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Prestorage Ultraviolet−White Light Irradiation Alters Apple Peel Metabolome

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Global metabolic profiling of 'Granny Smith' apple peel was employed for evaluating metabolomic alterations resulting from prestorage UV−white light irradiation. Apples were bagged midseason to restrict sunlight, harvested at the preclimacteric stage prior to bag removal, treated with fluorescent UV−white light for 0–48.5 h, and stored for 6 months at 0 °C. Trimethylsilyl (oxime) derivatized or underivatized aliquots of methanolic extracts from peel samples collected immediately after irradiation or following cold storage were evaluated using GC-MS and LC-UV/vis-MS, respectively. The profile, including more than 200 components, 78 of which were identified, revealed changes in the metabolome provoked by UV−white light irradiation and cold storage. Analyses of individual components selected using principal component analysis (PCA) models showed distinct temporal changes, before and after cold storage, related to prestorage irradiation in a diverse set of primary and secondary metabolic pathways. The results demonstrate metabolic pathways associated with ethylene synthesis, acid metabolism, flavonoid pigment synthesis, and fruit texture, are altered by prestorage irradiation, and many of the alterations are detectable after 6 months of cold storage in air.

KEYWORDS: *Malus pumila*, Mill; metabolomics; metabolic profiling; GC-MS; LC-UV/vis-MS; principal component analysis; fruit quality; *Malus sylvestris* var. *domestica* (Borkh.) Mansf; primary metabolism; trimethylsilyl (oxime); color; sugar; acid; amino acid; cold storage

INTRODUCTION

Exposure of apple peel to light provokes beneficial and detrimental preharvest and postharvest phenomena. Significant differences in peel appearance and morphology based on the degree of exposure to light during the growth period, even on the same fruit, may be observed. In many cultivars, light is required to stimulate desirable red color development (*1, 2*). During this process, red-colored anthocyanins (*3*) are synthesized and deposited in the vacuoles along with other flavonoids including hyperin, chlorogenic acid, and phloridzin (*4*). Similarly, concentrations of anthocyanins, hyperin, and chlorogenic acid (*5–7*) as well as chlorophyll *b* and β-carotene (*5*) increase in peel during artificial postharvest irradiation. Beyond changes in pigments, little is known about the underlying metabolic events in apple during and following light exposure, especially those that may affect the poststorage quality of the fruit.

Altered metabolism is apparent given the number of preharvest and postharvest conditions typically linked to light exposure. Preharvest disorders such as sunburn, where peel tissue is discolored leaving an orange to brown or bleached appearance, are characteristically associated with high-light and/or heat conditions leading to changes in gene expression (*8*), activity of proteins involved in antioxidative pathways (*9*), and pigment synthesis (*10*). These conditions may also affect changes in the cortex directly under the peel such as retention of firmness (*11*). During cold storage, disorders including delayed sunscald, a disorder similar to sunburn but with symptom expression occurring on the exposed side during cold storage (*12*), lenticel breakdown (necrotic lesions surrounding lenticels) (*13*), and superficial scald, a necrotic disorder that is more common on shaded fruit peel (*14*), can occur. Again, little is known about the metabolic origins of these disorders or how light may mediate their development.

Deciphering altered plant metabolism induced by environmental conditions has previously been conducted using narrowly focused analyses of targeted metabolites. This is certainly true with respect to postharvest metabolism in apple fruit where quality-related processes including ethylene and secondary metabolite biosyntheses receive considerable attention. Similarly, acid metabolism, specifically malic acid or titratable acidity, is addressed regularly in many apple postharvest studies given its relevance to fruit quality. Reports documenting more broad analyses of multiple pathways including primary sugars (*15–17*), acids (*15, 17*), and amino acids (*15*) are less common. More comprehensive metabolite profiling techniques may be required to get an accurate depiction of metabolism as affected by environmental or genotypic differences.

As the technical capacity of analytical instrumentation and methods for handling data improve, it is becoming more

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common to address biological phenomena using increasingly more metabolic information (*18, 19*). Converting large quantities of data into useable information can lead to a more complete understanding of a pathway, development of metabolic markers specific to a set of prescribed conditions, and more directed hypothesis development for further study (*18, 19*). Furthermore, metabolic profiling of constituents from multiple pathways is becoming increasingly important, given evidence demonstrating the significant function of allosteric modification of metabolic events in conjunction with posttranscriptional regulation (*20, 21*). Chemical phenotyping of different tissues of multiple plant species including potato (*22, 23*), *Arabidopsis* (*24*), and tomato (*20, 25, 26*) using global metabolic profiling can distinguish metabolomes affected by environmental conditions and/or genotype.

An established gas chromatography–mass spectroscopy (GC-MS) method for analysis of trimethylsilyl (oxime) derivatives in plants (*22*) has been optimized for apple peel analysis. In the present study, the GC-MS method combined with high-performance liquid chromatography−ultraviolet/visible mass spectroscopy (HPLC-UV/vis-MS) was used to study changes in primary and secondary metabolic pathways in apple peel related to light exposure and cold storage. Specifically, individual components were characterized and identified and metabolic fluctuations analyzed following different durations of artificial light treatment and following 6 months of cold-air storage. It was hypothesized that prestorage light exposure alters the poststorage metabolic profile of the exposed peel and that these metabolic alterations are detectable following the cold storage period.

MATERIALS AND METHODS

Fruit Source and Preharvest Treatment. 'Granny Smith' apples, a scald susceptible cultivar, conventionally grown at the Columbia View experimental plots near Orondo, WA, in 2006 were covered using commercial double-layer paper apple bags (Kobayashi Bag Manufacturing Co., Iida, Japan) 57 days after full bloom (DAFB) to delimit sunlight prior to postharvest irradiation. The outer bags were gray outside and black inside; the inner bags were green. The fruit were harvested 140 DAFB (1 month prior to commercial harvest) with bags intact and transported to the laboratory where bags were removed.

Light Treatment. Fruit (36 per treatment) were washed in water, allowed to air-dry, and then were placed approximately 15 cm (apple surface to lights) under two 2-outlet 1.22 m (length) fluorescent light banks, each containing one 40 W Sylvania (Versailles, KY) Cool White Deluxe fluorescent bulb and one 40 W Phillips (Somerset, NJ) fluorescent UV lamp. The irradiated area was delimited with aluminum foil. The intensity of key wavelength ranges was measured at various points within the chamber (*5*). The light treatment chamber temperature was 25 °C, and apples were irradiated for 0, 4, 18.5, 25, and 48.5 h. Additional fruit remained in the dark at 25 °C for the duration of the light treatment. Fruit were randomly removed from different positions in the light treatment chamber to account for any light variation within the treatment chamber; then the peel (0.5–1 mm thickness) was sampled from 18 fruit per treatment (six replications per treatment, three fruit per replication) using a potato peeler and then frozen in N_2(l). The remaining 18 fruit from each treatment were placed in air storage at 1 °C for 6 months and then at 20 °C for 4 days prior to peel sampling using the same scheme outlined previously. Peel from fruit stored for 6 months was also sampled from the unexposed side separately.

Metabolite Extraction. Frozen peel was cryogenically milled to a fine powder and stored at −80 °C prior to metabolite analysis. Frozen peel powder (100 mg per replication, six replications per treatment) was weighed into N_2(l)-cooled 1.5 mL screw-top microcentrifuge tubes to which ∼100 μL of 0.5 mm (diameter) soda lime glass beads (BioSpec Products, Inc., Bartlesville, OK) was added. The tissue/bead mixture remained N_2(l)-cooled until initiation of the extraction procedure when 1 mL of MeOH was added. Samples were then shaken vigorously using a Mini Beadbeater (BioSpec Products, Inc.) for 1 min and then floated in a 70 °C water bath for 15 min to inhibit any sample degradation resulting from intrinsic catalytic activity (*22*). Samples were then sonicated for 5 min at 25 °C in an ultrasonic bath followed by centrifugation for 2.5 min at 16200*g*.

Derivatization and GC-MS Analysis. Methoxyamination/trimethylsilylation followed a procedure similar to that described by Roessner et al. (*22*). Supernatant aliquots (10 or 100 μL) were placed into borosilicate glass test tubes followed by the addition of 45 μL of phenyl β-D-glucopyranoside (291 ng μL^{-1} in methanol) internal standard solution. The mixture was dried under a stream of N_2(g); then the residue was dissolved in 125 μL of methoxyamine (20 mg mL^{-1} in anhydrous pyridine) solution and incubated for 90 min at 30 °C. Next, 125 μL of *N*,*O*-bis(trimethylsilyl)trifluoroacetaminide (BSTFA) was added and the mixture incubated for 30 min at 37 °C. Samples were then transferred to glass vials containing deactivated glass inserts and analyzed by GC-MS.

Derivatized extract (0.2 μL) was injected into a 6890N GC coupled with a 5975B mass selective detector (MSD) using a 7683B automatic injector (Agilent Technologies, Palo Alto, CA). Samples were volatilized in a 230 °C splitless inlet lined with an unpacked 4 mm internal diameter, deactivated, tapered-bottom glass liner. Further focusing of the sample was accomplished using a pulsed injection technique that maintained a He carrier gas linear velocity of 66 cm s^{-1} for the first 0.25 min, reducing it to 40 cm s^{-1} thereafter. The GC column was a HP-5MS (Agilent Technologies) (30 m × 250 μm × 0.25 μm). The oven initial temperature was 40 °C held for 2 min followed by a 18 °C/min increase to a final temperature of 330 °C that was held for 6 min. The detector was operated in EI mode with transfer line, source, and quadrupole temperatures maintained at 250, 150, and 230 °C, respectively. Mass spectra ranging from *m*/*z* 30 to *m*/*z* 500 were recorded.

LC-UV/vis-MS Analysis. LC-UV/vis-MS analysis was used to analyze underivatized methanolic extracts for targeted anthocyanins and quercetin glycosides. Samples (10 μL of methanolic extract) were injected into a Series 1100 HPLC system (Agilent Technologies, Palo Alto, CA) equipped with a 5 μm Agilent Hypersil ODS (4.0 × 125 mm) reverse-phase column, a G1315B diode array detector (UV/vis), and a G1946D single quadrupole mass selective detector (MSD) using an APCI source. Elution solvents used for a linear gradient were (A) 0:95:5 and (B) 95:0:5 methanol/deionized water/formic acid. The column temperature and mobile phase flow rate were 20 °C and 0.5 $mL \cdot min^{-1}$, respectively. The mobile phase was comprised entirely of solvent A for the initial 2 min after sample injection, followed by a linear gradient of solvent A plus solvent B until reaching 100% solvent B at 25 min, and then remaining entirely solvent B until 40 min. The eluate was first analyzed by the DAD and then the MSD. The DAD continuously monitored and recorded spectra (200–700 nm) for the entire analysis.

The APCI spray chamber conditions were drying gas (N_2) flow 5 $L \cdot min^{-1}$, drying gas temperature 350 °C, nebulizer pressure 414 kPa, vaporizer temperature 425 °C, and coronal discharge 4 μA. The fragmentor and capillary potentials were 130 and 4000 V, respectively. The MSD was adjusted to monitor positive ions in the scanning mode, continuously monitoring and recording entire mass spectra within a *m*/*z* 100–1000 range.

Data Acquisition, Deconvolution, and Peak Identification. User-defined libraries were generated using the automated mass spectral deconvolution and identification system (AMDIS; National Institute of Standards,) to deconvolute GC-MS results and identify distinct chromatographic components. Retention indices (RI) were generated for each sequence by comparing the retention times of C10−C40 hydrocarbons evaluated under the same conditions as the samples with the retention times of sample components. Individual libraries made from samples from each treatment were compared and redundant components eliminated. From these libraries, mass spectral tags (MSTs) were cataloged using the RI coupled with key mass spectral features and calibration tables generated using Chemstation (G1701DA rev. D; Agilent, Palo Alto, CA). The Qedit macro was used to evaluate each compound and provide peak areas for components. Mass spectral

Table 1. Compounds Identified in Methanolic Granny Smith Apple Peel Extracts as Trimethylsilyl (Oxime) Derivatives Using GC-MS or Underivatized Using GC-MS or LC-UV/vis-MS

RI	compound	abbreviation	target ion	RI	compound	abbreviation	target ion
1054	pyruvic acid	pyr	174	1737	rhamnose	rhamn	117
1073	hexanoic acid	hex	173	1742	xylitol/arabinitol	xylOH/arabOH	307
1108	alanine	ala	116	1745	adonitol	ribOH	217
1217	1-aminocyclopropane-1-carboxylic acid	1-ACC	202	1750	fucose	fuc	231
1222	valine	val	144	1777	glycerol 3-phosphate	g 3-P	299
1245	norvaline	norval	144	1822	shikimic acid	shik	204
1250	benzoic acid	benz	179	1840	citric acid	cit	273
1276	leucine	leu	158	1890	quinic acid	quin	345
1280	phosphoric acid	PO_4	299	1901	fructose	fruct	364
1297	isoleucine	ile	158	1931	glucose	gluc	291
1302	proline	pro	142	1960	mannitol	mannOH	319
1307	maleic acid	maleic	245	1969	sorbitol	sorb	319
1308	glycine	gly	174	1970	ascorbic acid	asc	374
1317	succinic acid	succ	147	1972	galacturonic acid	galacturonic	333
1336	glyceric acid	glyceric	147	2079	mucic acid	mucic	333
1349	fumaric acid	fum	245	2132	inositol	inos	305
1365	serine	ser	204	2211	linoleic acid	C18:2	337
1394	threonine	thr	291	2214	oleic acid	C18:1	357
1433	β-alanine	β-ala	248	2247	tryptophan	trp	202
1456	homoserine	hser	218	2352	fructose 6-phosphate	f 6-P	315
1463	erythrose	eryth	205	2366	glucose 6-phosphate	g 6-P	387
1482	citramalic acid	citmal	247	2703	sucrose	suc	451
1495	malic acid	mal	235	2809	maltose	malt	361
1509	α-farnesene[a]	α-farn	93	2825	*S*-adenosylmethionine	SAM	236
1517	erythritol	eryOH	217	2839	squalene[a]	squal	69
1518	salicylic acid	salic	267	2886	(−)-epicatechin	epicat	368
1525	aspartic acid	asp	232	2906	catechin	cat	368
1527	methionine	met	250	3147	5′-caffeoylquinate	chlor	345
1530	5-oxoproline	oxopro	156	3152	α-tocopherol	vit E	430
1531	hydroxyproline	proOH	230	3396	β-sitosterol	sito	129
1536	γ-aminobutyric acid	GABA	304	3488	raffinose	raff	361
1566	cysteine	cys	220	3510	phloretin 2-glucoside	phlor	342
1572	threonic acid	threon	292	3655	ursolic acid	ursolic	203
1580	2-oxoglutaric acid	α-kg	198	NA	cyanidin 3-galactoside	cy3 gal	NA
1601	asparagine	asn	116	NA	cyanidin 3-glucoside	cy3glu	NA
1625	glutamic acid	glu	246	NA	quercetin 3-galactoside	q3 gal	NA
1640	phenylalanine	phe	218	NA	quercetin 3-xyloside	q3xyl	NA
1666	xylose/arabinose	xyl/arab	217	NA	quercetin 3-arabinoside	q3arab	NA
1696	ribose	rib	307	NA	quercetin 3-rhamnoside	q3rhamn	NA

[a] Underivatized using GC-MS.

comparison with spectra cataloged in NIST05 (National Institute of Standards) and mass spectral interpretation aided in tentative identification of many of the components. Compound identifications are based on comparison of sample compound spectra and RIs with those of authentic standards. All authentic standards were purchased from Sigma-Aldrich-Fluka (St. Louis, MO) except cyanidin 3-galactoside, cyanidin 3-glucoside, quercetin 3-*O*-galactoside (Indofine, Somerville, NJ), quercetin 3-*O*-arabinoside, and quercetin 3-*O*-xyloside (Plantech, Reading, U.K.).

For LC analytes including cyanidin 3-galactoside (idaein), cyanidin 3-glucoside (kuromanin), quercetin 3-*O*-galactoside (hypericin), quercetin 3-*O*-xyloside (reynoutrin), quercetin 3-*O*-arabinoside (avicularin), and quercetin 3-*O*-rhamnoside (quercetrin), UV/vis traces were used for semiquantification using wavelengths previously described (*5*).

Statistical Analysis. Raw chromatographic data were corrected by comparison of phenyl β-D-glucopyranoside (ISTD) in each sample with that in an external standard and then the sample fresh weight. These values were transformed by mean centering and log transformation prior to principal component analysis (PCA) using the SAS 9.1 software package (SAS Institute, Cary, NC). By modeling data using PCA, variance in a multivariate data set containing many instrument-derived component variables is reduced to a few orthogonal variables, called principal components, that each account for a portion of the variance in the data set. By plotting treatment scores or component loading values derived from PCA in the space defined by the largest principal components, associations between treatment and metabolic components can be uncovered. Data were analyzed together or separated by storage duration (initial or 6 months of storage) prior to analysis. Treatment differences were evaluated by plotting principal component scores against each other. Unpaired *t* tests were performed using Microsoft Excel (Redmond, WA).

RESULTS AND DISCUSSION

Identification and Classification of Metabolic Profile. Metabolic components (264) in apple peel were distinguished and evaluated using GC-MS and LC-UV/vis-MS. Most components detected using GC-MS were trimethylsilyl or trimethylsilyl (oxime) derivatives. A total of 78 components were identified using RI (GC-MS only) and spectral comparison with those of authentic standards (**Table 1**). Other MSTs, while contributing to statistical analysis, are not reported. Compounds detected in apple peel included many of the same components detected in potato tubers (*22*), tomato leaf (*26*), and tomato fruit (*20, 26*) using similar methods. Other compounds related to primary metabolism or purportedly present in apple fruit tissue were targeted but not detected in apple peel. These include the TCA metabolites isocitrate (RI = 1832) and aconitic acid (RI=1753). Also, caffeic acid (RI = 2149) and *p*-coumaric acid (RI = 1949), both components found in commercial apple juice (*27*), were not detected in this study. Trimethylsilyl derivatives of cyanidin 3-galactoside (cy3 gal) (RI = 3470) and quercetin 3-*O*-galactoside (q3 gal) (RI = 3725) were detected using GC-MS in long duration light-treated peel. As LC-UV/vis-MS was a more sensitive method for detecting these flavonoids, GC-MS data were not included in the statistical evaluation. Other

quercetin glycosides detected using LC-UV/vis-MS were not detected using GC-MS.

Hexanoic acid was detected in the method blank (no tissue) as well as in apple peel. For evaluation, hexanoic acid values obtained from method blanks, run alongside samples, were subtracted from component values acquired from samples. All components could be evaluated qualitatively and semiquantitatively using their specific RI and a target ion, a unique ion trace within a specific chromatographic environment, that represented the compound. The most abundant fragment for a specific compound was not always chosen to avoid interference from other components. Degradation of samples at all stages of processing was evaluated prior to these tests. The elimination of water from and timely evaluation of silylated samples was crucial to limit the presence of artifacts. Changes in samples were imperceptible within the time frame of our GC-MS and LC-UV/vis-MS evaluations. Silylated glutamic acid converted to 2-oxoproline, apparently in the GC inlet, and was monitored during analysis sequences by periodic injections of silylated glutamic acid standards. This phenomenon was rectified by timely replacement of deactivated glass inlet liners.

PCA Models of Metabolic Profile. Irradiation altered peel appearance on the exposed side of the fruit incrementally, with increasing reddening as treatment duration progressed. Altered appearance remained even following storage. Likewise, PCA scores indicate that metabolomic differences were present following light exposure and/or storage, demonstrating the influence of the evaluated components for estimating changes in the entire metabolome. Metabolic differences due to light exposure duration are present in models based on all samples as well as in samples collected at the end of light exposure (initial) or after storage (**Figure 1**). Increasing duration of light exposure generated more positive principal component 2 (PC2) (18.9%) scores when all observations were considered and more positive PC scores along the PC1 axis for models containing only initial (28.1%) samples or negative scores when only storage (39.1%) samples were considered. Where all of the samples were considered (**Figure 1A**), component scores for PC1 (36.1%) reflect differences resulting from storage duration, with initial samples having positive and cold-stored fruit negative scores. PC2 (29.0%) from the storage results model (**Figure 1C**) and from the initial results model (11.8%) (**Figure 1B**) had no obvious connection to treatment.

Sample differentiation due to light duration was pronounced in nonstored fruit where a clear separation between fruit receiving none or 4 h of light and those receiving 18.5, 25, and 48.5 h of light was observed. The effects of irradiation were still present following storage with fruit irradiated for 18.5, 25, or 48.5 h grouped together and separate from unexposed or dark control fruit (**Figure 1C**). The 4 h exposure was in between the long exposure and nonexposed fruit, indicating continuing physiological changes during storage provoked by irradiation. Component scores from the unexposed side of irradiated and then stored apples clustered together on the PC1 axis, segregating these samples from those obtained from fruit stored but not exposed to light. Differences in component scores due to light exposure represent transformation of individual metabolite levels within the metabolome. However, to recognize which components are responsible for these alterations, loading plots were evaluated.

Fruit ripening is reflected by physiological changes associated with alteration of gene expression, protein synthesis/activity, and metabolism (*28–30*). Tissue content of compounds identified in this study linked to apple fruit ripening increased during

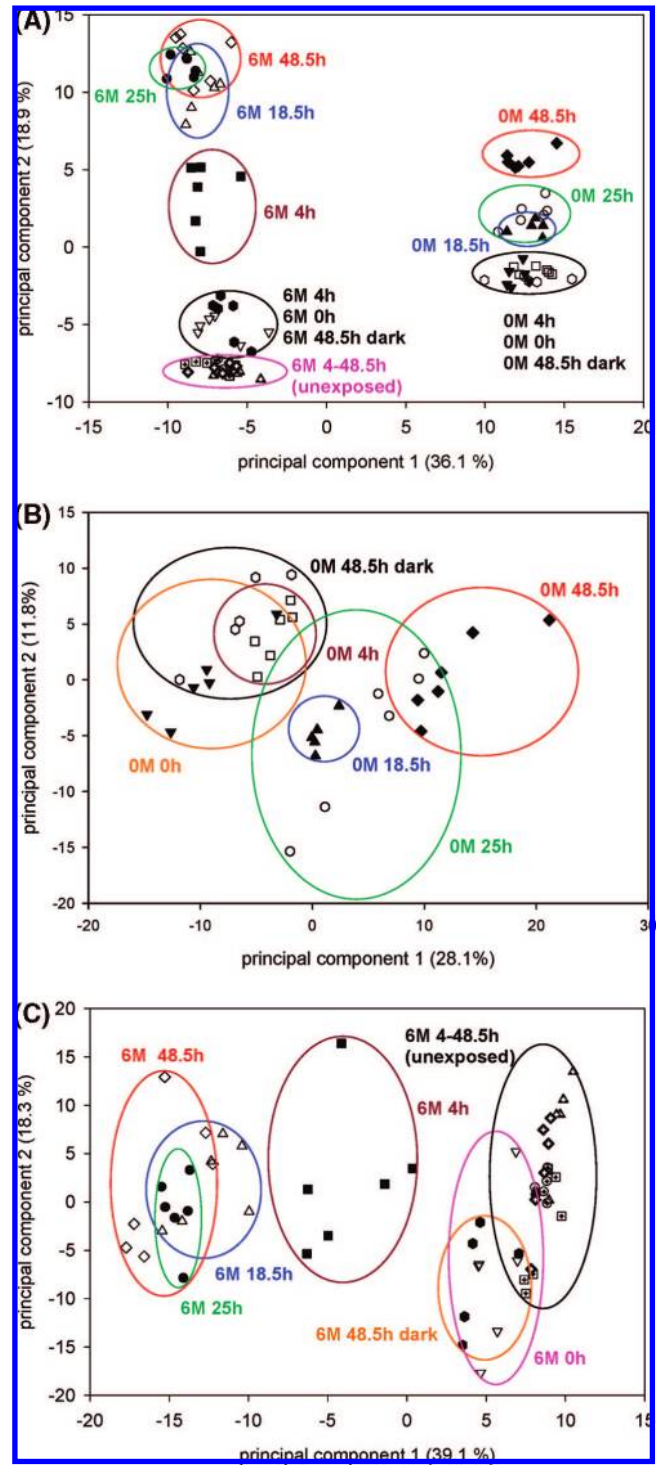

Figure 1. Principal component analysis of the metabolic profile of Granny Smith peel following irradiation for 0, 4, 18.5, 25, and 48.5 h or 48.5 h in the dark and prior to or after 6 months of storage at 0 °C. Peel was sampled from both the exposed and the unexposed side of cold-stored fruit. Component score plots from models containing all results (**A**), initial results (**B**), and 6 month storage results (**C**).

storage, including α-farnesene, a component that increases with ripening in Granny Smith peel (*31*), and galacturonic acid, a compound associated with ripening-related softening (*32*) (**Figure 2A**). Conversely, the concentration of malic acid, the primary substrate for apple fruit respiration (*33, 34*) and an important flavor component (*33*), decreases during apple ripening. Segregation of these compounds based on changes in concentration known to occur during apple ripening demon-

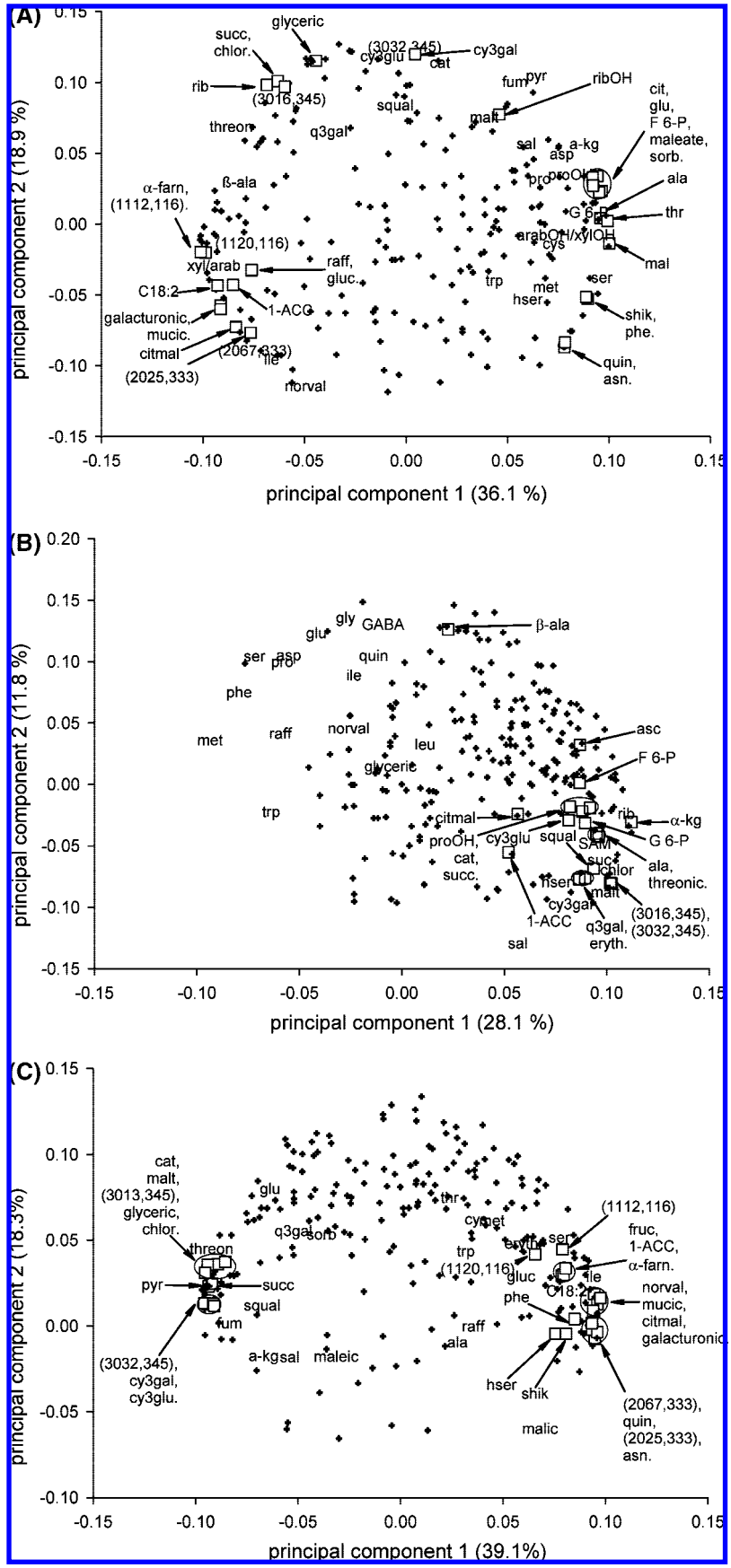

Figure 2. Principal component analysis of the metabolic profile of Granny Smith peel following irradiation for 0, 4, 18.5, 25, and 48.5 h or 48.5 h in the dark and prior to or after 6 months of storage at 0 °C. Peel was sampled from both the exposed and the unexposed side of cold-stored fruit. Loading plots from models containing all results (**A**), initial results (**B**), and 6 month storage results (**C**). Only selected unassociated variables are labeled, either in position or with a white square. Positions of other components are designated with black crosses.

strates the validity of these PCA models and their utility for evaluating other metabolites and unknown MSTs.

A number of ripening-related changes in Granny Smith apple peel components were identified using PCA modeling, including changes in compounds not reported previously to be present in apple fruit. Differences in amino acid content were apparent following ripening (**Figure 2A**). glu, phe, met, ser, ala, trp, asn, thr, pro, asp, hser, and cys were associated with unripe tissue, while ile, ostensibly a precursor for the 2-methylbutyl moiety of many apple volatiles (*35*), was linked to ripe fruit. Other amino acids linked to ripe fruit included the ethylene precursor 1-aminocylclopropane-1-carboxylic acid (1-ACC), norvaline, β-alanine, and two MSTs (RI, target ion: 1112, 116; 1120, 116) with mass spectra similar to that of ala. Ile increases in Granny Smith peel during ripening (*36*), although another report (*15*) demonstrates a general decline in the accessory tissue content of primary amino acids, including ile, during cold storage.

Alteration of organic acid and sugar contents was also identified using the storage PCA model. Peel levels of malic, maleic, pyruvic, α-ketoglutaric, citric, and quinic acids were associated with unripe fruit, while succinic, threonic, and citramalic acids were associated with stored fruit. Fructose 6-phosphate and glucose 6-phosphate were also associated with unripe peel. Some pentose sugars, including arabinose/xylose and ribose, were associated with stored fruit. Sorbitol was highest prior to storage. The relationship between sorbitol metabolism and storage is purportedly cultivar dependent (*16*). Glucose was associated with stored peel while sucrose and fructose were not strongly associated with storage contrary to previous reports (*16, 37, 38*). Also, while these were peel samples, other studies referenced assayed juice samples where metabolism during cold storage may differ.

Apple peel phenylpropanoid content of many apple cultivars, including Granny Smith (*39*), is altered in response to artificial (*6, 7, 40*) or natural (*4*) light exposure. Increases in cy3glu and cy3gal content, two constituents responsible for apple red peel color, as well as other phenylpropanoids including q3gal and chlorogenic acid (5′-caffeoylquinic acid) were observed. These changes were also reflected in PCA models describing all fruit, only unstored fruit (**Figure 2B**), or only stored fruit (**Figure 2C**) .

Sorting results by storage duration prior to modeling enabled irradiation-related changes to be identified that were not previously found. Evaluation of metabolic profiles of irradiated fruit prior to storage revealed a number of associations related to primary metabolism. trp, ile, leu, phe, asp, pro, glu, met, gly, β-alanine, γ-aminobutyric acid (GABA), quinic acid, and norvaline were more associated with unirradiated fruit while ala, homoserine, hydroxyproline, threonic acid, citramalic acid, succinic acid, and α-ketoglutaric acid were linked to light exposure (**Figure 2B**). *S*-adenosylmethionine (SAM) and 1-ACC, both ethylene precursors, were associated with increasing light exposure duration as were ascorbic and salicylic acids. Light treatment also affected peel sugar content, linking maltose with increasing and raffinose with decreasing light exposure. Higher ribose and erythrose content was also associated with longer light treatment duration.

Many light-induced metabolic profile changes were detectable following 6 months of air storage as were additional changes that occurred during storage. Compounds including cy3gal, cy3glu, q3gal, chlorogenic acid, α-ketoglutaric acid, salicylic acid, and maltose had a continued association with longer light treatment duration (**Figure 2C**). Raffinose remained linked with shorter light exposure duration. Light exposure was found to affect levels of many organic acids, amino acids, and sugars in stored fruit, some of which were not immediately altered during light treatment. Amino acids including ala, thr, cys, met, ser, homoserine, trp, and phe scores were positively loaded on the PC1 axis, while glu, 1-ACC, and norvaline scores were negatively loaded. Likewise, the content of citramalic, glyceric, succinic, threonic, pyruvic, fumaric, malic, quinic, shikimic, and maleic acid scores was altered during storage by prestorage irradiation on the PC1 axis. Sugar acids such as galacturonic and mucic acid as well as 2025, 333 and 2067, 333, two compounds with similar spectra, were closely associated in a positively loaded region of the PC1 axis.

Irradiation-Related Metabolite Fluctuation. PCA modeling is useful for directing consideration to germane metabolic components but does not illuminate temporal relationships during a time-course experiment. Effects of light exposure on specific compounds related to pigment biosynthesis, carbohydrate utilization, acid metabolism, and amino acid metabolism identified using PCA models were examined further by plotting relative response for individual compounds against light exposure duration (**Figures 3** and **4**).

Exposure of apple fruit to artificial light is known to elicit red coloration following an induction period (*6, 7, 41–43*). In the current study, the cy3gal biosynthesis rate increased between 4 and 18.5 h of light exposure, and this change was still detectable following storage. Similarly, light exposure enhanced cy3glu, q3gal, and chlorogenic acid levels although this pattern was not the same following storage. Continued anthocyanin biosynthesis in cold storage following fruit exposure to artificial light has been reported for 'Gala' apple fruit (*6*).

Peel content of many other components also changed with irradiation. Increased maltose levels following light exposure as well as after storage suggests that altered starch metabolism may be induced by artificial light exposure and this alteration persists for an extended period. Erythose content increased dramatically during light exposure, but altered levels were not detectable following 6 months of cold storage. Conversely, raffinose content diminished with increasing light exposure and remained lower compared with unexposed fruit following storage. As with maltose biosynthesis, raffinose metabolism during light exposure may result from alterations in carbohydrate metabolism induced by artificial light. Light exposure resulted in changes in sorbitol content only following storage. Alteration of sorbitol content, a major sugar alcohol component in apples (*16*), may affect fruit sweetness.

Amino acid fluctuation also varied with light exposure. While homoserine flux was very similar to that of cy3gal during light exposure, leu, ile, and phe exhibited a unique pattern with increasing light exposure. The content of leu, ile, and phe increased between 0 and 4 h and then decreased following the onset of the accumulation in cy3gal synthesis. This pattern suggests a role for these components during induction of anthocyanin synthesis. Previous work demonstrates upregulation of genes involved in flavonoid biosynthesis during this period (*43*). As phe is a precursor for the phenylpropanoid pathway (*1*), this pattern may indicate allosteric regulation where substrate builds up to a critical amount and then depletes once product biosynthesis begins.

Light-altered amino acid fluctuations were also evident following storage. Glu increased with light exposure duration while norvaline levels decreased. Phe levels remained altered by light treatment following storage, and, as with ala, ser, and thr, a distinctive poststorage fluctuation pattern was evident. Levels of these amino acids diminished in peel treated with 4 h

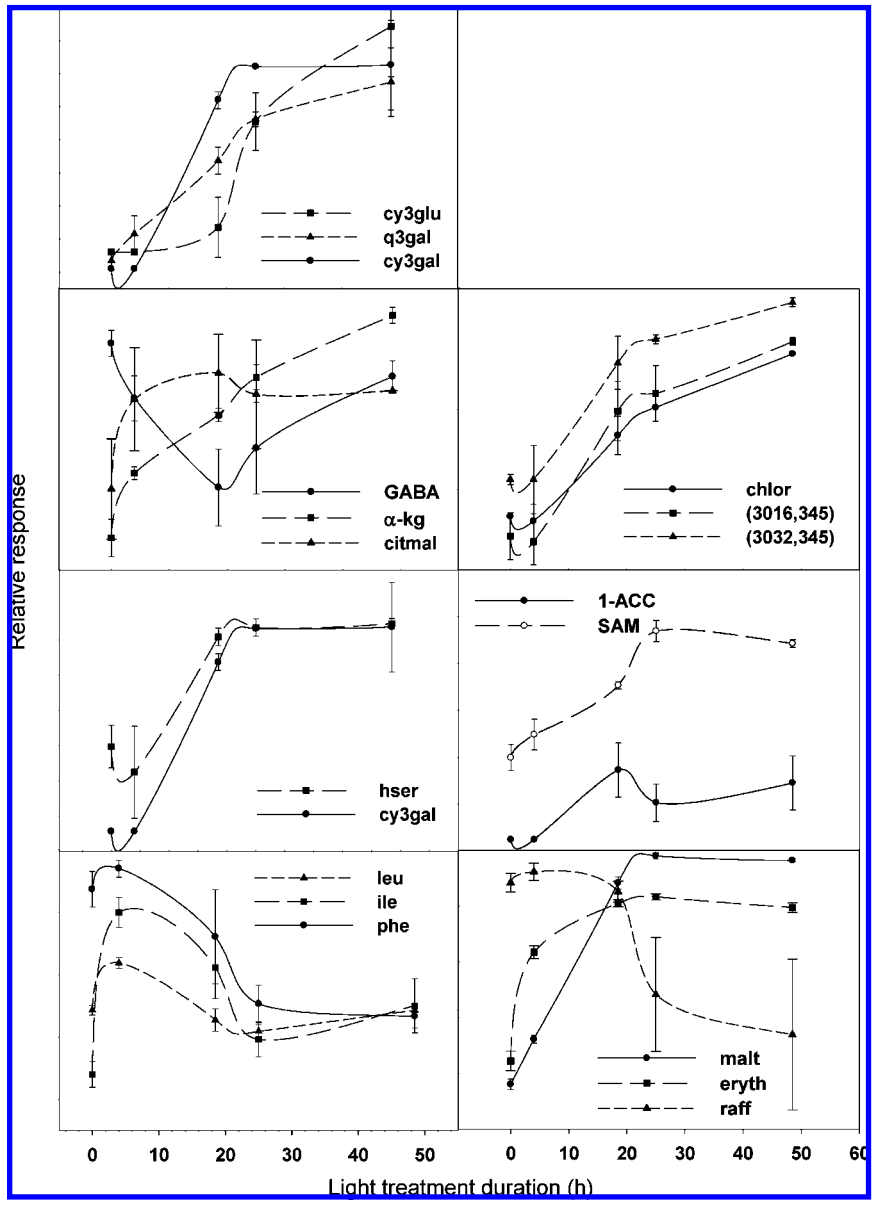

Figure 3. Fluctuation of selected metabolites from Granny Smith peel treated with 0–48 h UV−white irradiation. Peel was sampled immediately following irradiation. Error bars designate standard error ($n = 6$).

of light but were more abundant in peel exposed to artificial light for 18.5 h. Given the temporal association between content of these amino acids and cy3gal synthesis, this pattern suggests that these amino acids contribute to anthocyanin synthesis during storage or metabolic perturbation triggered by initiation of anthocyanin production.

During the first 18.5 h of irradiation, 1-ACC, along with its precursor, SAM, increased, indicating rising ethylene pathway activity. Increased 1-ACC content and ethylene evolution from foliar tissues, provoked using ultraviolet B light, have been reported previously (*44–46*), and ethylene generating compounds enhance anthocyanin synthesis in apple peel (*47*). Surprisingly, the opposite trend in 1-ACC content was present following storage, suggesting that prestorage light exposure may continue to modulate ethylene biosynthesis in affected tissue following storage.

Poststorage malic and citramalic acid levels decrease as light exposure duration increases. Other evidence indicates that increased natural light exposure, related to fruit on-tree position, reduces malic acid levels or titratable acidity, albeit following shorter periods of or no cold storage (*48, 49*). Conversely, poststorage levels of other acids including pyruvic, fumaric, glyceric, threonic, and α-ketoglutaric acid were elevated with increasing light exposure duration, suggesting differences in acid metabolism between these compounds. Two primary acid metabolites influenced by light treatment duration prior to harvest were citramalic and α-ketoglutaric acid for which content increased with increasing light exposure duration.

Triggered by the dissolution of the middle lamella and cell-wall degradative processes, flesh softening is another ripening-related process (*29*). Free pectic acids are among the products of middle lamella degradation (*32*). In the current study, poststorage galacturonic acid and mucic acid, both pectic acids, as well as two components (2025, 333; 2067, 333) with similar mass spectra, were altered by light exposure, and all of these compounds decreased with increased light exposure duration. These results suggest that prestorage light exposure may actually impede the breakdown of the middle lamella and, thereby, softening. Lending support to this inference is previous work demonstrating that flesh firmness is higher on the sunburned portions of apple fruit relative to nonsunburned areas (*11*). Additional evidence indicates that associations exist between

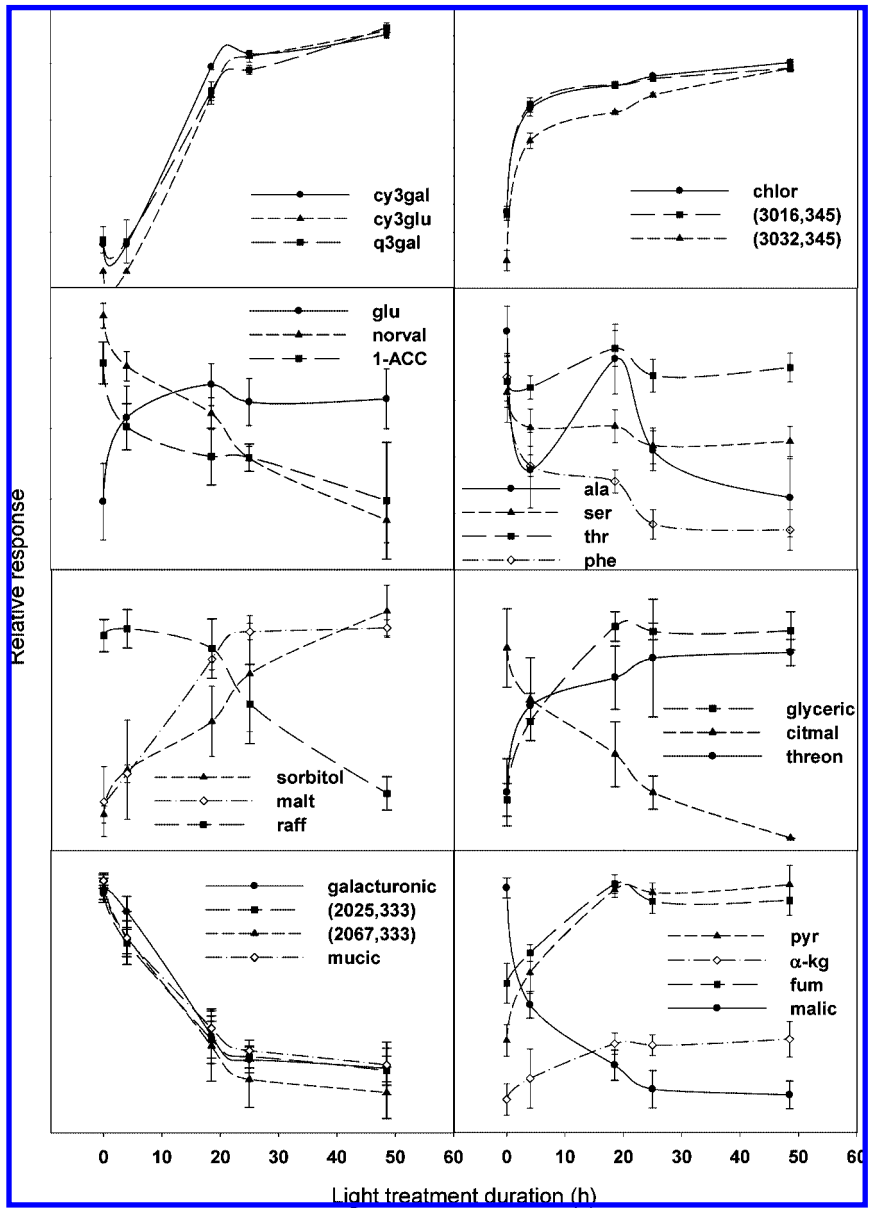

Figure 4. Fluctuation of selected metabolites from Granny Smith peel treated with 0–48 h UV−white irradiation and stored for 6 months at 0 °C. Peel was sampled immediately following removal from storage. Error bars designate standard error ($n = 6$).

firmness and nondamaging preharvest light exposure based on tree position or orientation (*48, 50*), although no relationship was apparent immediately after UV irradiation (*7*).

Postharvest Metabolomics. Apple maturation is a complex event incorporating intricate physiological processes represented by changes in the transcriptome, proteome, and the metabolome. Addition of preharvest factors such as light environment transforms this process, creating a new phenotype, even within the same fruit, that may translate into changes affecting management and marketability of the product.

In this case, profiling a portion of the metabolome and subsequent statistical evaluation provided evidence of metabolic alterations prior to and following storage and then pointed to a number of less conspicuous poststorage metabolic alterations in important pathways related to prestorage irradiation. Along with perturbations in other pathways, changes in metabolism of components involved in fruit quality such as flavonoids, ethylene, malic acid, and pectic acids were demonstrably modified by irradiation. Not only does this show how metabolic profiling can be a useful evaluation tool for further understanding of the biochemical basis of postharvest physiology, but its employment could also yield metabolic markers useful for chemical phenotyping or even storage and marketing decisions.

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