Effect of postharvest ultraviolet-C treatment on the proteome changes in fresh cut mango (Mangifera indica L. cv. Chokanan)

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Abstract

BACKGROUND: Postharvest treatments of fruits using techniques such as ultraviolet-C have been linked with maintenance of the fruit quality as well as shelf-life extension. However, the effects of this treatment on the quality of fruits on a proteomic level remain unclear. This study was conducted in order to understand the response of mango fruit to postharvest UV-C irradiation.

RESULTS: Approximately 380 reproducible spots were detected following two-dimensional gel electrophoresis. Through gel analysis, 24 spots were observed to be differentially expressed in UV-C treated fruits and 20 were successfully identified via LCMS/MS. Postharvest UV-C treatment resulted in degradative effects on these identified proteins of which 40% were related to stress response, 45% to energy and metabolism and 15% to ripening and senescence. In addition, quality and shelf-life analysis of control and irradiated mangoes was evaluated. UV-C was found to be successful in retention of quality and extension of shelf-life up to 15 days. Furthermore, UV-C was also successful in increasing antioxidants (total flavonoid, reducing power and ABTS scavenging activity) in mangoes.

CONCLUSION: This study provides an overview of the effects of UV-C treatment on the quality of mango on a proteomic level as well as the potential of this treatment in shelf-life extension of fresh-cut fruits.

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Keywords: postharvest; UV-C treatment; fruit quality; allergens; proteomic changes; Chokanan mango

INTRODUCTION

Mango (Mangifera indica L.) is classified as a climacteric fruit which ripens at elevated rates following harvest.1 This characteristic of the mango leads to disease susceptibility, sensitivity to low storage temperature and perishability, which results in the short shelf-life of mangoes.2 Although preharvest metabolic processes lead to changes in texture, pigmentation, taste and aroma of the fruits that are highly beneficial,3 these processes which continue following harvest can cause undesirable outcomes which eventually results in the short shelf-life of mangoes of less than 10 days under ambient tropical conditions.4 Several postharvest treatments have been successful in extending the shelf-life of mangoes through retardation of these metabolic changes. The treatments include heat disinfestations,5 chitosan coating,6 hot water, chemicals, wax and film packaging,6 as well as ultraviolet-C (UV-C) light irradiation.7

The UV-C irradiation is a dry and non-chemical treatment that has emerged as an effective and more feasible alternative postharvest treatment of fruits for commercial application. The UV-C treatments have been used for maintaining the quality as well as reducing postharvest losses in fruits.8,9 Fruits that were exposed to UV-C were found to exhibit delayed ripening,10 delayed senescence11,12 as well as reduced spoilage.13,14 Several researchers have reported on the immediate effects of UV-C treatment on the quality of fruits while the underlying changes at a proteomic level that occurs as a result of UV-C irradiation remains unclear.15–20 UV-C irradiation has been known to cause either generation of reactive species, which results in protein thiol oxidation, or protein damage through photolysis. Therefore, proteomic analysis of the effects of UV-C irradiation is of significant importance.21 As publications on the effect of postharvest UV-C irradiation on the proteome of mango with relation to quality maintenance remains scarce, detailed molecular information on protein expression changes following treatment remains unclear.

Proteomics has emerged as a highly reliable tool for the screening of a large set of proteins which eventually enables the identification of important proteins as well as their functionality and the roles that they play in various biological processes.22 This leads to an enhanced understanding of the molecular basis of fruit quality. Despite the complexity of an organism’s proteome, the use of differential proteomics enables one to discriminate the specific proteins affected in a certain physiological conditions.23 Up to date,
proteomics has been exploited to study fruit development and ripening, differences between cultivars, stress resistance, postharvest conditions and physiological disorders. The effects of UV-C treatment on the proteomic changes in mango fruit has not been reported, hence this study will offer new insights on the physiological changes that occur in mango fruit as a result of UV-C irradiation on a proteomic level.

MATERIALS AND METHODS
Sample preparation
Mature mango (Mangifera indica L. cv. Chokanan) fruits free from external defects were harvested from a mango plantation in Kangar, Perlis, Malaysia. The fruits were rinsed under tap water, dipped in a 5% benomyl solution for 1 min, rinsed again with water, air dried and left to ripen at room temperature (25 ± 2 ºC). Maturity of mangoes was determined based on visual assessment of the peel colour according to the maturity indices standard specified by the Federal Agricultural Marketing Authority (FAMA) Malaysia. Fruits were peeled, cleaned, and thinly sliced (5 mm) with the aid of a dial thickness gauge (Mitutoyo MI 7305, Kawasaki, Japan). The fruits were then cut into uniform discs with a borer (diameter of 2 cm). Twelve fruit disc samples from a single fruit were placed on a sterilised Petri dish (15 × 100 mm; 10 mm depth) and exposed to a UV light (germicidal lamp with a peak emission of 254 nm, Biological Safety Cabinet Class II, 240 V, 50 Hz, 10 A) with a distance of 15 cm from the lamp to the surface of the samples. The samples were irradiated for 15, 30 and 60 min with samples receiving UV doses of 1.764 J m⁻², 3.525 J m⁻² and 7.056 J m⁻² respectively. For protein analysis, untreated (control) and treated samples were stored at 4 ºC for 15 days before analysis. Sample discs from nine different fruits were pooled and considered as one biological replicate. Nine different batches of biological replicates were used for each treatment in this study (n = 9).

Quality analysis
Quality analysis of treated fruit samples were carried out to evaluate the effects of UV-C treatment on the quality of fruits during storage at 4 ºC. Quality analysis was carried out immediately after treatment (Day 0), and after storage at 4 ºC for up to 15 days (Days 5, 10 and 15). Untreated Chokanan mango samples were used as controls.

Physicochemical analysis (pH, sugar acid ratio and sugar content)
The pH of samples was determined using a pH meter (Hanna Microprocessor pH 211; Hanna, Sarmeola di Rubano, Italy) at 25 ± 1 ºC. The sugar acid ratio was calculated by first determining total soluble solids value via a digital refractometer (Atago PR-1 digital refractometer, Tokyo, Japan) at 25 ± 1 ºC according to the manufacturer’s protocol and results expressed in standard °Brix unit followed by determination of titratable acidity through the method described by Sadler and Murphy and results expressed in %TA 100 g⁻¹ of sample. The sugar acid ratio was calculated according to the formula: sugar acid ratio = °Brix value/% acid. The sugar content was determined by high-performance liquid chromatography as described by Roha et al. Samples were crushed in a food processor with ratio of sample to purified water of 1:1 to obtain sample extract. The extract was then filtered through muslin cloth and centrifuged at 360 × g for 10 min. The resulting supernatant was filtered through a Millipore 0.45 µm membrane and the filtrate was used for analysis of total sugar.

Extraction of phenolic compounds and antioxidants
Mango samples were mashed into a paste using a mortar and pestle. Equal parts of mango paste were added to 80% methanol. The mixture was placed in a shaking incubator (Shelllab Orbital Shaking Incubator S14; Shellab, Cornelius, OR, USA) at 250 rpm for 30 min at room temperature, and then centrifuged. The supernatant of the centrifuged sample was used for subsequent analysis.

Total flavonoid content
Flavonoid content of samples was determined using a colorimetric method as described previously. A standard curve of (+)-catechin was prepared and results were reported as milligrams of catechin equivalent (CE) 100 mL⁻¹ sample extract.

Total carotenoid content
Carotenoid was extracted and the total carotenoid content calculated as described by Santhirasegaram et al. with minor modifications. The ABTS radical cation (ABTS⁺) stock solution was prepared by mixing equal parts of 7.4 mmol L⁻¹ ABTS⁺ solution and 2.6 mmol L⁻¹ potassium persulfate solution and adjusted to absorbance of 0.880 ± 0.05 units at 734 nm (UV-200-RS Spectrophotometer; MRC, Tel-Aviv, Israel). A standard curve of ascorbic acid was prepared and results were reported as micrograms of ascorbic acid equivalent (AAE) mL⁻¹ sample extract for the reducing power assay.

Reducing power assay
A spectrophotometric method, as described by Santhirasegaram et al, was used for measuring the reducing power of samples. A standard curve of ascorbic acid was prepared and results were reported as micrograms of ascorbic acid equivalent (AAE) mL⁻¹ juice extract.

2,2-Azino-bis-3-ethylbenzothiazoline-6-sulfonic acid
The 2,2-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) assay was conducted according to the method of Arnao et al. with minor modifications. The ABTS radical cation (ABTS⁺) stock solution was prepared by mixing equal parts of 7.4 mmol L⁻¹ ABTS⁺ solution and 2.6 mmol L⁻¹ potassium persulfate solution and allowed to stand at room temperature (25 ± 1 ºC) for 12 h (in the dark). This ABTS⁺ stock solution was diluted by mixing 3.5 mL of stock solution with 30 mL methanol to obtain an absorbance of 0.880 ± 0.05 units at 734 nm (UV-200-RS Spectrophotometer; MRC, Tel-Aviv, Israel). A standard curve of ascorbic acid was prepared and results were reported as micrograms of ascorbic acid equivalent (AAE) mL⁻¹ juice extract.

Shelf-life analysis
Shelf-life of treated samples was determined as described by George et al. Changes in microbial counts (aerobic bacteria) in Chokanan mango stored at refrigeration temperature (4 ± 1 ºC) for 15 d were determined using Petrifilm plates (3M Center, Maplewood, MN, USA) and were calculated as colony forming units. The results were expressed as log(CFU mL⁻¹). Shelf life of the samples was determined according to the Public Health Laboratory Service, PHLS (2000) where the acceptable maximum microbial load including aerobic bacteria, and total yeast and mold in fresh-cut fruits are 7 log CFU mL⁻¹. Samples with microbial loads of below log 7 CFU during storage period of up to 15 days at 4 ºC were perceived as being fit for consumption.
Protein sample preparation for two-dimensional electrophoresis
Proteins were extracted from mango fruit pulp tissues according to the phenol extraction protocol\textsuperscript{34} with modifications. Mango fruit tissue were finely powdered using mortar and pestle under liquid nitrogen and 150 mg of tissue were transferred into a micro-centrifuge tube containing 500 μL of extraction buffer (0.1 mol L\textsuperscript{−1} Tris–HCl, 0.05 mol L\textsuperscript{−1} EDTA, 0.1 mol L\textsuperscript{−1} KCl, 1% Dithiothreitol (DTT), 30% sucrose) and vortexed for 30 s. Buffered phenol (500 μL) was then added and the mixture was vortexed for a further 10 min at 4 °C. The sample mixture was then centrifuged for 3 min, 7800 \times g at 4 °C and the phenolic phase (upper layer) was collected and transferred to a new Eppendorf tube. This phenolic phase was then back extracted with equal volume of extraction buffer via vortex. Centrifugation for phase separation was then repeated for 3 min, 7800 \times g at 4 °C. The phenolic phase was once again collected and was precipitated overnight with five volumes of 100 mmol L\textsuperscript{−1} ammonium acetate in methanol at −20 °C. The precipitated protein mixture was then centrifuged for 60 min, 20,600 \times g, at 4 °C. The supernatant was removed and the resulting pellet was rinsed twice in 2 mL of rinsing solution (0.13 mol L\textsuperscript{−1} KCl, 1% Dithiothreitol (DTT), 30% sucrose) followed by 15 min in 2% (w/v) SDS. The strips were sealed by 0.5% molten agarose in running buffer. Isoelectric focusing was performed on an Ettan IPGphor IEF system (GE Healthcare) at 20 °C. The sample mixture was then centrifuged for 60 min, 20,600 \times g, at 4 °C. The supernatant was removed and the resulting pellet was rinsed twice in 2 mL of rinsing solution (0.13 mol L\textsuperscript{−1} of DTT in acetone). The pellet was dried briefly before it was suspended in 100 μL of freshly prepared lysis buffer (7 mol L\textsuperscript{−1} urea, 2 mol L\textsuperscript{−1} thiourea, 4% (w/v) CHAPS, 1% (w/v) DTT and 0.5% (v/v) pH 4–7 IPG buffer) pH 4–7. The samples were cleared by centrifugation for 30 min, 20,600 \times g at 18 °C twice and stored at −80 °C for further analysis. The concentration of protein in the samples was determined using the 2D Quant kit (GE Healthcare, Uppsala, Sweden) according to manufacturer’s protocol.

Two-dimensional electrophoresis and staining
Sample aliquots containing 50 μg of proteins were applied to 24 cm pH 4–7 IPG strips via cup loading, small volumes of rehydration buffer (7 mol L\textsuperscript{−1} urea, 2 mol L\textsuperscript{−1} thiourea, 4% (w/v) CHAPS, 1% (w/v) DTT) and 0.5% (v/v) pH 4–7 IPG buffer) were added to the sample aliquots in order to achieve a final volume of 150 μL. To which dry strips were rehydrated for 12 h with the rehydration buffer. Isoelectric focusing was performed on an Ettan IPGphor IEF system (GE Healthcare) at 20 °C. The voltage was set at 100 V for 1 h, 200 V for 1 h and then 500 V for 1 h, 1000 V for 1 h, 4000 V for 2 h, slow mode ramped to 8000 V over 2 h and then run at 8000 V until final volt-hours (60 kVh) were reached. Subsequently, the strips were equilibrated for 15 min in 2% (w/v) DTT in equilibration buffer (50 mmol L\textsuperscript{−1} Tris–HCl pH 8.8, 6 mol L\textsuperscript{−1} urea, 20% (v/v) glycerol and 2% (w/v) sodium dodecyl sulfate (SDS)) followed by 15 min in 2.5% (w/v) iodoacetamide in equilibration buffer. Following equilibration, the strips were sealed by 0.5% molten agarose in running buffer on 12%, self-casted gels and run on the Ettan DALTSix electrophoresis system (GE Healthcare) in a buffer of 25 mmol L\textsuperscript{−1} Tris, 192 mmol L\textsuperscript{−1} glycine, 0.1% SDS, at 15 °C with a cooling device (GE Healthcare). The gels were run at 1 W gel\textsuperscript{−1} for 1.5 h, and then at 15 W gel\textsuperscript{−1} for 4 h. Staining was performed by placing gels into fixative solutions (40% ethanol, 10% acetic acid) overnight. Then the gels were stained with Plus one silver staining kit (GE Healthcare) according to manufacturer’s protocol. Each treatment comprised of three batches of three biological replicates with three technical replicates each giving a total sample size of (n = 9) for gel analysis.

Image acquisition and data analysis
The silver-stained 2-DE gels were imaged by an Image Scanner III at GE Healthcare. Images were properly cropped and optimised, and then gel-to-gel matching of the standard protein maps was performed. The spot detection parameters were optimised by checking different protein spots in certain regions of the gel which were then automatically detected, followed by visual inspection for removal or addition of undetected spots. Spot detection was refined by manual spot editing when needed. Only spots that were present on all gels of one treatment or control based on the image analysis were identified as reproducible expressed protein spots. The abundance of each protein spot was estimated as the percentage volume (vol.%), that is, the spot volumes were normalised as a percentage of the total volume in all the spots present in the gel to correct the variability because of loading and gel staining. The percentage volumes were used to designate the significant differentially expressed spots (at least two-fold increase/decrease and statistically significant as calculated through missing value imputation via K-nearest neighbours analysis, followed by log transformation of the imputed data and comparison of control and treated values to evaluate corresponding variance (ANOVA), with a non-linear mixed-effects model P values < 0.05).

Protein in-gel digestion
Spots showing changes statistically significant (at P < 0.05) and above a two-fold threshold were excised from the gels and washed with double-distilled water and then transferred to sterilised Eppendorf tubes. Protein samples were trypsin digested and peptides extracted according to standard techniques prior to protein identification through MS.\textsuperscript{35}

Protein identification by MS and database search
Peptides were analysed by electrospray ionisation mass spectrometry using the Shimadzu Prominance nano HPLC system (Shimadzu, Kyoto, Japan) coupled to a 5600 Triple TOF mass spectrometer (ABSciex, Framingham, MA, USA). Tryptic peptides were loaded onto an Agilent Zorbax 300SB-C18, 3.5 μm (Agilent Technologies, Santa Clara, CA, USA) and separated with a linear gradient of water/acetonitrile/0.1% formic acid (v/v). Spectra were analysed to identify proteins of interest using Mascot sequence matching software (Matrix Science, London, UK) with Ludwig NR database (non-identical sequences from GenBank CDS translations, PDB, Swiss-Prot, PRF, and PRF). The identity of the spot was selected as the protein that produced the highest score and, consequently, the best match with its peptide sequence. For the MS peptides identified, database searches (BLAST; National Center for Biotechnology Information, Rockville Pike, Bethesda, MD, USA) were used to create protein sequence alignments. Searches were performed over the full molecular weight and pI range and no species restriction was applied. Functional classification was performed according to the primary biological function of the protein and was based on information found in databases (NCBI, CDD, PantherDB, ExPASy–UniProt/ PROSITE, Gene Ontology, EMBL-EBI Interpro, KEGG) and on available data in the literature.

RESULTS AND DISCUSSION
Effects of UV-C irradiation on the quality of Chokanan mango
The effects of UV-C irradiation on the quality of Chokanan mango is shown in Table 1. UV-C irradiation was found to have no adverse effects on the quality of mangoes with respect to the parameters evaluated. No significant changes were observed for pH, sugar acid
Table 1. Effects of UV-C treatment on the quality and shelf-life of Chokanan mango

<table>
<thead>
<tr>
<th>Day no. and UV-C treatment</th>
<th>pH</th>
<th>Sugar acid ratio (*Brix) (% acid)</th>
<th>Total sugar content (g kg⁻¹)</th>
<th>Total carotenoid content (µg 100 mL⁻¹)</th>
<th>Total flavonoid content (mg CAE 100 mL⁻¹)</th>
<th>Reducing power (µg AAE mL⁻¹)</th>
<th>ABTS radical scavenging activity (µg AAE mL⁻¹)</th>
<th>Shelf-life (log CFU mL⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
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</tr>
<tr>
<td>Control</td>
<td>4.68 ± 0.04a</td>
<td>44.80 ± 2.29a</td>
<td>1688 ± 3.3a</td>
<td>85.03 ± 1.10a</td>
<td>7.74 ± 0.10a</td>
<td>250.71 ± 3.20a</td>
<td>7.70 ± 0.15a</td>
<td>3.92 ± 0.02a</td>
</tr>
<tr>
<td>UV-C 15 min</td>
<td>4.67 ± 0.05a</td>
<td>43.10 ± 2.11a</td>
<td>1685 ± 3.5a</td>
<td>85.11 ± 1.09a</td>
<td>7.81 ± 0.13a</td>
<td>255.02 ± 2.23a</td>
<td>7.85 ± 0.12a</td>
<td>3.33 ± 0.01b</td>
</tr>
<tr>
<td>UV-C 30 min</td>
<td>4.59 ± 0.04a</td>
<td>43.59 ± 2.35a</td>
<td>1691 ± 3.2a</td>
<td>85.23 ± 1.12a</td>
<td>7.91 ± 0.09ab</td>
<td>260.32 ± 1.99b</td>
<td>7.90 ± 0.11b</td>
<td>3.11 ± 0.02c</td>
</tr>
<tr>
<td>UV-C 60 min</td>
<td>4.64 ± 0.05a</td>
<td>41.73 ± 2.09a</td>
<td>1689 ± 3.7a</td>
<td>85.32 ± 1.07a</td>
<td>7.99 ± 0.11b</td>
<td>266.18 ± 2.02b</td>
<td>7.92 ± 0.13b</td>
<td>2.85 ± 0.03d</td>
</tr>
<tr>
<td>Day 5</td>
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</tr>
<tr>
<td>Control</td>
<td>4.65 ± 0.05a</td>
<td>37.80 ± 1.08b</td>
<td>149.4 ± 3.9b</td>
<td>83.03 ± 1.25b</td>
<td>7.56 ± 0.08c</td>
<td>242.30 ± 2.15d</td>
<td>7.46 ± 0.12c</td>
<td>6.42 ± 0.03c</td>
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<tr>
<td>UV-C 15 min</td>
<td>4.68 ± 0.04a</td>
<td>42.25 ± 2.05a</td>
<td>1685 ± 3.5a</td>
<td>85.64 ± 1.16a</td>
<td>7.82 ± 0.10b</td>
<td>256.15 ± 3.02a</td>
<td>7.84 ± 0.09b</td>
<td>5.88 ± 0.02d</td>
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<tr>
<td>UV-C 30 min</td>
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<td>41.99 ± 2.11a</td>
<td>1691 ± 3.2a</td>
<td>85.55 ± 1.09a</td>
<td>7.92 ± 0.10ab</td>
<td>262.19 ± 2.55b</td>
<td>7.91 ± 0.12b</td>
<td>5.02 ± 0.03e</td>
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<tr>
<td>UV-C 60 min</td>
<td>4.63 ± 0.05a</td>
<td>42.65 ± 1.95a</td>
<td>1689 ± 3.7a</td>
<td>85.20 ± 1.20a</td>
<td>7.96 ± 0.12b</td>
<td>267.46 ± 1.98e</td>
<td>7.91 ± 0.10b</td>
<td>4.01 ± 0.03h</td>
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<td>34.80 ± 1.23c</td>
<td>113.3 ± 5.0c</td>
<td>81.03 ± 2.20b</td>
<td>7.51 ± 0.10c</td>
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<td>8.33 ± 0.01i</td>
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<td>38.63 ± 1.10b</td>
<td>150.0 ± 2.0b</td>
<td>85.12 ± 1.07a</td>
<td>7.81 ± 0.05a</td>
<td>255.00 ± 2.75a</td>
<td>7.85 ± 0.11b</td>
<td>6.99 ± 0.02j</td>
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<td>1689 ± 2.9a</td>
<td>85.78 ± 1.15a</td>
<td>7.90 ± 0.11ab</td>
<td>261.60 ± 3.12b</td>
<td>7.91 ± 0.08b</td>
<td>6.16 ± 0.01k</td>
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<td>85.51 ± 1.11a</td>
<td>7.98 ± 0.10b</td>
<td>267.11 ± 2.04c</td>
<td>7.90 ± 0.15b</td>
<td>5.03 ± 0.03c</td>
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<tr>
<td>Day 15</td>
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</tr>
<tr>
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<td>29.21 ± 2.26d</td>
<td>115.8 ± 1.9c</td>
<td>78.15 ± 2.15c</td>
<td>7.46 ± 0.08d</td>
<td>235.22 ± 3.06d</td>
<td>7.38 ± 0.09d</td>
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<td>135.5 ± 2.0d</td>
<td>83.76 ± 1.25b</td>
<td>7.55 ± 0.08c</td>
<td>246.78 ± 1.98d</td>
<td>7.47 ± 0.10f</td>
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<tr>
<td>UV-C 30 min</td>
<td>4.59 ± 0.05a</td>
<td>43.47 ± 2.19a</td>
<td>1689 ± 2.9a</td>
<td>85.88 ± 1.09a</td>
<td>7.90 ± 0.07ab</td>
<td>260.52 ± 3.40b</td>
<td>7.89 ± 0.10b</td>
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<td>UV-C 60 min</td>
<td>4.65 ± 0.05a</td>
<td>41.99 ± 1.10a</td>
<td>1688 ± 3.1a</td>
<td>85.85 ± 1.18a</td>
<td>7.99 ± 0.08b</td>
<td>266.25 ± 1.66d</td>
<td>7.92 ± 0.09b</td>
<td>6.38 ± 0.03n</td>
</tr>
</tbody>
</table>

Values followed by different superscript letters within the same column are significantly different (P < 0.05, n = 9).
Effects of postharvest UV-C on mango

2855

AB

CD

Figure 1. Representative spot maps of ‘Chokanan’ mango. (A) Control; (B) UV-C irradiated for 15 min; (C) UV-C irradiated for 30 min and (D) UV-C irradiated for 60 min. 2-DE was carried out using 50 μg of proteins, linear 24 cm IPG strips (pH 4–7) and 12% SDS-PAGE gels for second dimension electrophoresis. Gels were stained with silver stain.

ratios, sugar content and total carotenoid content following UV-C irradiation. In addition, total flavonoid content, reducing power and ABTS activity increased up to 3%, 7% and 3%, respectively, in mangoes following UV-C irradiation. An increase of antioxidant activities in fruits are highly desirable and therefore, gives added value to treated fruits. UV-C irradiation was also observed to be successful in maintenance of mango quality with regards to pH, sugar acid ratio, total sugar content and total carotenoid content. No significant changes were observed in these parameters in UV-C treated samples during storage at 4 °C. As for total flavonoid content, reducing power and ABTS radical scavenging activity, initial increases following UV-C treatment were maintained during storage without significant changes observed. In controls, significant changes were observed in all the parameters tested except for pH, indicating reduced quality of controls during storage. Results from quality analysis indicate that postharvest UV-C treatment is a valuable tool for maintenance of fruit quality. Furthermore, UV-C irradiation was found to confer shelf-life extension of up to a maximum of 15 days when stored at 4 °C. Shelf-life of the samples was evaluated according to the Public Health Laboratory Service, PHLS (2000) where the acceptable maximum microbial load (aerobic bacteria) in fresh-cut fruits are 7 log CFU mL⁻¹. The shelf-life of fresh-cut mango was extended from 6 days to 10 days after 15 min of UV-C irradiation and for up to 15 days following irradiation of 30 and 60 min. UV-C irradiation was found to be successful in extending the shelf-life of mangoes exponentially and therefore, it could be exploited for preservation techniques of postharvest produce. Fresh fruits and vegetables are highly perishable and the perishability is attributed to various factors which include composition of commodity, morphological structure, development stages as well as physiology of horticultural products. Yet, continuous metabolism and growth, water loss, physiological disorders, mechanical damage as well as microbial activity has been attributed as the major causes of fresh produce deterioration. Results of this study are in agreement with previous researches on the effects of UV-C irradiation on quality of fruits which includes mandarin, strawberry, pepper, fig, kiwifruit and mango during storage. Proteomic analysis was carried out along with quality analysis to document the changes that occur at a proteomic level to better understand the molecular mechanisms that entails quality maintenance and shelf-life extension in mangoes.

Proteomic analysis of UV-C irradiated Chokanan mango

Results from the software guided comparative visual analysis of the representative 2-DE proteome profiles of Chokanan mango following UV-C treatment of 15, 30 and 60 min are represented by Fig. 1. An average of 450 spots was detected in the gels while only 380 spots out of the 450 detected could be reproducibly detected. Spots detected were mainly in the pH range of 4 to 7 and molar mass range of 14 to 97 kDa. Gel analysis of the treated samples against untreated samples (control) via the ImageMaster 2-D Platinum software Version 7.0 (GE Healthcare) revealed 24 spots that were differentially expressed (reduced in expression) above the two-fold threshold following UV-C treatment (Fig. 2). Spots...
that were differentially expressed were excised, in gel digested via trypsin, and finally subjected to mass spectrometry. Out of the 24 spots that were differentially expressed, 20 were successfully identified using the Ludwig NR database. The 20 differentially expressed spots that were successfully identified were divided into respective functional groups according to the homologies of the proteins and references (Table 2). Eight identified spots were found to correspond to sequences from *Mangifera indica* while the remaining 12 identified spots were found to be similar to sequences of other plants. Proteins that were differentially expressed following UV-C treatments were found to be related to stress and defence (45%), energy and metabolism (45%) and ripening and senescence (10%) (Fig. 3). In addition, several proteins that were known to cause allergic reactions in patients were also identified (Putative Allergen Pru P, Allergen Pyr c 3, Allergen Profilin 1 and Birch Pollen Allergen Bet V 1 Mutant).

Changes in postharvest treated mangoes are attributed to the disruption of various aspects which includes biological processes. It is evident that biological processes are catalysed by proteins; however, information regarding the proteome associated to these biological processes remains scarce. This study therefore serves as a means of understanding the molecular mechanisms that underlines the changes that take place in postharvest UV-C treated mangoes. The comprehensive analysis of this study revealed that the proteins affected by UV-C treatment were those that regulate biological processes which contribute to changes in fruits leading to deterioration of postharvest crops. Therefore, observations of this study are highly relevant to the postharvest industry as well as to consumers. Our most significant observations are discussed in the following segments.
Table 2. Identification of spots differentially expressed following postharvest UV-C treatment

<table>
<thead>
<tr>
<th>Spot (#)</th>
<th>Protein name</th>
<th>Accession no.</th>
<th>Matched peptide</th>
<th>Molecular weight (Da)</th>
<th>pI</th>
<th>UV-C 15 min</th>
<th>UV-C 30 min</th>
<th>UV-C 60 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Putative allergen Pru P (Prunus dulcis x Prunus persica)</td>
<td>ACE80950</td>
<td>4</td>
<td>17437</td>
<td>5.10</td>
<td>2.12</td>
<td>2.24</td>
<td>2.36</td>
</tr>
<tr>
<td>2</td>
<td>Allergen Pyr c 3 (Pyrus communis)</td>
<td>Q9XF38</td>
<td>3</td>
<td>14064</td>
<td>5.19</td>
<td>2.01</td>
<td>2.16</td>
<td>2.33</td>
</tr>
<tr>
<td>3</td>
<td>Allergen profilin 1 (Mangifera indica)</td>
<td>ABD62998.1</td>
<td>3</td>
<td>14070</td>
<td>4.89</td>
<td>1.98</td>
<td>2.15</td>
<td>2.43</td>
</tr>
<tr>
<td>4</td>
<td>Birch Pollen Allergen Bet V 1 Mutant (Betula pendula)</td>
<td>1QMR_A</td>
<td>2</td>
<td>17310</td>
<td>5.37</td>
<td>2.33</td>
<td>2.47</td>
<td>2.62</td>
</tr>
<tr>
<td>5</td>
<td>Thaumatin-like protein 1 (Solanum lycopersicum)</td>
<td>XP_004231024</td>
<td>2</td>
<td>36363</td>
<td>4.31</td>
<td>2.08</td>
<td>2.15</td>
<td>2.28</td>
</tr>
<tr>
<td>6</td>
<td>Polygalacturonase inhibiting protein (Prunus persica)</td>
<td>AFW93253</td>
<td>3</td>
<td>36198</td>
<td>5.63</td>
<td>2.07</td>
<td>2.16</td>
<td>2.27</td>
</tr>
<tr>
<td>7</td>
<td>Superoxide dismutase (Zea mays)</td>
<td>AFD86132</td>
<td>3</td>
<td>36258</td>
<td>6.30</td>
<td>2.11</td>
<td>2.43</td>
<td>3.18</td>
</tr>
<tr>
<td>8</td>
<td>Rubisco complex protein (Glycine max)</td>
<td>20194381A</td>
<td>2</td>
<td>19507</td>
<td>5.77</td>
<td>1.96</td>
<td>2.16</td>
<td>2.22</td>
</tr>
<tr>
<td>9</td>
<td>β-1,3-Glucanase (Mangifera indica)</td>
<td>AAU035700</td>
<td>2</td>
<td>49898</td>
<td>5.01</td>
<td>2.12</td>
<td>2.38</td>
<td>2.59</td>
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<tr>
<td>10</td>
<td>6-Phosphogluconolactonase (Mangifera indica)</td>
<td>ABG23393</td>
<td>3</td>
<td>29986</td>
<td>5.16</td>
<td>1.95</td>
<td>2.17</td>
<td>2.34</td>
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<td>11</td>
<td>Methionine γ-lyase-like (Cicer arietinum)</td>
<td>XP_004502061</td>
<td>4</td>
<td>72498</td>
<td>6.28</td>
<td>2.12</td>
<td>2.87</td>
<td>3.43</td>
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<tr>
<td>12</td>
<td>Alcohol dehydrogenase 3 (Vitis vinifera)</td>
<td>NP_001268071</td>
<td>2</td>
<td>41241</td>
<td>6.76</td>
<td>2.09</td>
<td>2.67</td>
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<td>13</td>
<td>Sucrose synthase (Mangifera indica)</td>
<td>AEQ30069</td>
<td>4</td>
<td>30019</td>
<td>6.27</td>
<td>2.06</td>
<td>2.88</td>
<td>3.97</td>
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<tr>
<td>14</td>
<td>Neutral invertase (Mangifera indica)</td>
<td>AEQ30068</td>
<td>4</td>
<td>27812</td>
<td>5.13</td>
<td>4.12</td>
<td>4.88</td>
<td>5.38</td>
</tr>
<tr>
<td>15</td>
<td>Isoflavone reductase homologue (Solanum tuberosum)</td>
<td>XP_006353700</td>
<td>3</td>
<td>35258</td>
<td>5.82</td>
<td>2.07</td>
<td>2.98</td>
<td>3.41</td>
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<td>16</td>
<td>ATP synthase β subunit (Mangifera indica)</td>
<td>ADY90124</td>
<td>3</td>
<td>52594</td>
<td>5.04</td>
<td>3.79</td>
<td>4.37</td>
<td>4.58</td>
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<tr>
<td>17</td>
<td>Putative malate dehydrogenase (Trypanosoma grayi)</td>
<td>XP_009312008</td>
<td>5</td>
<td>37408</td>
<td>5.49</td>
<td>2.22</td>
<td>3.05</td>
<td>3.55</td>
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<tr>
<td>18</td>
<td>Citrate synthase (Mangifera indica)</td>
<td>AEQ30066</td>
<td>3</td>
<td>30536</td>
<td>5.57</td>
<td>3.03</td>
<td>3.87</td>
<td>4.32</td>
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<tr>
<td>19</td>
<td>1-Aminocyclopropane-1-carboxylate oxidase (Mangifera indica)</td>
<td>AAM6261</td>
<td>5</td>
<td>10573</td>
<td>5.60</td>
<td>2.16</td>
<td>2.38</td>
<td>2.59</td>
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<tr>
<td>20</td>
<td>Aminocyclopropane carboxylate synthase (Glycine max)</td>
<td>2019442A</td>
<td>3</td>
<td>54730</td>
<td>5.84</td>
<td>2.56</td>
<td>3.19</td>
<td>3.87</td>
</tr>
</tbody>
</table>

*Spot numbers correspond to the 2-DE in Fig. 2.*

**Molecular weight.**

**Fold change of protein expression (decrease in expression) as compared to controls following different durations of UV-C irradiation.**

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injury. It has also been speculated that they may be involved in other processes related to fruit quality which includes pulp softening. Although PG-inhibiting proteins were observed to be lower in the UV-C treated fruits as compared to controls, the lower decay found may be related with germicidal effects of UV-C and activation of others defence responses. Furthermore, UV-C treatment has been reported to reduce the rate of senescence, maturation, softening and decay in fruits. A lowered rate of senescence would preserve tissue integrity which may be beneficial in terms of delaying deterioration of fruits caused by softening. Superoxide dismutase plays an important role in a plants’ defence mechanism whereby it constitutes the first line of defence against reactive oxygen species (ROS). Reactive oxygen species (ROS) are produced in both unstressed and stressed cells which may lead to oxygen radical mediated toxicity. The ability of superoxide dismutase to catalyse the dismutation of superoxide into oxygen and hydrogen peroxide therefore is vital for plant development in stressed conditions. The protein β-1,3-glucanase is more commonly known for its antimicrobial activity in infected plants. However, there are strong evidences that this enzyme is also involved in diverse physiological and developmental processes in uninfected plants or fruits. Based on our observations, the efficacy of UV-C treatment in reducing the proteins mentioned above is beneficial for maintenance of...
Nine protein spots affected by UV-C treatments were found to be related to energy and metabolism which were 6-phosphogluconolactonase (spot#10), methionine gamma-lyase-like (spot#11), alcohol dehydrogenase 3 (spot#12), sucrose synthase (spot#13), neutral invertase (spot#14), isoflavone reductase homolog (spot#15), ATP synthase beta subunit (spot#16), putative malate dehydrogenase (spot#17) and citrate synthase (spot#18). The protein 6-phosphogluconolactonase is an enzyme which is responsible for the hydrolysis of 6-phosphogluconolactone to 6-phosphogluconate, the second step in the pentose phosphate pathway.\(^{50}\) Methionine gamma-lyase-like protein catalyses the reaction: L-methionine = methanethiol + NH\(_3\) + 2-oxobutanoate.\(^{51}\) Alcohol dehydrogenase 3 is an enzyme that catalyses the fourth step in the metabolism of fructose before glycolysis. NAD\(^+\) is regenerated along with a limited amount of ATP during the process.\(^{52}\) Both the glycolytic and the pentose phosphate pathway have been reported to provide ATP for respiration burst in the increased energy demand during a climacteric phase.\(^{53}\) Our observation in this study suggests that a reduced expression of proteins involved in these pathways may reduce the rate of respiration in mangoes following treatment. A reduced rate of respiration may in turn translate to quality maintenance in mangoes. Sucrose synthase has been reported to be involved in the catalysis of the cleavage of sucrose in the presence of uridine diphosphate (UDP) into UDP-glucose and fructose. Furthermore, it has also been reported the enzyme sucrose synthase exhibits biochemical properties which allows it to function in the direction of both sucrose cleavage and synthesis.\(^{54}\) Based on recent findings, neutral invertase is an enzyme that is involved in catalysing the breakdown of sucrose into glucose and fructose which renders it as an essential protein in plants. Invertases are also known to be present in multiple locations as isoforms for the coordination of metabolic processes which further highlights the importance of this enzyme in plants.\(^{55}\) Isoflavone reductase homologues are known to be involved in the biosynthesis of isoflavonoid phytoalexins. Several homologues are also known to catalyse distinct reductase reactions.\(^{56}\) ATP synthase is made up of two main parts, which are the F1 and Fo. The F1 is made up of five subunits: three alphas, three betas, one gamma, one delta, and one epsilon. The synthesis of ATP typically occurs in the beta sub-unit of the ATP synthase.\(^{57}\) Besides its role in synthesis of ATPs, the ATP synthase beta sub-unit has been reported to be involved in plant cell death regulation.\(^{58}\) The ability of UV-C treatments to reduce the expression of this protein may therefore reduce cell death regulation which may translate to extended shelf-life in treated fruits. Malate dehydrogenase catalyses the conversion of oxaloacetate and malate via the NAD/NADH coenzyme system. This reaction is pivotal in the malate/aspartate shuttle across the mitochondrial membrane and in the tricarboxylic acid cycle.\(^{59}\) The protein citrate synthase is an enzyme which is known for catalysing the first reaction of the citric acid cycle which involves the condensation of acetyl-CoA and oxaloacetate to form citrate. Citrate synthase has also been reported to be the rate determining enzyme in the citric acid cycle.\(^{60}\) As stated above, a majority of proteins that were affected by UV-C treatments were found to play important roles in energy and metabolism with several proteins being rate determining enzymes in certain pathways. These processes bring about desirable changes in fruits as they develop and ripen; however, the ongoing metabolic processes lead to deterioration of fruits and limited shelf-life. Fruit ripening can also be classified as a senescence process due to the breaking down of the cellular integrity of tissues.\(^{61}\) The quality of horticultural commodities can be maintained by lowering metabolic activities during storage. Therefore, a reduced expression of proteins involved in metabolism following UV-C treatments might prove to be invaluable in the maintenance of fruit quality and extension of shelf-life.

Two proteins that were related to ripening and senescence were found to be reduced in expression following UV-C treatments which are 1-aminocyclopropane-1-carboxylate oxidase (spot#19) and aminocyclopropane carboxylate synthase (spot#20). Both aminocyclopropane carboxylate synthase and 1-aminocyclopropane-1-carboxylate oxidase has been reported to be involved in the biosynthesis of ethylene.\(^{62,63}\) Aminocyclopropane carboxylate synthase catalyses the conversion of S-adenosyl-L-methionine (SAM) into 1-aminocyclopropane-1-carboxylate (ACC) which is a direct precursor of ethylene.\(^{61}\) On the other hand, the enzyme 1-aminocyclopropane-1-carboxylate oxidase which is a non-heme iron-containing enzyme is known to catalyse the final step in the biosynthesis of ethylene through its oxidation at the expense of O\(_2\) giving yield to ethylene (HCN), CO\(_2\), and water.\(^{64}\) Ethylene is a plant hormone which is known to control various physiological processes in plants. Although these physiological processes are beneficial during ripening, it can induce negative effects during postharvest storage of fruits and vegetables which includes over-ripening, accelerated quality loss, increased susceptibility to pathogen and physiological disorders.\(^{65}\) A reduced expression of 1-aminocyclopropane-1-carboxylate oxidase, which is involved in the biosynthesis of ethylene, may have led to the reduced adverse effects of ethylene and hence an improved quality and extended shelf-life. Although the physiological changes that are brought about by ethylene are beneficial during fruit ripening, ethylene is still a major concern to crop producers due to its detrimental effects on quality of crops during storage. The efficacy of UV-C irradiation in reducing ethylene in postharvest storage is therefore of significant importance to both producers and consumers.

**CONCLUDING REMARKS**

To the best of our knowledge, this work reports the first proteomic study of the effects of postharvest UV-C irradiation on the proteomic changes in Chokanan mango. Some of the identified proteins were found to be involved in various biochemical processes that are important for fruit quality. The identified proteins...
in this study are related to energy and metabolism, stress and response as well as ripening ad senescence. Response patterns of Chokanan mango to UV-C irradiation are complex, as the differentially abundant proteins are involved in multiple metabolic pathways. Differential analyses of protein maps from UV-C irradiated mango using 2D-DIGE technology provided new information on the changes in protein levels as a response to UV-C irradiation. It can be inferred that the effects of UV-C irradiation on these proteins and the role that they play may be partly responsible for the maintenance of postharvest fruit quality and shelf-life extension. In this regard, the differential proteomics of UV-C has broadened our understanding of the effects of postharvest treatments on mangoes.

ACKNOWLEDGEMENT

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REFERENCES