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Correlation between Changes in Polyphenol Composition of Peels and Incidence of CO_2 Skin Burning of 'Cameo' Apples As Influenced by Controlled Atmosphere Storage

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ABSTRACT: 'Cameo' apples stored under high CO_2 levels suffer from "skin burning". Accordingly, this study is aimed to correlate the incidence of skin burning with different polyphenols. After harvest, apples were sorted into bad- and good-colored fruit and further stored under either high (3%) or low (0.7%) CO_2 level. At frequent intervals, fruit were assessed for incidence of skin burning and relative concentrations of various polyphenols. Results clearly show that bad-colored apples stored under high CO_2 level had the highest incidence percentage. Concerning the polyphenol profile, good-colored and healthy apples had significantly higher concentrations of certain polyphenols, including cyanidin-3-galactoside and rutin. However, bad-colored and injured apples had significantly higher concentrations of another set of polyphenols, including phloridzin, epicatechin, and (epi)catechin→(epi)catechin isomers. Taking into account that quercetins and cyanidins account for more than 80% of antioxidants, it is logical to assume that these polyphenols might give protection to good-colored apples against skin burning.

KEYWORDS: *apples, Malus, antioxidants, skin burning, CA storage, LC-MS*

INTRODUCTION

Apples are consumed widely at any time of the year and, consequently, are considered one of the major sources of dietary polyphenols.[1] Polyphenols are important for the human diet mainly due to their capacity to scavenge free radicals.[2] This antioxidative capacity reduces oxidative stress-related chronic diseases and age-related disorders (e.g., atherosclerosis, carcinogenesis, neurodegeneration, and skin deterioration) substantially.[3] In this sense, consumers are highly interested in purchasing fruit with the highest possible concentrations of such phytochemicals. However, fruit developmental changes, fruit parts, storage conditions, and genotypes highly affect the actual concentrations of various polyphenols. Concerning genotypes, the mean concentration of total polyphenols ranged, among assessed apple varieties, between 66.2 and 211.9 mg·100 g^{-1} fresh weight.[4] As for developmental changes, strong reductions in quercetin glycosides and proanthocyanidins were reported from early to midseason in skins of red-colored 'Splendour' apples.[5] Furthermore, it was also reported that the capacity of apples to scavenge radicals decreased during ripening.[6] Concerning fruit parts, it is well documented that peels, compared to flesh, have significantly much higher concentrations of polyphenols, with quercetin glycosides as the main polyphenols.[7,8] In addition, it was reported that seeds are very rich in these phytochemicals.[6] In this context, it is worth mentioning that apple pomace contains numerous polyphenols including epicatechin, caffeic acid, phloridzin, avicularin, hyperin, and quercitrin.[9]

Concerning storage and postharvest treatments, studies show two major trends. The first trend is that long-term storage, whether cold or in a controlled atmosphere, had no influence on polyphenol concentrations or the antioxidant activity of stored apples.[10−12] The second trend is that storage increased the antioxidative capacity and polyphenol concentrations.[13,1] Moreover, 1-methylcyclopropene (1-MCP) treatment, which is widely used to suppress the ethylene action of stored apples, maintained some of the intrinsic polyphenols[14] and anthocyanins[15] of red apples. Despite the new technologies (e.g., 1-MCP treatment), storage of apples still relies heavily on increasing the CO_2 level inside the store, mainly to suppress ethylene biosynthesis and action and subsequently maintaining the firmness of stored fruit.[16] However, high CO_2 levels proved to be stressful for certain apple genotypes, resulting in external injury symptoms.[17−19] These injuries can be prevented or markedly reduced by keeping apples in cold air storage for a few weeks before application of high CO_2 levels.[20,21] One of these injuries is "skin burning", which was recorded in our lab, mostly with poorly colored 'Cameo' apples that were stored under high CO_2 levels. Typically, injured fruit showed slightly sunken, slightly colored, and visible blotches just beneath the exocarp. In this respect, the

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connection between fruit color and incidence of external CO_2 injuries is not well studied. It is worth mentioning here that the color of red apple is directly related to their concentration of anthocyanins,[22,23] whose biosynthesis is highly influenced by light[24,25] and ethylene.[26]

On the basis of the above-mentioned findings and trends, the aim of this study is to connect the incidence of skin burning of both good-colored and bad-colored 'Cameo' apples, either freshly harvested or stored, with their concentrations of polyphenols. In addition, the connection between the delay in establishing the controlled atmosphere (CA)-storage conditions, concentrations of various polyphenols, and incidence of skin burning is also investigated.

MATERIALS AND METHODS

Plant Material and Storage Conditions. 'Cameo' apples were picked from the orchard of the Competence Centre for Fruit Growing (KOB), Ravensburg, Southern Germany. Directly on the same day, apples were sorted visually by two trained panelists into various blush color intensities. For bad coloration treatments, apples with <25% of surface with red coloration were selected, whereas apples with >50% of surface with red coloration were selected for good coloration treatments. Apples with 25−50% red coloration were discarded. After sorting, uniform apples from each group were stored separately at 3 °C under two CA conditions, namely, 1 kPa of O_2 and <0.7 kPa of CO_2 (designated 0.7% hereafter), and 1 kPa of O_2 and 3 kPa of CO_2 (designated 3% hereafter). Moreover, the establishment of CA storage was either direct, in which CA-storage conditions were achieved on the harvest day, or with a delay of 21 days. During the delay period, harvested apples were kept at 3 °C under normal air-conditioning. After storage periods of four and seven months plus seven days shelf life at 20 °C, fruit were assessed for CO_2 injuries, fruit flesh firmness, total soluble solids, and titratable acidity. For each treatment and sampling date, there were three replicates. Each replicate consisted of eight fruit for each parameter. Skin burning, as an external CO_2 injury of stored apples, was assessed against reference photographs. The following scale, which reflects percentage of injured peel surface, was used: 0 = no injury; 1 = 1−5%; 2 = 6−15%; 3 = 16−40%; and 5 = >40% of the total peel surface. Flesh firmness was measured using a semiautomated penetrometer (Guess fruit texture analyzer; Strand, South Africa) that is equipped with 8 mm probe at two points from the equatorial region of each peeled apple. Both points were between the green-yellow (shaded side) and red blush (sun-exposed side) regions. Values are in $kg \cdot cm^{-2}$. Total soluble solids (TSS) of the juice were determined using a digital refractometer (PR-1; Atago Co. Ltd., Tokyo, Japan) and expressed as degrees Brix (°Bx). Titratable acidity was determined through titration of 10% (v/v) juice with 0.1 N NaOH to pH 8.1 using a Metrohm pH meter (Filderstadt, Germany).

Polyphenol Analysis. Peels from eight fruits, for each replicate and sampling date, were obtained, directly shock frozen in liquid nitrogen, and further freeze-dried to a powder. A 500 μL amount of absolute methanol and 250 μL of the internal standard solution (50 mg of biochanin A in 250 mL of absolute methanol) were added to 100 mg of lyophilized fruit powder in 1.5 mL tubes. The solution was then vortexed for 1 min and further sonicated for 5 min. After centrifugation for 10 min at 13 200 rpm, the supernatants were collected in new tubes, and the pellets were extracted, vortexed, sonicated, and centrifuged once again as mentioned above. Supernatants were pooled and placed in a Speed-Vac for 2 h. The dried residue from each replicate was dissolved in 35 μL of water (LC-MS quality), sonicated for 10 min, and finally centrifuged for 10 min at 13 200 rpm. A 20 μL sample of the clear supernatant was placed in HPLC vials for analysis using an LCMS instrument (Agilent HPLC 1100; MS: Bruker Daltonics Esquire 3000 Plus) equipped with a Phenomenex column (Luna 3u C18 (2) 100A′′, 150 × 2.0 mm (part no. 00F-4251-B0). The LCMS analysis conditions were as follows: column temperature, 28 °C; injection volume, 5 μL; flow rate, 0.2 $mL \cdot min^{-1}$; solvents: A, 0.1% formic acid in water; B, 0.1% formic acid in methanol; gradient, 0−30 min, 0−50% B; 30−35 min, 50−100% B; 35−50 min, 100% B; 50−55 min, 100−0% B; 55−65 min, 0% B; detection wavelength, 280 nm; MS: dry gas: nitrogen at 330 °C and flow rate of 10 $L \cdot min^{-1}$; capillary, −4000 V; end plate offset, −500 V; collision gas, helium; collision voltage, 1 V. The electrospray ionization voltage of the capillary was set to −4000 V and the end plate to −500 V. The full scan mass spectra were measured in a scan range from m/z 100 to 800. Tandem mass spectrometry was carried out using helium as collision gas (3.5−6 mbar) with the collision voltage set at 1 V. Spectra were acquired in the positive and negative ionization modes, and data analysis was performed using the DataAnalysis 5.1 software (Bruker Daltonics, Bremen, Germany). Relative concentrations of polyphenols were calculated based on an internal standrad (biochanin A).

Statistical Analysis. Analysis of variance (ANOVA) was performed using the CoStat statistical package (CoHort Software, Monterey, CA, USA). The factors considered are fruit color and storage condition. Mean separations were conducted using Student−Newman−Keuls test at $p \leq 0.05$. SE values were also calculated and included ($n = 3$).

RESULTS

Incidence of Skin Burning. The incidence of skin burning among different treatments is shown in Figure 1. It is obvious

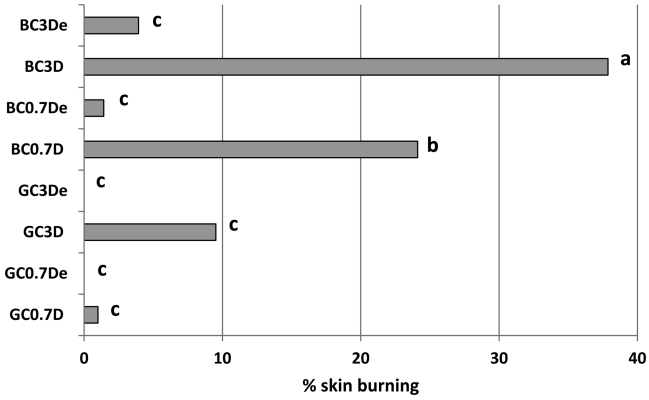

Figure 1. Percentage of skin burning of 'Cameo' apples in connection with fruit color at harvest and storage conditions. GC = good-colored (>50% blush); BC = bad-colored (<25% blush); HT = harvest time; 0.7 = storage at 0.7% CO_2; 3 = storage at 3% CO_2; D = direct establishment of CA-storage conditions; De = delayed establishment of CA-storage conditions. Storage period was seven months. Mean separation by Student−Newman−Keuls test, and means follwed by different letters are significantly different at $p \leq 0.05$.

that good-colored 'Cameo' apples had a significantly lower incidence of the disorder compared to bad-colored fruit. Furthermore, the 3% CO_2 storage condition proved to be injurous to 'Cameo' apples. However, the delayed establishment of CA-storage conditions, whether 3% or 0.7% CO_2, resulted in drastic reductions in the percentages of skin burning.

Quality Parameters. Table 1 shows the effect of fruit coloring and storage conditions on various quality parameters. Flesh firmness of 'Cameo' apples was reduced over prolonged storage periods, irrespective of coloring at harvest or storage conditions. Moreover, it is clear that apples stored under 3% CO_2 maintained flesh firmness significantly better than apples stored under 0.7% CO_2. Concerning the delay in establishing the CA-storage conditions, significant differences in favor of direct CA-storage on firmness can be noticed in the first sampling date, which diminished, however, by the second sampling date. With respect to changes in sugar content, no obvious trends can be seen. Concerning titratable acidity (TA), results show that prolonged storage resulted in significant decreases in TA with all treatments. However, clear significant differences between

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Table 1. Flesh Firmness, Sugar Content, and Titratable Acidity of 'Cameo' Apple in Connection with Fruit Color at Harvest and Storage Conditions[a]

fruit color at harvest	% CO_2	storage conditions establishement	storage period (months)	firmness ($kg \cdot cm^{-2}$)	soluble solids content (°Bx)	titratable acidity ($g \cdot L^{-1}$)
good	0.7	direct CA	4	8.4 ± 0.07	14.3 ± 0.06	4.4 ± 0.01
		delayed CA		7.4 ± 0.07	14.2 ± 0.10	4.2 ± 0.01
		direct CA	7	6.1 ± 0.09	14.5 ± 0.08	3.5 ± 0.03
		delayed CA		5.9 ± 0.04	14.8 ± 0.08	3.6 ± 0.02
bad		direct CA	4	8.2 ± 0.06	13.4 ± 0.06	4.5 ± 0.08
		delayed CA		8.1 ± 0.11	14.0 ± 0.09	4.5 ± 0.03
		direct CA	7	6.4 ± 0.04	13.5 ± 0.18	3.8 ± 0.05
		delayed CA		6.2 ± 0.17	12.9 ± 0.07	3.1 ± 0.10
good	3	direct CA	4	8.1 ± 0.01	14.4 ± 0.13	4.3 ± 0.03
		delayed CA		7.8 ± 0.09	14.4 ± 0.10	4.1 ± 0.05
		direct CA	7	7.0 ± 0.09	13.9 ± 0.06	3.6 ± 0.04
		delayed CA		7.0 ± 0.01	14.4 ± 0.12	3.7 ± 0.08
bad		direct CA	4	8.4 ± 0.02	14.6 ± 0.08	4.7 ± 0.04
		delayed CA		8.3 ± 0.08	14.0 ± 0.05	4.6 ± 0.05
		direct CA	7	7.0 ± 0.06	14.2 ± 0.10	3.7 ± 0.05
		delayed CA		6.9 ± 0.03	13.3 ± 0.01	3.6 ± 0.02
ANOVA	color			*	***	*
	storage			***	**	***
	color * storage			***	*	***

[a]Means ± SE (n = 3) are shown.

Table 2. Relative Concentrations of Cyanidin 3-Galactoside, Cyanidin 3-Arabinoside, and Kaempferol-3-glucuronide of 'Cameo' Apple Peels in Connection with Fruit Color at Harvest, Storage Conditions, Storage Period, and the Delay in Establishing CA Conditions[a]

fruit color	peel condition	CA-storage condition and establishment	storage period (months)	cyanidin 3-galactoside	cyanidin 3-arabinoside	kaempferol-3-glucuronide
good	healthy	harvest time	0	153.34 ± 11.60	14.08 ± 01.40	0.88 ± 0.05
		0.7% CO_2 ; direct CA	4	124.80 ± 07.98	9.80 ± 0.25	0.57 ± 0.13
		0.7% CO_2 ; delayed CA	4	121.91 ± 08.15	9.09 ± 0.45	0.76 ± 0.09
		0.7% CO_2 ; direct CA	7	78.70 ± 01.98	5.28 ± 0.43	0.91 ± 0.12
		0.7% CO_2 ; delayed CA	7	70.02 ± 21.20	5.17 ± 02.43	0.75 ± 0.05
		3.0% CO_2 ; direct CA	4	116.61 ± 02.59	8.71 ± 01.00	0.61 ± 0.06
		3.0% CO_2 ; delayed CA	4	122.62 ± 00.61	10.17 ± 0.48	0.71 ± 0.10
		3.0% CO_2; direct CA	7	86.57 ± 04.02	6.37 ± 0.44	0.66 ± 0.01
		3.0% CO_2; delayed CA	7	84.76 ± 07.18	5.39 ± 0.71	0.62 ± 0.03
	injured	3.0% CO_2; direct CA	4	44.58 ± 11.19	2.98 ± 0.95	0.49 ± 0.20
bad	healthy	harvest time	0	30.77 ± 04.35	1.88 ± 0.18	0.46 ± 0.06
		0.7% CO_2; direct CA	4	34.25 ± 02.36	2.05 ± 0.17	0.65 ± 0.06
		0.7% CO_2 ; delayed CA	4	30.77 ± 02.55	1.95 ± 0.13	0.44 ± 0.07
		0.7% CO_2; direct CA	7	23.64 ± 02.31	1.29 ± 0.13	0.57 ± 0.06
		0.7% CO_2; delayed CA	7	42.89 ± 23.23	3.13 ± 2.07	0.61 ± 0.01
		3.0% CO_2; delayed CA	4	28.63 ± 03.07	1.74 ± 0.42	0.61 ± 0.05
		3.0% CO_2 ; delayed CA	7	22.26 ± 00.39	1.09 ± 0.03	0.66 ± 0.02
	Injured	3.0% CO_2; direct CA	4	22.53 ± 01.53	1.37 ± 0.17	0.74 ± 0.14
		3.0% CO_2; delayed CA	4	13.47 ± 00.44	0.90 ± 0.18	0.19 ± 0.01
		3.0% CO_2; direct CA	7	22.12 ± 04.07	0.99 ± 0.21	0.82 ± 0.02
		3.0% CO_2; delayed CA	7	10.73 ± 0 1.53	0.45 ± 0.12	0.28 ± 0.03
		0.7% CO_2; direct CA	4	17.88 ± 0 2.17	1.21 ± 0.18	0.36 ± 0.14
		0.7% CO_2; direct CA	7	8.71 ± 00.75	0.54 ± 0.13	0.12 ± 0.02
ANOVA	color			***	***	**
	storage			***	***	***
	color * storage			***	***	**

[a]Means ± SE (n = 3) are shown.

treatments are not evident, and differences between 3% and 0.7% CO_2 treatments are almost negligible.

Antioxidant Composition of Peels. Tables 2–4 explore relative changes in concentrations of selected polyphenols, which are calculated on the basis of values of the internal standard (biochanin A). Since the number of polyphenols assessed is large, only a selected number of these polyphenols will be presented.

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Table 3. Relative Concentrations of Rutin (Quercetin-3-*O*-rutinoside), Hyperin (Quercetin-3-galactoside), and *p*-Cumarylglucoside of 'Cameo' Apple Peels in Connection with Fruit Color at Harvest, Storage Conditions, Storage Period, and the Delay in Establishing CA Conditions[a]

fruit color at harvest	peel condition	CA-storage condition and establishment	storage period (months)	rutin (quercetin-3-*O*-rutinoside)	hyperin (quercetin-3-galactoside)	*p*-cumaryl-glucosid
good	healthy	harvest time	0	0.42 ± 0.06	7.33 ± 1.01	0.65 ± 0.03
		0.7% CO_2; direct CA	4	0.29 ± 0.02	2.89 ± 0.28	0.75 ± 0.04
		0.7% CO_2; delayed CA	4	0.44 ± 0.02	5.64 ± 0.08	0.98 ± 0.12
		0.7% CO_2; direct CA	7	0.3 ± 0.04	4.06 ± 0.74	1.1 ± 0.15
		0.7% CO_2; delayed CA	7	0.24 ± 0.05	2.78 ± 1.00	0.93 ± 0.06
		3.0% CO_2; direct CA	4	0.3 ± 0.10	5.39 ± 0.91	0.97 ± 0.30
		3.0% CO_2; delayed CA	4	0.22 ± 0.04	3.98 ± 0.81	0.76 ± 0.01
		3.0% CO_2; direct CA	7	0.17 ± 0.03	2.68 ± 0.46	0.87 ± 0.07
		3.0% CO_2; delayed CA	7	0.3 ± 0.12	5.02 ± 1.26	0.82 ± 0.06
	injured	3.0% CO_2; direct CA	4	0.15 ± 0.06	1.99 ± 0.62	0.69 ± 0.13
bad	healthy	harvest time	0	0.1 ± 0.01	2.48 ± 0.19	0.52 ± 0.04
		0.7% CO_2; direct CA	4	0.09 ± 0.03	1.6 ± 0.13	0.76 ± 0.07
		0.7% CO_2; delayed CA	4	0.16 ± 0.02	1.3 ± 0.07	1.04 ± 0.02
		0.7% CO_2; direct CA	7	0.14 ± 0.02	1.8 ± 0.56	1.01 ± 0.07
		0.7% CO_2; delayed CA	7	0.09 ± 0.06	2.59 ± 1.10	0.92 ± 0.03
		3.0% CO_2; delayed CA	4	0.11 ± 0.02	2.45 ± 0.26	1.07 ± 0.04
		3.0% CO_2; delayed CA	7	0.08 ± 0.03	1.9 ± 0.38	0.96 ± 0.05
	injured	3.0% CO_2; direct CA	4	0.14 ± 0.03	2.31 ± 0.48	0.83 ± 0.04
		3.0% CO_2; delayed CA	4	0.04 ± 0.00	1.55 ± 0.19	0.9 ± 0.08
		3.0% CO_2; direct CA	7	0.08 ± 0.02	2.06 ± 0.62	0.95 ± 0.03
		3.0% CO_2; delayed CA	7	0.01 ± 0.01	1.3 ± 0.17	1.85 ± 0.21
		0.7% CO_2; direct CA	4	0.03 ± 0.00	0.92 ± 0.02	0.88 ± 0.15
		0.7% CO_2; direct CA	7	0 ± 0.00	0.46 ± 0.04	1.37 ± 0.10
ANOVA	color			***	***	ns
	storage			**	***	***
	color * storage			*	***	ns

[a]Means ± SE (n = 3) are shown.

The relative changes in profiles of others, which have similar trends, will be mentioned briefly.

It is clear from Table 2 that freshly harvested good-colored apples had much higher concentrations of cyanidin-3-galactoside (Cy3G) and cyanidin-3-arabinoside (Cy3A) than freshly harvested bad-colored apples. This significant difference continued during storage, irrespective of storage conditions, sampling dates, or CA-establishment techniques. Moreover, it is obvious that good-colored 'Cameo' apples achieved their peak concentrations of both Cy3G and Cy3A at harvest time, and their concentrations decreased later by all storage conditions. Prolonged storage, under most storage conditions, led to further significant decreases. In addition, it is clear that skin burning rendered the affected apples unable to synthesize and/or accumulate higher concentrations of both Cy3G and Cy3A, either before harvest or during storage; bad-colored apples with injured peels had significantly the lowest concentrations. Concerning the delay in establishing CA-storage conditions, consistent trends cannot be observed.

With respect to the relative concentrations of kaempferol-3-glucuronide (Kaem3G), peels of freshly harvested good-colored apples had significantly much higher concentrations of this compound than freshly harvested bad-colored apples. Another obvious trend is related to CO_2 level during storage. The relative concentrations of Kaem3G by good-colored apples, which were stored under the least stressful CO_2 level (0.7%), were significantly higher than those of apples that were stored under the most stressful CO_2 level (3%). Another interesting trend is related to apples that exhibited skin-burning injuries. For all these treatments, it is clear that the delayed establishment of CA conditions led to drastic reductions in the concentrations of Kaem3G. In addition to these trends, a slight but significant recovery can be seen by bad-colored apples that maintained healthy peels over the entire storage period.

Table 3 shows changes in relative concentrations of rutin (Rut), hyperin (Hyp), and *p*-cumarylglucoside (*p*-Cum). Like both Cy3G and Cy3A, good-colored apples had their peak concentrations of both Rut and Hyp already at harvest time. The peak concentration of Rut was maintained only by good-colored apples that were stored under 0.7% CO_2 after a delayed establishment of CA-storage conditions. Hyp concentration was also the highest under the same treatment, although its concentration remained below harvest time level. In contrast to this trend, bad-colored apples and apples that showed skin-burning injuries had significantly much lower concentrations of Rut. Hyp with the same treatments was less affected. It is interesting to note that Rut diminished almost completely in injured peels of bad-colored apples that were stored for seven months under 0.7% or 3% CO_2.

In addition to the above-mentioned compounds, similar trends were also recorded for avicularin (quercetin-3-arabinoside), quercitrin (quercetin-3-rhamnoside), and several unknown metabolites (m/z 359 M^+ at t_R 21.4 min, m/z 505 M^+ at t_R 25.7 min, m/z 481 M^- at t_R 25.7 min, m/z 417 M^- at t_R 38.3 min) (data not shown).

Concerning changes in *p*-cumarylglucoside (*p*-Cum) concentrations, it is obvious that these changes give different trends in comparison to other polyphenols (Table 3). At harvest time,

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Table 4. Relative Concentrations of (epi)Catechin→(epi)Catechin (Isomer 1), Chlorogenic Acid, and Phloridzin of 'Cameo' Apple Peels in Connection with Fruit Color at Harvest, Storage Conditions, Storage Period, and the Delay in Establishing CA Conditions[a]

fruit color at harvest	peel condition	CA-storage condition and establishment	storage period (months)	(epi)catechin→(epi)catechin (isomer 1)	chlorogenic acid	phloridzin
good	healthy	harvest time	0	16.54 ± 0.58	0.91 ± 0.09	6.47 ± 0.47
		0.7% CO_2; direct CA	4	15.89 ± 0.89	0.86 ± 0.20	6.2 ± 0.51
		0.7% CO_2; delayed CA	4	16.1 ± 0.73	1.22 ± 0.13	6.98 ± 0.69
		0.7% CO_2; direct CA	7	17.08 ± 3.11	1.1 ± 0.21	7.87 ± 0.20
		0.7% CO_2; delayed CA	7	13.95 ± 2.27	0.71 ± 0.09	5.91 ± 1.06
		3.0% CO_2; direct CA	4	16.75 ± 2.05	1.16 ± 0.26	7.44 ± 1.37
		3.0% CO_2; delayed CA	4	14.81 ± 0.75	0.98 ± 0.22	6.91 ± 0.15
		3.0% CO_2; direct CA	7	13.82 ± 0.76	0.70 ± 0.06	5.24 ± 0.38
		3.0% CO_2; delayed CA	7	14.4 ± 0.62	0.79 ± 0.02	5.52 ± 0.30
	injured	3.0% CO_2; direct CA	4	19.33 ± 2.52	1.13 ± 0.32	7.39 ± 1.04
bad	healthy	harvest time	0	15.42 ± 0.96	0.61 ± 0.05	6.36 ± 0.18
		0.7% CO_2; direct CA	4	18.47 ± 1.00	1.24 ± 0.23	7.58 ± 0.66
		0.7% CO_2; delayed CA	4	21.65 ± 0.35	1.52 ± 0.13	7.08 ± 0.19
		0.7% CO_2; direct CA	7	14.87 ± 0.64	0.84 ± 0.12	5.9 ± 0.07
		0.7% CO_2; delayed CA	7	13.95 ± 0.64	0.68 ± 0.05	5.52 ± 0.19
		3.0% CO_2; delayed CA	4	24.32 ± 0.63	1.54 ± 0.01	8.85 ± 0.40
		3.0% CO_2; delayed CA	7	16.07 ± 0.24	0.71 ± 0.03	5.99 ± 0.23
	injured	3.0% CO_2; direct CA	4	22.09 ± 0.79	1.56 ± 0.05	8.83 ± 0.39
		3.0% CO_2; delayed CA	4	20.27 ± 0.26	2.09 ± 0.10	8.19 ± 0.55
		3.0% CO_2; direct CA	7	16.0 ± 1.02	0.73 ± 0.08	6.11 ± 0.40
		3.0% CO_2; delayed CA	7	24.09 ± 3.29	2.37 ± 0.36	10.36 ± 0.70
		0.7% CO_2; direct CA	4	20.82 ± 3.05	1.55 ± 0.43	8.39 ± 2.29
		0.7% CO_2; direct CA	7	24.37 ± 1.40	1.80 ± 0.09	11.13 ± 0.57
ANOVA	color			**	ns	ns
	storage			***	***	***
	color * storage			*	ns	ns

[a]Means ± SE (n = 3) are shown.

Table 5. Pearson Product Moment Correlation Coefficients (r) between Polyphenols Assessed[a]

	Cy3G	Cy3A	Rut	Hyp	Kaem3G	Epi1	Chlo	*p*-Cum	Phlo
Cy3G	1	0.98 ***	0.83 ***	0.82 ***	0.48 ***	−0.47 ***	−0.38 **	−0.38 **	−0.35 **
Cy3A	0.98 ***	1	0.79 ***	0.80 ***	0.44 ***	−0.46 ***	−0.36 **	−0.35 **	−0.34 **
Rut	0.83 ***	0.79 ***	1	0.85 ***	0.54 ***	−0.39 ***	−0.33 **	−0.24 ns	−0.30 *
Hyp	0.82 ***	0.80 ***	0.85 ***	1	0.53 ***	−0.43 ***	−0.36 **	−0.29 *	−0.32 **
Kaem3G	0.48 ***	0.44 ***	0.54 ***	0.53 ***	1	−0.45 ***	−0.57 ***	−0.30 *	−0.41 ***
Epi1	−0.47 ***	−0.46 ***	−0.39 ***	−0.43 ***	−0.45 ***	1	0.87 ***	0.61 ***	0.88 ***
Chlo	−0.38 **	−0.36 **	−0.33 **	−0.36 **	−0.57 ***	0.87 ***	1	0.67 ***	0.83 ***
p-Cum	−0.32 **	−0.35 **	−0.24 ns	−0.29 *	−0.30 *	0.61 ***	0.67 ***	1	0.65 ***
Phlo	−0.35 **	−0.34 **	−0.30 *	−0.32 **	−0.41 ***	0.88 ***	0.83 ***	0.65 ***	1

[a]Cyanidin-3-galactoside = Cy3G, cyanidin-3-arabinoside = Cy3A, rutin (quercetin-3-*O*-rutinoside) = Rut, hyperin (quercetin-3-galactoside) = Hyp, kaempferol-3-glucuronide = Kaem3G, (epi)catechin→(epi)catechin (isomer 1) = Epi1, chlorogenic acid = Chlo, *p*-cumarylglucoside = *p*-Cum, and phloridzin = Phlo. For correlations: ns = nonsignificant, *$p \leq 0.05$, **$p \leq 0.01$, and ***$p \leq 0.001$.

peels of both good-colored and bad-colored apples had significantly lower concentrations compared to stored apples. During harvest, no consistent trends can be seen. Moreover, statistical analysis reveals that fruit color at harvest time had no significant influence on the concentrations of this compound.

In contrast to polyphenols illustrated above, the concentrations of a second set of polyphenols (Table 4) are either slightly affected by bad-coloration or completely unaffected. Moreover, peels of apples that exhibited skin-burning injuries had significantly higher concentrations of these polyphenols. With the first compound, namely, (epi)catchin→(epi)catechin (isomer 1) (Epi1), significant differences were recorded throughout the storage period. The highest concentration of Epi1 was recorded in peels of bad-colored apples that showed skin-burning injuries. Furthermore, delayed establishment of CA-storage conditions led, in most cases, to further significant increases in the concentrations of Epi1.

The profile of changes with chlorogenic acid (Chlo) gave different trends. Freshly harvested good-colored apples had significantly higher concentrations than freshly harvested bad-colored apples. However, peels of stored apples that exhibited injuries had significantly higher concentrations of Chlo, and delayed establishment of CA-storage conditions tended to intensify this trend. For phloridzin (Phlo), both freshly harvested good- and bad-colored apples had almost the same concentrations. However, the concentration of Phlo increased

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significantly by bad-colored apples that exhibited skin-burning injuries.

In addition to the above-mentioned compounds, similar trends were also recorded for (epi)catchin→(epi)catechin (isomer 2), phloretin-2′-xyloglucoside, epicatechin, and several unknown compounds (m/z 379 M^+ at t_R 24.4 min, m/z 379 M^+ at t_R 26.2 min, m/z 505 M^+ at t_R 26.7 min, m/z 697 M^- at t_R 27.95 min, m/z 363 M^- at t_R 29.6 min, m/z 488 M^+ at t_R 35.2 min (data not shown).

In addition to trends mentioned above, correlations between the above-illustrated polyphenols reveal several interesting trends (Table 5). The first trend is the strong positive correlations between Cy3G, Cy3A, Rut, Hyp, and Kaem3G. The second trend is the strong positive correlations between Epi1, Phlo, Chlo, and *p*-Cum. The third trend is the negative correlations between the first group of polyphenols (Cy3G, Cy3A, Rut, Hyp, and Kaem3G) and the second group of polyphenols (Epi1, Phlo, Chlo, and *p*-Cum).

DISCUSSION

Bad coloration of 'Cameo' apples and storage of such apples under high CO_2 level (3%) led to drastic reductions in concentrations of cyanidin-3-galactoside, cyanidin-3-arabinoside, rutin, hyperin, avicularin, quercitrin, and several unknown compounds in peels of stored apples. In addition, concentrations of (epi)catchin→(epi)catechin (isomer 1), phloridzin, chlorogenic acid, (epi)catchin→(epi)catechin (isomer 2), phloretin-2′-xyloglucoside, epicatechin, and several unknown compounds either were unaffected by bad coloration or were higher in injured peels. Taking into account that cyanidin 3-galactoside is the main glycoside in peels of red apple varieties,[27] such severe reduction in its concentrations may indirectly compromise the membrane integrity of injured peels, leading subsequently to skin burning. It is well known that cyanidins and quercetins are powerful antioxidants,[28,29] and the presence of these polyphenols together induces the well-known synergistic effect of anthocyanin mixtures.[30] Furthermore, good coloration of apples appears to be crucial to counteract the stressful high CO_2 level during storage. In this respect, various studies proved that illumination of fruit before harvest induces higher rates of anthocyanin synthesis, mainly in skins.[31−34] On the basis of our results, the negative effect of reduced illumination on flesh tissues is expected to be minimal, since the main polyphenols in these tissues are chlorogenic acid, epicatechin, and procyanidin B1.[35,36] On the basis of these studies,[35,36] it is possible to predict that bad coloration led to severe skin burning, due to the highly reduced antioxidative capacity of peels. The severity of this physiological disorder intensified further upon subjecting bad-colored apples to an additional external abiotic stress, namely, storage under high CO_2 level.

Studies that addressed storage impact on the metabolism of the various polyphenols gave inconsistent trends. On one hand, Nga et al.[37] reported that the concentrations of both quercetin 3-galactoside and quercetin 3-glucoside increased significantly under CA storage of 'Cripps Pink' apples. On the other hand, van der Sluis et al. (2001)[10] found that CA storage did not lead to drastic changes in the concentrations of phloridzin, cyanidin 3-galactoside, and chlorogenic acid in 'Cox Orange', 'Jonagold', 'Elstar', and 'Golden Delicious' apples. However, the major effect may be because of the stressful CO_2 levels in the store. In this sense, it was reported that among postharvest factors associated with increased incidence of skin spots are higher CO_2 concentrations[38] and prolonged storage duration.[39] It is possible here to assume that high CO_2 represses certain biochemical pathways, which are responsible for the synthesis of more quercetin and cyanidin glycosides. In addition to that, it is also possible to predict that prolonged storage depletes fruit of their powerful polyphenols. In this respect, it is reported that prestorage dipping of 'Elstar' apples in ascorbic acid led to a lower incidence of skin spots. [40] In the same study, researchers found, however, that 1-MCP treatment and poststorage H_2O_2 incubation of apples increased the incidence of skin spots.[40] The results with 1-MCP indicate a possible interaction between ethylene metabolism and skin injuries. Leja et al. (2003)[41] attributed the significant increase in total phenolics observed with cold-stored apples to the ethylene action. Ethylene is known to stimulate the activity of phenylalanine ammonia lyase, which is considered a key enzyme in the biosynthesis of phenolics.[42,43] In another study with 'Pink Lady' apples, positive and significant correlations were found between ethylene, color development, and total anthocyanins.[44]

The positive impact of delayed establishment of CA-storage conditions cannot be explained with changes in polyphenols, as these changes give no consistent trends. It is most propable that ethylene biosynthesis and its perception increased during the delay period, which rendered stored apples less susceptible to high CO_2 stress. In a comparable study, Ju et al. (1996)[45] reported that delaying harvest protected stored apples from scald, which was attributed to an increased anthocyanins accumulation.

In conclusion, the most probable cause of skin burning is the bad coloration of fruit at harvest time. In this sense, low polyphenol content might cause both bad coloration and skin burning. However, this a a correlative study and cannot attribute the incidence of skin burning solely to differences in polyphenol concentrations mentioned above. Accordingly, further investigation is needed to elucidate possible molecular mechanisms that lead to the development of skin burning in susceptible apple genotypes, such as 'Cameo' apples.

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After this paper was published ASAP on April 3, changes were made to the title and affiliations. The corrected version was reposted April 5, 2013.