin burning susceptible) cultivars. Fruit was left in contact with the solutions for 1, 15, 30, and 45 min. Data are means of 10 replications. Vertical bars represent SDs.

Influence of Phenolics Profile on the Skin Burning Susceptibility. After the results obtained in the previous experiments showing the different skin burning susceptibilities among the 21 peach and nectarine cultivars, we studied their skin phenolic composition to look for any correlation between the phenolic profile of the fruit skin and its susceptibility to skin burning. Previous studies have shown that phenolic compounds are involved in the development of similar types of postharvest skin disorders.[12−14] It has also been reported that phenolic compounds play an important role in other postharvest disorders such as chilling injury in chilling-sensitive commodities [21] or "scald" damage observed in apples.[22]

Table 2. Basic Statistics for Skin Burning Susceptibility and Phenolics Profile for the 21 Peach and Nectarine Cultivars Studied[a]

	N	minimum	maximum	mean	SD
skin burning susceptibility[b]	63	1.0	3.0	2.1	0.5
CA (μg/g FW)	63	17.9	466.4	162.5	101.2
NCA (μg/g FW)	63	4.0	106.3	34.9	23.4
C3G (μg/g FW)	63	38.1	724.6	207.1	173.5
Q3R (μg/g FW)	63	11.3	355.6	118.0	85.5
Q3Glu (μg/g FW)	63	9.8	253.0	85.5	61.6
Q3Gal (μg/g FW)	63	7.6	58.8	27.9	12.0
catechin (μg/g FW)	63	4.4	72.1	31.9	17.0
CA/C3G molar ratio	63	0.2	4.1	1.4	1.0
TPC (μg GAE/g FW)	63	690.9	2875.8	1563.4	518.8
AOC (μg TEAC/g FW)	63	861.3	7248.8	3469.9	1502.0
BP (ΔA420/h)	63	0.1	1.2	0.5	0.3

[a] For each trait, the number of samples analyzed (*N*), minimum, maximum, and mean values, and standard deviation (SD) are presented. [b] Skin burning susceptibility scored on a categorical scale of 1 (non-susceptible), 2 (susceptible), and 3 (very susceptible).

Values of TPC, AOC, and browning potential (BP) found among the samples are within the range reported for peach skin in the literature (Table 2).[23−25] Lower values of TPC are reported when only flesh is included in the sample,[24,26] due to the unequal distribution of phenolic compounds in the flesh (~30%) and the skin (~70%) of the peach fruit.[19,20] Regarding hydroxycinnamates, in all cases, the amount of chlorogenic acid (CA) was higher than that of neochlorogenic acid (NCA) (averaged 162.5 and 34.9 μg/g FW, respectively), as previously reported for other peach and nectarine cultivars.[24] Cyanidin-3-glucoside (C3G) was identified as the main anthocyanin pigment

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Table 3. Skin Phenolics Profile, TPC, AOC, and BP of 21 Different Peach and Nectarine Cultivars[a]

		μg/g FW									
cultivar	fruit type[b] (P/N)	CA	NCA	C3G	Q3R	Q3Glu	Q3Gal	catechin	TPC (μg GAE/g FW)	AOC (μg TEAC/g FW)	BP (ΔA420/h)
August Lady	P	172.3 d	28.4 ef	173.4 h	102.1 gh	68.9 ef	27.5 fg	49.4 c	2051.5 bcd	4829.3 bc	0.60 def
August Pearl	N	445.4 a	102.8 a	238.5 f	239.2 bc	192.0 b	41.5 c	68.1 a	2754.5 a	6977.8 a	1.01 ab
Autumn Snow	P	121.9 e	23.3 efg	30.0 klm	26.7 k	20.4 h	17.3 k	20.3 fg	1096.0 kl	2538.9 hij	0.65 de
Bright Pearl	N	254.4 c	49.9 c	129.3 ij	162.2 e	146.3 c	23.5 hij	40.0 d	1656.6 fgh	3942.2 def	0.61 def
Diamond Bright	N	155.1 de	30.6 fg	367.2 c	251.7 b	84.5 e	33.5 de	10.7 hi	1211.1 jkl	2016.3 ijk	0.28 gh
Early Elegant Lady	P	134.2 e	25.1 efg	109.0 ijk	80.2 hi	51.7 fg	18.9 jk	30.2 e	1438.4 hij	3164.7 fgh	0.33 g
Fire Pearl	N	178.6 d	46.6 c	131.1 i	133.9 f	125.7 d	26.3 fg	24.5 ef	1308.1 ijk	2664.7 ghi	0.49 f
Fire Sweet	N	172.6 d	53.6 c	58.8 lm	60.1 ij	48.3 g	9.3 m	29.2 e	1350.5 ijk	3406.7 fg	0.89 bc
Glacier	P	30.2 gh	5.1 i	85.1 jklm	29.1 k	26.4 h	22.4 hij	8.9 i	776.8 m	1296.9 kl	0.31 g
Grand Sweet	N	184.7 d	49.1 c	286.8 de	216.8 cd	161.9 c	34.4 d	39.9 d	1750.5 efg	3758.3 ef	0.65 de
Honey Blaze	N	355.5 b	75.5 b	695.6 a	305.9 a	220.1 a	52.3 a	28.8 e	2175.8 bc	4468.0 cde	0.11 i
Honey Fire	N	284.5 c	50.6 c	634.1 b	204.9 d	193.4 b	45.1 bc	25.7 ef	1875.8 def	3855.1 ef	0.29 gh
Kaweah	P	155.2 de	20.1 fgh	191.1 gh	65.8 ij	36.0 gh	19.8 ijk	41.3 d	1904.0 cdef	4739.0 bc	0.56 ef
O'Henry	P	166.9 d	41.2 de	131.5 fg	79.5 hi	55.1 fg	30.1 def	60.1 b	2271.7 b	5442.3 b	0.82 c
Rich Lady	P	57.0 fg	14.5 h	252.0 ef	106.4 gh	75.2 e	48.1 ab	20.1 fg	1433.3 hij	2555.0 hij	0.13 i
Snow King	P	129.4 e	11.9 hi	75.7 klm	41.9 jk	48.1 g	24.3 g	40.3 d	1574.7 ghi	3780.9 ef	0.76 cd
Snow Princess	P	89.2 f	23.9 efg	39.6 m	30.5 k	38.6 gh	15.4 kl	50.5 c	1738.4 efg	4858.4 bc	1.11 a
Spring Bright	N	125.7 e	36.7 ef	257.1 ef	215.9 cd	83.1 e	41.9 c	11.7 hi	1098.0 kl	1703.3 k	0.13 i
Summer Bright	N	48.9 gh	47.5 c	78.2 klm	124.1 fg	118.4 d	25.5 fg	8.1 i	732.3 m	929.1 l	0.18 ghi
Summer Lady	P	127.7 e	18.0 gh	308.6 d	52.5 ijk	28.7 h	16.6 k	51.1 c	1987.9 cde	4655.1 cd	0.32 g
Sweet Dream	P	18.3 h	9.7 i	88.6 ijkl	27.7 k	19.8 h	11.2 lm	16.3	952.5 lm	1884.0 jk	0.14 hi

[a] Data are means of three replicates. In each column, means with the same letter are not significantly different according to Duncan's test ($P \leq 0.01$).
[b] Fruit type: P, peach; N, nectarine.

present in the skin of peaches and nectarines, with a range of 38−725 μg/g FW. Three different flavonols were identified and quantified in the samples: quercetin-3-rutinoside (Q3R), quercetin-3-glucoside (Q3Glu), and quercetin-3-galactoside (Q3Gal). A large variation was found for Q3R and Q3Glu among the samples (11−356 and 10−253 μg/g FW, respectively), whereas Q3Gal showed smaller variability than on other phenolic metabolites analyzed (8−59 μg/g FW), as previously reported by other authors.[24] Finally, catechin was the main flavan-3-ol found in the samples, showing a wide range among samples (4−72 μg/g FW). Large variation was also found among the samples for TPC (691−2876 μg GAE/g FW), AOC (861−7249 μg/g FW), and BP (0.10−1.23 ΔA420/h).

Considerable variation was found as well in the amount of individual skin phenolic compounds, AOC, and BP, among the fruit from different cultivars (Table 3), as found by other authors.[19,24,26] August Pearl showed the statistically highest content of hydroxycinnamates (CA and NCA) among the 21 cultivars. On the other hand, Glacier and Sweet Dream showed the lowest content of CA and NCA, although they were not significantly different from Summer Bright and Rich Lady or Snow King, respectively. Regarding C3G, Honey Blaze and Honey Fire yellow-fleshed nectarines had the statistically highest values among the 21 cultivars studied. It is well-known that, in general, yellow-fleshed cultivars contain more anthocyanin pigments in the skin than white flesh cultivars,[24] as observed in this work. Among the flavonols, Q3R was the most abundant compound for all of the cultivars studied, except in Snow King and Snow Princess, in which the most abundant flavonol in the skin was Q3Glu. In general, white-fleshed cultivars contained similar amounts of Q3R and Q3Glu, whereas the skin of yellow-fleshed cultivars contained higher amounts of Q3R than of Q3Glu. This result is in agreement with previous studies on the skin of different peach and nectarine cultivars.[24] Honey Blaze had the highest amount of Q3R, Q3Glu, and Q3Gal but was not significantly different from Rich Lady in the case of Q3Gal. The lowest contents of Q3R and Q3Glu were shown by white-fleshed cultivars such as Autumn Snow, Glacier, Snow King, and Snow Princess, with the exception of the yellow flesh cultivars Summer Lady and Sweet Dream, which also had low values. Fire Sweet had the lowest content of Q3Gal, without being significantly different from Sweet Dream.

August Pearl showed the highest TPC and AOC among the 21 cultivars tested (Table 3), followed by August Lady, Honey Blaze, and O'Henry, all of them with values higher than 2000 μg GAE/g FW. A similar trend was seen for AOC, where values higher than 4000 μg TEAC/g FW were found in August Lady, August Pearl, Honey Blaze, Kaweah, O'Henry, Snow Princess, and Summer Lady. Regarding BP, Snow Princess had the highest value, although it was not significantly different from August Pearl. The BP lowest values were found for Honey Blaze, Rich Lady, and Spring Bright, without being significantly different from those of Summer Bright and Sweet Dream.

Skin burning susceptibility was significantly higher ($P \leq 0.01$) for nectarine than for peach fruit (Table 4). This result could be explained by the significantly higher contents of hydroxycinnamate, anthocyanin, and flavonol compounds found in the skin of nectarine when compared to the peach fruit (Table 4), in agreement with previous data reported for other peach and nectarine cultivars.[24] Conversely, no significant differences in skin burning

Table 4. Skin Burning Susceptibility, Skin Phenolics Profile, TPC, AOC, and BP Associated with Fruit Type (Peach/Nectarine) and Flesh Color (Yellow/White) Qualitative Traits, Analyzed in 21 Different Peach and Nectarine Cultivars

		μg/g FW									
quality trait	skin burning susceptibility[a] (1−3)	CA	NCA	C3G	Q3R	Q3Glu	Q3Gal	catechin	TPC (μg GAE/g FW)	AOC (μg TEAC/g FW)	BP (ΔA420/h)
peach	1.9 b	114.1 b	18.7 b	141.1 b	56.8 b	42.3 b	12.4 b	34.5 a	1540.2 a	3551.3 a	0.5 a
nectarine	2.3 a	220.5 a	54.3 a	287.7 a	191.5 a	137.4 a	33.3 a	28.7 a	1591.3 a	3372.1 a	0.5 a
yellow	2.0 a	155.5 a	34.7 a	266.6 a	135.2 a	88.9 a	29.6 a	30.2 a	1588.1 a	3386.1 a	0.4 b
white	2.1 a	174.7 a	35.1 a	104.7 b	87.9 b	79.6 a	25.0 a	34.8 a	1520.2 a	3616.3 a	0.7 a

[a] Skin burning susceptibility scored on a categorical scale of 1 (non-susceptible), 2 (susceptible), and 3 (very susceptible). For each pair of traits (peach/nectarine, yellow/white fleshed), in each column, means with the same letter are not significantly different according to the *t* test ($P \leq 0.01$).

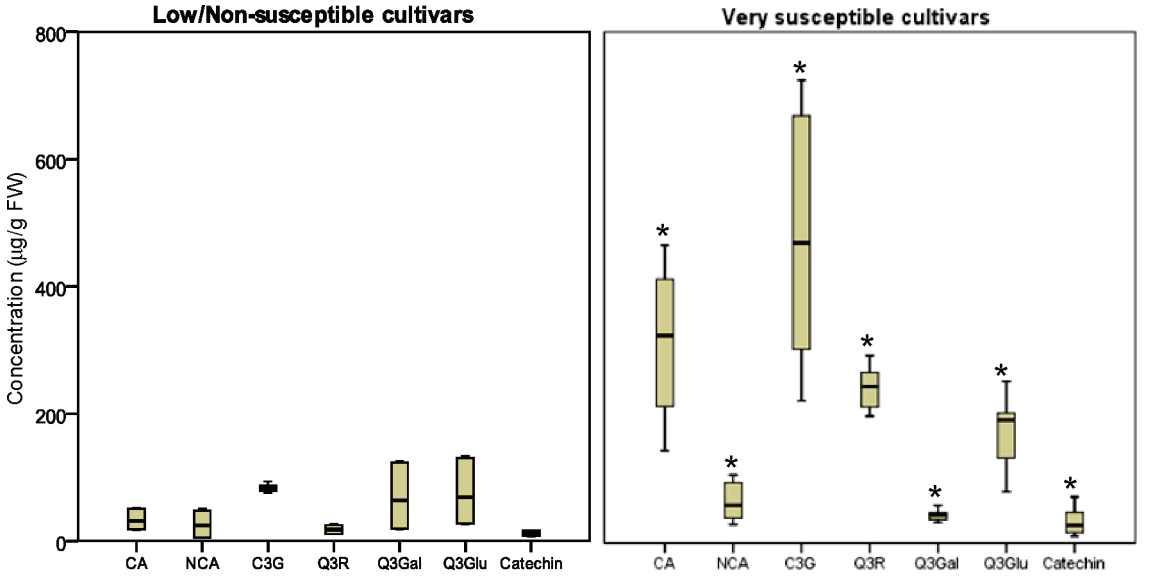

Figure 4. Range and distribution of skin phenolic compounds on the skin of 21 peach and nectarine cultivars low/non-susceptible (left) and very susceptible (right) to skin burning. The horizontal line in the interior of each box is the median value. The height of each box is equal to the interquartile distance, indicating the distribution for 50% of the data. Approximately 99% of the data fall inside the whiskers (the line extending from the top and bottom of each box). The data outside these whiskers are indicated by horizontal lines. *Represents significant differences for each phenolic compound between low/non-susceptible and very susceptible cultivars at $P \leq 0.01$. Abbreviations: Q3R, quercetin-3-*O*-rutinoside; Q3Glu, quercetin-3-*O*-glucoside; Q3Gal, quercetin-3-*O*-galactoside.

susceptibility were found in relation to the flesh color (yellow/white) quality trait (Table 4).

Significant differences ($P \leq 0.01$) were found between skin burning low or non-susceptible cultivars and the very susceptible cultivars for their phenolics profile (Figure 4). The concentration of all of the phenolic compounds identified in the fruit skin extract was significantly lower in the low or non-susceptible peach and nectarine cultivars than in the very susceptible ones. Additionally, significant differences among these groups of cultivars were also found for TPC, AOC, and BP (Table 5). Fruit from low or non-susceptible cultivars had significantly lower TPC, AOC, and BP than fruit from susceptible and very susceptible cultivars. These results were expected, since TPC, AOC, and BP are related to the concentrations of different phenolic compounds existing in the sample.[23,26]

These results show that cultivars with higher amounts of phenolic compounds, TPC, AOC, and/or BP in their fruit skin cells tend to be more susceptible to the development of skin burning when exposed to triggering conditions. This could be due to the higher amount of phenolic compounds available to undergo potential structural transformations triggered by high pH, which will ultimately lead to skin burning disorder development. This is an important result to consider, since the demonstrated beneficial effects of antioxidant compounds on health[27,28] are making the AOC of fruits an important trait to boost in current peach and nectarine breeding programs, and this could be causing a higher susceptibility to skin burning.

Table 5. TPC, AOC, and BP Associated with Different Skin Burning Susceptibility Peach and Nectarine Cultivars[a]

skin burning susceptibility	TPC (μg GAE/g FW)	AOC (μg TEAC/g FW)	BP (ΔA420/h)
low/non-susceptible	842.4 b	1406.5 b	0.16 b
susceptible	1543.3 a	3521.9 a	0.55 a
very susceptible	2004.3 a	4328.8 a	0.42 ab

[a] In each column, means with the same letter are not significantly different according to the Duncan's test ($P \leq 0.01$).

The differences in skin burning susceptibility found for different peach and nectarine cultivars could also be due to the

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Table 6. Pearson's Correlation Coefficients between Skin Burning Susceptibility and Different Sources of Variation Such as Fruit Type, Flesh Color, Concentration of Different Phenolic Compounds in the Fruit Skin, TPC, AOC, and BP

	skin burning susceptibility
fruit type	0.25[a]
flesh color	0.05 ns
CA	0.75[a]
NCA	0.52[a]
C3G	0.70[a]
Q3R	0.64[a]
Q3Glu	0.55[a]
Q3Gal	0.59[a]
catechin	0.24 ns
TPC	0.55[a]
AOC	0.45[a]
BP	0.11 ns

[a] $P \leq 0.01$; ns, not significant.

occurrence of different rates of copigmentation. Copigmentation is a phenomenon in which the pigments and other organic compounds (usually non-colored) form molecular associations, generating an increment in the color intensity.[6] The copigments can be flavonoids, alkaloids, amino acids, organic acids, nucleotides, polysaccharides, or other anthocyanins, and when they are mixed with an anthocyanin solution, an interaction takes place, producing an increase in the absorption intensity (UV−visible region) and in its wavelength.[7] Therefore, not only the concentration of specific phenolics, but the relative amounts of them and the presence of other copigments in the fruit skin cells, could influence the chance to undergo the copigmentation interaction, which will lead to fruit skin darkening. The magnitude of the copigmentation effect is pH dependent, which would explain the differences observed in the spectrum absorbance of our skin extract samples when incubated at different pH values.

Among the studied attributes, CA and C3G contents showed the highest correlation with the skin burning susceptibility of peach and nectarine cultivars (0.75 and 0.70, respectively) (Table 6). This result corroborates our hypothesis of copigmentation being the main reaction beyond the skin burning disorder, since C3G is the main anthocyanin in peach and nectarine fruit that can undergo copigmentation, whereas CA is reported as one of the most common copigment in this reaction.[8] It has been demonstrated that the concentration of pigments and copigments as well as the copigment-to-pigment molar ratio are determinant parameters in the extent of copigmentation.[8] On the other hand, no significant correlation to skin burning susceptibility was found for fruit flesh color, catechin content, or BP. The susceptibility of structurally different plant phenolic compounds to pH has been reported to highly depend on the molecule structure.[3] Multiring aromatic structures such as catechin and flavonols are less susceptible to the effects of pH than monoring phenolic compounds such as CA.[29] Therefore, this monoring phenolic compound could have an important role in the development of skin burning when the fruit is exposed to high pH. On the other hand, the lack of correlation between skin burning susceptibility and BP is expectable, since BP measures mainly enzymic oxidation of phenolics via polyphenol oxidase (PPO),[30] which is different that the non-enzymic skin burning disorder that we studied in this work.

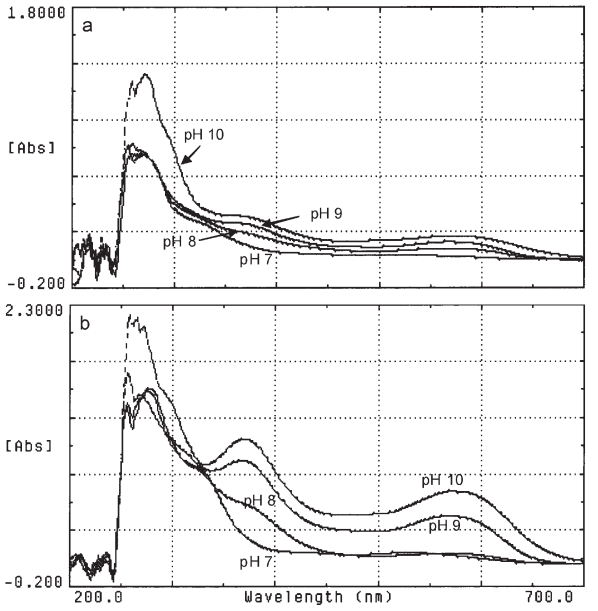

Figure 5. Effect of pH (7−10) on the absorption spectra of phenolics in skin extracts: (a) Sweet Dream (low/non-susceptible to skin burning) and (b) Honey Fire (very susceptible to skin burning) cultivars. The spectra were recorded immediately after solution mixing.

Stability of Skin Extracts Phenolics at Different pH Values. The effect of pH 7−10 (0.5 M) on the absorption spectra of skin phenolics from Sweet Dream and Honey Fire (skin burning low/non-susceptible and very susceptible cultivars, respectively) at time 0 are illustrated in Figure 5. The spectrum of the phenolics in Sweet Dream skin extract slightly shifted in contact with different pH solutions (Figure 5a). However, the absorption spectrum of phenolics in Honey Fire skin extract shifted dramatically in contact to pH 9 and pH 10 (Figure 5b). The absorption maximum of the spectrum changed in both position and intensity. Differences in the spectrum were especially relevant in two regions (325−425 and 550−650 nm) where absorbance increased gradually with pH. The second region on the spectrum where absorbance shifted with pH (550−650 nm) corresponds to anthocyanins maximum absorbance region, which shows distinctive band I peaks in the 450−560 nm region, due to the B ring hydroxyl cinnamoyl system.[27] Previous studies by Sun et al.[31] on anthocyanins extracts from fruits of *Kadsura coccinea* (Lem.) also reported a shift in the maximum absorbance region and an increase of absorbance at 520 nm as long as the pH raised. The changes seen on the absorption spectra are the changes that define the occurrence of an anthocyanin−copigment interaction. The formation of the new complex causes changes in the spectral properties of the molecules increasing the absorption intensity (hyperchromic effect) and its wavelength (bathochromic shift).[32] Among the factors that affect the copigment effect are pigment and copigment structures and concentrations, pH, solvent, and temperature.[5] Therefore, differences in the structure and composition of phenolics and copigment-to-pigment molar ratio may influence the extent of copigment interactions that we propose as the cause of the skin burning disorder.

The shift in absorption intensity and its wavelength when compared to the spectrum at neutral pH (pH 7) was obvious at pH 9 and 10, whereas less pronounced changes were observed at pH 8 (Figure 5b). Previous studies on anthocyanins stability and color variation with pH concluded that changes in the color of

these compounds are more significant in the alkaline region due to their instability in those conditions.[33,34] Moreover, the magnitude at what the copigmentation reaction occurs is pH dependent, which explains the different results in the absorption spectra of the skin extracts phenolics exposed to different pH values.

It is also important to note that the spectra changes observed at high pH values are time-dependent. The spectra changes occurred at pH 9 are more evident after 12 and 24 h than after 1 h after mixing with the high pH solution for both August Pearl and Summer Lady cultivars (Figure 6). This result confirms what was observed in the experiments carried out with the whole piece of fruit and suggests that the time that the susceptible abraded fruit is in contact with the high pH solution during the post-harvest operations might influence the intensity of the skin damage that will appear afterward on the fruit surface.

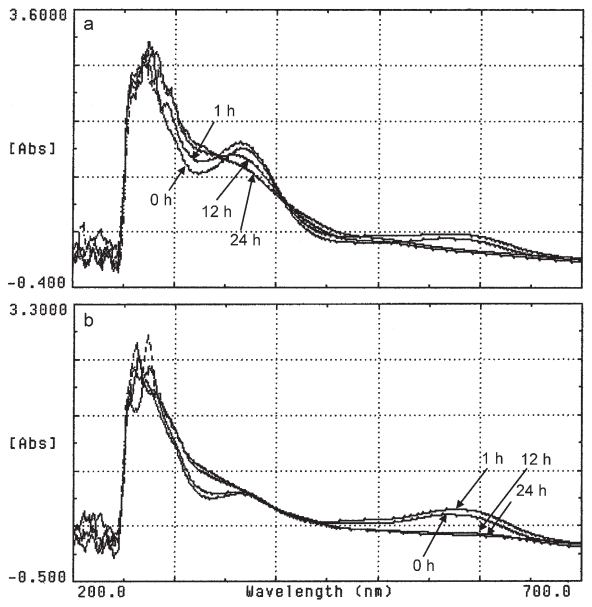

Figure 6. Effect of exposure time to pH 9 on the absorption spectra of phenolics in skin extracts from (a) August Pearl (very susceptible to skin burning) and (b) Summer Lady (susceptible to skin burning) cultivars. Absorption spectra were measured immediately after mixing skin extracts with pH 9 solution (0 h) and after 1, 12, and 24 h.

To probe our hypothesis of copigmentation in the susceptible samples when subjected to high pH, and therefore the formation of a new compound, we analyzed skin extracts at different pH solutions for phenolic compounds by HPLC-DAD (Figure 7). A new compound was detected at pH 9 (called peak A), and its peak is much more apparent at higher pH values. This peak was not detectable in the skin extracts subjected to solutions with pH values lower than 8.5. Peak A eluted 2.6 min before than NCA, and its UV−vis spectrum, recorded using a diode array detector, had a λ_{max} of 275 nm. On the basis of its retention time in the UV−vis chromatogram, this new compound formed in the samples subjected to high pH has higher polarity than the rest of phenolic compounds identified in the samples, since the elution sequence on reversed phases is polarity dependent.

To test the reversibility of the spectral shifts observed on the phenolic skin extract from our samples, we neutralized (to pH 7) the skin extract solutions after incubation at pH 10 for 2 h. After the solutions were neutralized to pH 7, the original spectrum at pH 7 was regenerated (results not shown), showing the reversibility of the transformations occurred in the phenolics in the skin extract due to high pH. It has been reported that when the copigment is another phenolic compound, the anthocyanin copigment interaction is transitory due to the lack of chemical bonds.[7] However, the skin burning damage developed in the fruit skin in the in vivo experiments shown above was not reversible. This could be explained by the disruption of the fruit tissues and

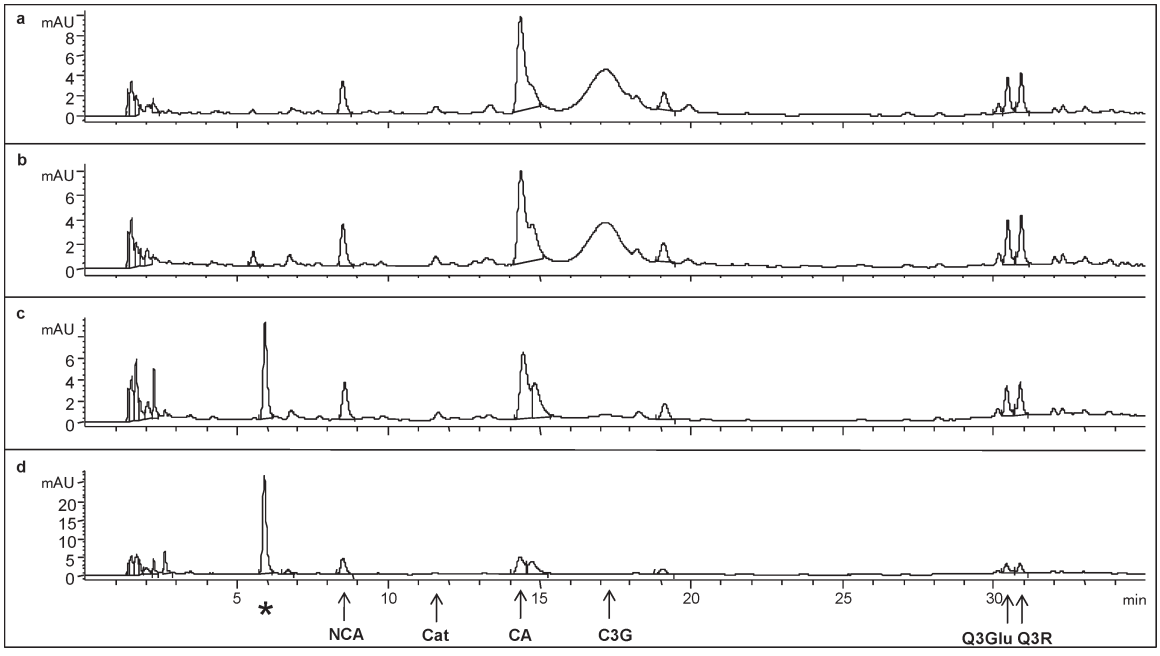

Figure 7. Effect of pH (8−10) on the chromatogram of Honey Fire skin extracts (very susceptible to skin burning). The phenolic composition of the skin extract was analyzed by HPLC-DAD at pH 8 (a), pH 9 (b), pH 9.5 (c), and pH 10 (d). *Indicates the retention time of the new peak found at high pH. The arrows indicate the retention time for the main phenolic compounds found in the peach skin extract. Abbreviations: Cat, catechin; C3G, cyanidin-3-*O*-glucoside; Q3Glu, quercetin-3-*O*-glucoside; Q3R, quercetin-3-*O*-rutinoside.

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the influence of other compounds different to skin phenolics present on the fruit affected by the whole process that leads to the development of skin burning.

In conclusion, the results of this work characterize the symptoms and causes of an economically very important postharvest skin disorder affecting the peach and nectarine industry worldwide. This work confirms the key role of phenolics in the development of skin burning and points out the copigmentation reaction between anthocyanins and other phenolic compounds as the main mechanism that leads to the change in the skin color. The different composition and concentrations of phenolic compounds on the skin cells may explain the differences in skin burning susceptibility existing among different cultivars. The demonstration that abrasion damage is the first condition to trigger skin burning disorder on the fruit surface indicates the importance of minimizing the physical damage pre- and postharvest on these skin burning susceptible peach and nectarine cultivars to reduce the incidence of skin burning. Finally, the proven influence of contact time with the high pH solution on the intensity of the skin discoloration indicates that the exposure to high pH solution during the postharvest washing−waxing operations should be minimized, if not avoided, to reduce the incidence of the skin burning disorder.

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