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ARTICLE

Maintaining Quality of Litchi Fruit with Acidified Calcium Sulfate

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The effect of acidified calcium sulfate (ACS) on the quality of litchi (*Litchi chinensis* Sonn. cv. 'Brewster') fruit after harvest was evaluated. ACS at 1.25% or higher concentrations significantly inhibited the activities of polyphenol oxidase and peroxidase in the pericarp during storage at both 5 and 10 °C. These treatments also effectively prevented browning and retained the red color of the outer shell of the fruit. Total phenolic and total anthocyanin contents in pericarp were increased by ACS treatments in a dose-dependent manner. The radical scavenging activities for $ROO^{\bullet}$, $DPPH^{\bullet}$, $^{\bullet}OH$ and $O_2^{\bullet-}$ were also enhanced by ACS, particularly by 2.5 and 5% concentrations. The activities of several antioxidant enzymes and enzymes of ascorbate−glutathione cycle including catalase, ascorbate peroxidase, glutathione peroxidase, glutathione reductase, dehydroascorbate reductase, and monodehydroascorbate reductase gradually declined during storage. However, ACS enhanced the activities of these enzymes, especially at the beginning of the storage. Samples treated with ACS generally had higher flavonoid levels than the control. The three major flavonoids, cyanidin-3-rutinoside, cyanidin-3-glucoside and quercetin-3-rutinoside, were found to be significantly increased by 2.5 and 5.0% ACS at both 5 and 10 °C. No differences were detected among various treatments in soluble solids content or sugar and organic acid levels in the pulp of litchi fruit, indicating that the internal quality of the fruit was not adversely affected by ACS treatment.

KEYWORDS: *Litchi chinensis*; pericarp browning; antioxidant enzymes; anthocyanins; phenolics; flavonoids

INTRODUCTION

It has long been recognized that litchi fruit is highly perishable; its pericarp turns brown within a few days under ambient conditions (*1*, *2*). Because the red color is an important factor in the attraction of litchi fruit, pericarp browning leads to undesirable appearance and low retail value. Oxidation of anthocyanins by polyphenol oxidase (PPO) and peroxidase (POD) was found to be the main cause of browning in litchi (*3*,*4*). Various techniques have been used in an effort to inhibit the activities of PPO and POD and to prevent or delay this color change. Most methods involve the use of sulfur dioxide fumigation, primarily because this gas treatment blocks the enzymatic activities and stops the oxidative reactions (*5*). However, this treatment also bleaches the fruit; therefore, a second treatment with acid is necessary to help restore the red color (*6*). This two-step procedure has been commonly adopted as a standard treatment for litchi after harvest (*7*). However, this procedure was still found to be not sufficient to prevent the development of fungal decay, particularly the infection by *Penicillium* spp. (*5*). A simpler and more effective treatment is needed for maintaining the postharvest quality of litchi fruit.

Acidified calcium sulfate (ACS) is a clear liquid with no off-odors. This compound was found to have the capability to control microbial growth in apple wine (*8*) and to inhibit the proliferation of pathogens such as *Listeria monocytogenes*, *Staphylococcus aureus*, *Salmonella serotype*, and *Escherichia coli* (*9*, *10*). The diluted solution of ACS has also been approved by the U.S. FDA as a generally regarded as safe (GRAS) compound and safe to be used for preventing enzymatic browning and reducing mold growth in whole and fresh-cut fruits and vegetables (*11*). Because low-pH conditions help in the retention of anthocyanins and restoration of red color on litchi shell (*6*), this acidified solution may have such an effect. Positive relationships between calcium content and litchi fruit firmness have also been shown (*12*). High calcium content also enhanced skin color and resistance to fruit cracking (*13*, *14*). However, calcium concentration in the pericarp was negatively correlated to litchi fruit deterioration rate and membrane leakage (*15*). This study was undertaken to test if a single treatment with ACS could be effective in retaining the red color and retarding the microbial growth of litchi fruit. The effects of this treatment on antioxidant properties and other quality attributes in peel and pulp of litchi fruit were also evaluated.

MATERIALS AND METHODS

Chemicals. 2′,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was obtained from Wako Chemicals USA Inc. (Richmond VA). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) and fluorescein

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disodium were obtained from Aldrich Chemical Co. (Milwaukee, WI). Acetonitrile, methanol, acetone, and water were of HPLC grade and were purchased from Baxter (Muskegon, MI). Quercetin-3-rutinoside, quercetin-3-glucoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, malvidin-3-acetylglucoside, and malvidin-3-arabinoside were obtained from Indofine Chemical Co., Inc. (Somervill, NJ). Pelargonidin-3-rhamnoside and pelargonidin-3,5-diglucoside were obtained from Polyphenols Laboratories AS (Sandnes, Norway). Other authentic standards were obtained from Sigma Chemical Co. (St. Louis, MO) and Fisher Scientific (Suwanee, GA).

Fruit Material and Treatment. Litchi (*Litchi chinensis* Sonn. cv, 'Brewster') were harvested at commercial maturity in Homestead, FL, and were transportated to our laboratory overnight. Fruits were sorted for uniform size and pericarp color. Selected fruits were randomized and used for the experiments. Fruit samples were then divided into five groups and immersed for 2 min into aqueous solutions of ACS with concentrations of 0, 0.625, 1.25, 2.5, or 5%, respectively. Tween-20 was added to each solution as a wetting agent (final concentration = 0.05%). A wetting agent is a substance that reduces the surface tension of a liquid, causing the liquid to spread across and penetrate more easily the surface. After treatment, each group was further divided into two lots for 5 or 10 °C storage, respectively. Eight fruits were placed in a 1 L plastic tray, which was then covered with a perforated clear plastic cover. Twelve trays were used for each concentration. Six trays from each concentration were stored at 5 °C, whereas the other six trays were kept at 10 °C. Samples were taken for evaluation and chemical analyses on days 5 and 15 during storage. Three replicates were used, and the experiment was repeated two times.

Soluble Solids Content (SSC), Color Measurement, and Decay Evaluation. The SSC of the fruit was determined at 20 °C on a digital refractometer (PR-101, Spectrum Technologies, Plainfield, IL). Surface color of 10 fruits from each replicate was measured using a colorimeter (CR 200 Minolta, Ramsey, NJ), which provided L^*, a^*, and b^* values according to the system established by the Commission Internationale de L'Eclairage (CIE, International Commission on Illumination) in 1978 (*16*). Negative a^* values indicate green and higher positive a^* values red color. Higher positive b^* values indicate a more yellow skin color and negative b^* values a blue color. These values could then be used to calculate hue degree (h° = arctangent[b^*/a^*]), where 0° = red-purple, 90° = yellow, 180° = bluish-green, and 270° = blue, and chroma ($C^* = [a^{*2} + b^{*2}]^{1/2}$), which indicates the intensity or color saturation. The development of decay was evaluated after 5 and 15 days of storage at 5 and 10 °C and expressed as percent of fruit showing any fungal infection.

Analysis of Sugars and Organic Acids. Two grams of litchi pulp was homogenized with a Polytron homogenizer (Brinkmann Instruments, Westbury, NY) in imidazole buffer (20 mM, pH 7.0). The extracts were centrifuged, and the supernatants were dried in vacuo in vials that were used during derivatization. Procedures described by Li and Schuhmann (*17*) were modified for the derivatization of sugars and organic acids. A known amount of β-phenyl-D-glucopyranoside was included in all samples as an internal standard. One milliliter of Trisil reagent (Pierce, Rockford, IL) was mixed vigorously with each sample and then heated at 75 °C for 30 min. After silylation, 1 μL of each derivatized sample was injected into a Hewlett-Packard 5890 gas chromatograph (Palo Alto, CA) equipped with a flame ionization detector and a 25 m cross-linked methyl silicon gum capillary column (Hewlett-Packard, HP-1, 0.2 mm i.d., 0.33 μm film thickness) and using helium as carrier gas. Temperatures were as follows: injector, 250 °C; detector, 275 °C; column temperature, programmed to increase from 100 to 250 °C at 10 °C min^{-1} and then held constant at 250 °C for 23 min. Organic acids were analyzed after extraction with imidazole buffer (20 mM, pH 7.0) and purification with a Baker-10 solid phase extraction system. Supernatants from the extract were passed through quaternary amine columns, which were previously conditioned with hexane and methanol. The samples were then eluted from the columns with 0.1 N HCI. The eluates were concentrated to dryness in vacuo in derivatized vials. Procedures of derivatization and chromatography for organic acids were the same as those for sugars except that column temperature was held at 180 °C for 3 min, then increased to 250 °C at 10 °C min^{-1}, and held at 250 °C for 12 min. The sugars and organic acids were quantified by comparison with derivatized standards.

Antioxidant Enzyme Measurements. *Catalase* (*CAT, EC 1.11.1.6*) was extracted from 1 g of pericarp sample with 5 mL of 50 mmol L^{-1} sodium phosphate buffer (pH 7) at 4 °C. CAT activity was determined according to the method described previously (*18*). One unit of CAT activity was defined as the amount of enzyme that decomposed 1 μmol of H_2O_2 min^{-1} at 30 °C.

PPO (*EC 1.14.18.1*) *Assay*. One gram of frozen pericarp tissue was homogenized in 0.2 mol L^{-1} sodium phosphate buffer (pH 6.5) containing 1% polyvinylpyrrolidone (PVP). PPO activity was assayed following the method of Murr and Morris (*19*). One unit of PPO activity was defined as the amount of enzyme that caused the increase in absorbance of 0.01 at 410 nm in 1 min under the specified conditions.

POD (*EC 1.11.1.7*) activity was assayed using guaiacol as a donor and H_2O_2 as a substrate according to the method of Kochba et al. (*20*). One unit of POD activity was defined as an increase of 0.01 in absorbance per minute at 470 nm under the assay conditions.

Glutathione Peroxidase (*GSH-POD, EC 1.11.1.9*) *and Glutathione Reductase* (*GR, EC 1.6.4.2*). Litchi pericarp tissue (2 g of fresh weight) was homogenized in 4 mL of 0.1 M Tris-HCl buffer (pH 7.8) containing 2 mM EDTA-Na and 2 mM dithiothreitol (DTT). The homogenate was centrifuged at 20000*g* for 30 min at 4 °C, and the supernatant was used for the GSH-POD and GR assays. GSH-POD activity was determined using the method of Tappel (*21*) with a slight modification. The reaction mixture contained 0.1 M Tris-HCl buffer (pH 8.0), 0.4 mM EDTA, 1.0 mM NaN_3, 1.0 mM H_2O_2, 1.0 mM glutathione (GSH), 0.15 mM NADPH, 1 unit of glutathione reductase, and 100 μL of enzyme extract. The total reaction volume was 1.0 mL. The reaction was started by adding H_2O_2. GSH-POD activity was determined by the rate of NADPH oxidation at 340 nm via a spectrophotometer.

GR activity was assayed according to the method of Smith et al. (*22*). The activity of GR was determined by monitoring glutathione-dependent oxidation of NADPH at 340 nm. The reaction was started by adding oxidized glutathione (GSSG), and the rate of oxidation was calculated using the extinction coefficient of NADPH (6.22 mM^{-1} cm^{-1}).

Ascorbate Peroxidase (*AsA-POD, EC 1.11.1.11*). Litchi pericarp tissue (2 g) was pulverized with a cold mortar and pestle with 5 mL of 50 mM sodium phosphate buffer (pH 7.0) containing 0.1 mM ethylenediaminetetraacetic acid (EDTA), 1 mM ascorbic acid, and 1% PVP at 4 °C. The homogenate was centrifuged at 10000*g* for 20 min at 4 °C and the supernatant used for the AsA-POD assays. The activity was determined according to the method of Nakano and Asada (*23*). One unit of AsA-POD was defined as the amount of enzyme that oxidized of 1 μmol of ascorbate per minute at 30°C.

Dehydroascorbate Reductase (*DHAR, EC 1.8.5.1*). DHAR activity was assayed by measuring the rate of NADPH oxidation at 340 nm. The reaction mixture contained 50 mM potassium phosphate (pH 6.1), 0.2 mM NADPH, 2.5 mM dehydroascorbate, 2.5 mM glutathione, 0.6 unit of glutathione reductase (GR, EC 1.6.4.2), and 0.1 mL of pericarp extract (2 g homogenized with 4 mL of 50 mM potassium phosphate, pH 6.1). The reaction was started by adding dehydroascorbate.

Monodehydroascorbate Reductase (*MDAR, EC 1.6.5.4*). MDAR activity was assayed by measuring the rate of NADH oxidation at 340 nm. The reaction mixture contained 50 mM potassium phosphate buffer (pH 7.3), 0.2 mM NADH, 1.0 mM ascorbate, 1.0 unit of ascorbate oxidase, and 0.1 mL of 50 mM potassium phosphate buffer (pH 7.3) pericarp extract in a total volume of 1.0 mL. The reaction was started by adding ascorbate oxidase (EC 1.10.3.3).

Free Radical Scavenging Activity Assay. For the assays of oxygen radical absorbance capacity (ORAC), hydroxyl radical scavenging capacity ($^{\bullet}OH$; HOSC), 2,2-di-(4-*tert*-octylphenyl)-1-picrylhydrazyl (DPPH) assay, and superoxide radicals ($O_2^{\bullet -}$) scavenging activity, three 5-g composite pericarp samples were extracted twice with 50% acetone using a Polytron (Brinkmann Instruments). The homogenized samples from the acetone extraction were then centrifuged at 14000*g* for 20 min at 4 °C. The supernatants (final volume of 25 mL) were transferred to vials, stored at −80 °C, and later used for ORAC, HOSC, DPPH, and $O_2^{\bullet -}$ analysis.

ORAC Assay. The ORAC assay was carried out according to the method of Huang et al. (*24*) using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system. A, FL800 microplate fluorescence reader (Bio-Tek Instruments, Inc., Winooski, VT)

was used with fluorescence filters for an excitation wavelength of 485 ± 20 nm and an emission wavelength of 530 ± 25 nm. The plate reader was controlled by software KC4 3.0 (revision 29) (Bio-Tek Instruments). Sample dilution was accomplished by a Precision 2000 automatic pipetting system managed by precision power software (version 1.0) (Bio-Tek Instruments). The ORAC values were determined by calculating the net area under the curve (AUC) of the standards and samples (*24*). The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final ORAC values were calculated using the regression equation between Trolox concentration (6.25−50 μM) and the net AUC and were expressed as micromoles of Trolox equivalents per gram of fresh weight (*24*).

Hydroxyl Radical Scavenging Capacity (•OH; HOSC) Assay. The •OH in aqueous media is generated through the Fenton reaction. The HOSC assay was conducted with acetone solutions according to a previously published protocol (*25*) with some modifications. The assay was carried out using a high-throughput instrument platform consisting of a robotic eight-channel liquid handling system and a microplate fluorescence reader with an FL800 microplate fluorescence reader (Bio-Tek Instruments). Fluorescence was measured every minute for 3 h with an excitation wavelength of 485 nm and an emission wavelength of 535 nm. The plate reader was controlled by software KC4 3.0 (revision 29). Sample dilution was accomplished by a Precision 2000 automatic pipetting system managed by precision power software (version 1.0) (Bio-Tek Instruments). Reaction mixtures consisted of 170 μL of 9.28×10^{-8} M FL prepared in 75 mM sodium phosphate buffer, 30 μL of standard or sample or blank, 40 μL of 0.1990 M H_2O_2, and 60 μL of 3.43 mM $FeCl_3$. Trolox prepared in 50% acetone at concentrations of 20, 40, 60, 80, and 100 μM was used to prepare the standard curve for HOSC quantification. The HOSC values were determined by calculating the net AUC of the standards and samples. The standard curve was obtained by plotting Trolox concentrations against the average net AUC of the two measurements for each concentration. Final HOSC values were calculated using the regression equation between Trolox concentration and the net AUC and were expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight.

2,2-Di-(4-tert-octylphenyl)-1-picrylhydrazyl (DPPH•) assay. To determine the antioxidant activity of different extracts, DPPH• radicals were used. In the radical form, this molecule has an absorbance at 515 nm that disappears with acceptance of an electron from an antioxidant compound to become a stable diamagnetic molecule. The method described by Cheng et al. (*26*) was used with some modifications. A high-throughput instrument platform consisting of a robotic eight-channel liquid handling system and a microplate with an FL800 microplate UV−visible spectrometer reader (Bio-Tek Instruments) was used. The automated sample preparation was performed using a Precision 2000 instrument and automatic pipetting system managed by precision power software (version 1.0) (Bio-Tek Instruments). The plate reader was controlled by software KC4 3.0 (revision 29). Five grams of fruit sample was extracted with 25 mL of 50% acetone, and 50 μL of this extract was diluted with 150 μL of 50% acetone. Then, 40 μL of this diluted extract was used for assay. An aliquot (160 μL) of the DPPH• solution (3.3 mg/50 mL 100% ethanol) was used in each well. The mixtures were shaken gently and allowed to stand for 40 min in the dark. The decrease in absorbance was measured at 515 nm against a blank (50% acetone) without extract using an FL800 microplate UV−visible spectrometer reader (Bio-Tek Instruments). The DPPH• values were determined by calculating the end point of the standards (gallic acid) and samples. Final DPPH• values were calculated using the regression equation between standard gallic acid concentrations (10−60 mg) and were expressed as milligrams of gallic acid equivalents per gram of fresh weight.

Superoxide Radicals ($O_2^{\bullet -}$) Scavenging Activity. The assay for $O_2^{\bullet -}$ was performed using the methods of Gutteridge (*27*). Final $O_2^{\bullet -}$ scavenging capacity was calculated using the regression equation between standard ascorbate concentrations (10−100 mg) and was expressed as milligrams of ascorbate equivalents per gram of fresh weight.

Total Anthocyanin and Total Phenolic Content. Five grams of pericarp tissues was extracted with 25 mL of 50% acetone. Total anthocyanin content in pericarp tissue extracts was determined using the pH differential method (*28*). Absorbance was measured in a Shimadzu spectrophotometer (Shimadzu UV-160) at 510 nm and at 700 nm in buffers at pH 1.0 and 4.5, using $A = [(A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}]$ with a molar extinction coefficient of cyanidin-3-glucoside (29600). Results were expressed as milligrams of cyanidin-3-glucoside equivalents per 100 g of fresh weight. Total soluble phenolics in the pericarp tissue extracts were determined with Folin−Ciocalteu reagent according to the method of Slinkard and Singleton (*29*) using gallic acid as a standard. Results were expressed as milligrams gallic acid equivalents (GAE) per 100 g of fresh weight.

High-Performance Liquid Chromatography (HPLC) Analysis of Flavonoids in Litchi Pericarp. HPLC was used to separate and determine individual anthocyanins and phenolic compounds in litchi pericarp samples. The supernatants (18 mL) from the above extracts were concentrated to dryness using a Buchler Evapomix (Fort Lee, NJ) in a water bath at 35 °C and were dissolved in 4 mL of acidified water (3% formic acid) and then passed through a C_{18} Sep-Pak cartridge (Waters), which was previously activated with methanol followed by water and then 3% aqueous formic acid. Anthocyanins and other phenolics were adsorbed onto a column, whereas sugars, acids and other water-soluble compounds were eluted with 10 mL of 3% formic acid. Anthocyanins and other phenolics were then recovered with 2.0 mL of acidified methanol containing 3% formic acid. The methanol extract was passed though a 0.45 μm membrane filter (Millipor, MSI, Westboro, MA), and 20 μL was analyzed by HPLC. The samples were determined using a Waters (Milford, MA) HPLC system coupled with a photodiode array detector (Waters 996 series) and equipped with two pumps (600E system controller). Samples were injected at ambient temperature (20 °C) into a reverse phase Nova-Pak C_{18} column (150 × 3.9 mm, particle size = 4 μm) with a guard column (nova-pak C_{18}, 20 × 3.9 mm, particle size = 4 μm) (Waters). The mobile phase consisted of 5% aqueous formic acid (A) and HPLC grade acetonitrile (B). The flow rate was 1 mL/min, with a gradient profile consisting of A with the following proportions (v/v) of B: 0−1 min, 4%; 1−10 min, 4−6% B; 10−15 min, 6% B; 15−35 min, 6−18% B; 35−40 min, 18−20% B; 40−42 min, 20−45% B; 42−45 min, 45−100% B; 45−50 min, 100% B. The phenolic compounds in pericarp extracts were identified by their UV spectra, recorded with a diode array detector, and by chromatographic comparison with authentic markers (*30*). Retention times and spectra were compared to those of the pure standards, and the results were confirmed by co-injection with authentic standards. Individual phenolic acids, flavonols, and anthocyanins were quantified by comparison with an external standard of quercetin-3-rutinoside, quercetin-3-glucoside, pelargonidin-3-rhamnoside, pelargonidin-3,5-diglucoside, cyanidin-3-glucoside, cyanidin-3-rutinoside, malvidin-3-acetylglucoside, and malvidin-3-arabinoside. Each standard was dissolved in methanol at a concentration of 1 mg/mL, and five dilute solutions from these stock solutions were used to prepare calibration curves of each standard. Recoveries were measured by extracting the recovered amounts of pure substances added to frozen litchi samples before the experiment. Three replicates from each sample were used for HPLC analyses. Scanning between 250 and 550 nm was performed, and data were collected by the Waters 990 3-D chromatography data system.

Statistical Analysis. Data presented are the mean ± SD values. All statistical analyses were performed with the NCSS Statistical Analysis System (Statistical Analysis and Graphics, Kaysville, UT) (NCSS, 2007) (*31*). One- and two-way analyses of variance (ANOVA) were used to compare the means. Differences were considered to be significant at $p \leq 0.05$. Correlations were determined between antioxidant activity, total phenolics, and total anthocyanins in litchi by using NCSS Statistical Analysis System (NCSS, 2007). Results are reported as R^2 values.

RESULTS AND DISCUSSION

Changes in Color and Decay Development During Storage. The most dramatic effect of ACS was the retention of red color and the prevention of browning. These effects were shown by the objective measurements of the pericarp color with a colorimeter using the L^*, a^*, b^* system established by the Commission Internationale de L'Eclairage (CIE, International Commission on Illumination) in 1978. The a^* values, which indicate the degree of the red color, were much higher in fruit that received 1.25% and higher concentrations than in those treated with 0.625% or the control in both 5 and 10 °C storage after 5 days of storage

Table 1. Changes of Color and Decay in Litchi Pericarp Treated with Acidified Calcium Sulfate during Storage at 5 and 10 °C[a]

days in storage	storage temp (°C)	concn (%)	L^*	a^*	b^*	decay (%)
0		initial	35.09 ± 2.97	43.25 ± 2.42	20.46 ± 1.19	0
5	5	control	28.33 ± 1.79	30.61 ± 4.27	19.30 ± 1.74	0
		0.625	28.87 ± 1.47	31.56 ± 2.82	20.02 ± 1.33	0
		1.25	38.86 ± 1.77	41.46 ± 3.87	20.72 ± 2.33	0
		2.5	37.25 ± 2.49	44.92 ± 2.37	20.53 ± 0.91	0
		5.0	37.58 ± 2.48	41.54 ± 2.51	20.96 ± 2.08	0
5	10	control	32.79 ± 1.97	29.62 ± 3.97	22.25 ± 1.83	0
		0.625	30.45 ± 1.47	31.44 ± 3.57	23.02 ± 2.13	0
		1.25	37.06 ± 2.30	40.51 ± 3.17	18.75 ± 2.60	0
		2.5	38.27 ± 1.91	42.70 ± 2.35	20.25 ± 1.32	0
		5.0.	33.87 ± 2.31	41.39 ± 2.90	19.71 ± 1.76	0
15	5	control	28.30 ± 3.59	28.02 ± 3.35	18.14 ± 3.34	23 ± 1.6
		0.625	27.35 ± 2.36	31.28 ± 2.65	17.33 ± 2.65	16 ± 1.2
		1.25	36.50 ± 2.70	35.79 ± 2.71	16.59 ± 3.25	0
		2.5	35.89 ± 2.45	38.47 ± 1.90	20.50 ± 3.19	0
		5.0	35.25 ± 2.60	39.52 ± 1.49	17.69 ± 1.30	0
15	10	control	28.10 ± 2.53	22.24 ± 4.74	13.50 ± 2.47	48 ± 3.1
		0.625	28.34 ± 2.64	24.38 ± 2.34	14.68 ± 1.68	42 ± 2.6
		1.25	33.09 ± 1.85	28.31 ± 3.29	17.00 ± 2.21	27 ± 1.8
		2.5	36.01 ± 2.90	35.38 ± 1.75	17.11 ± 3.07	0
		5.0	33.47 ± 2.67	35.13 ± 2.79	15.63 ± 1.25	0

[a] Data expressed as mean ± SD.

(**Table 1**). The degree of redness decreased in all fruits with time in storage. However, after 15 days of storage, the differences in a^* values among various concentrations were still striking. The differences in the lightness measurement (L^*) were also significant among treatments receiving different concentrations of ACS. The L^* values were much lower in litchi treated with 0.625% or control than fruit treated with 1.25% or higher, indicating that fruit received 1.25, 2.5, or 5% ACS treatment were not as dark and had less or no browning. This pattern was consistent for different temperatures (5 and 10 °C) and durations (5 and 15 days) of storage (**Table 1**). However, storage temperatures and durations greatly influenced the decay development in litchi fruit. No decay was found after 5 days of storage at either 5 or 10 °C (**Table 1**). After 15 days of storage, however, control fruit and fruit treated with 0.625% of ACS had 23 and 16% decay, respectively, whereas fruit treated with 1.25% or higher concentrations remained free from decay at 5 °C storage. Mold growth on litchi stored at 10 °C increased to 48% on nontreated fruit and to 42% on fruit treated with 0.625%. Even fruit treated with 1.25% had 27% decay after 15 days of storage at 10 °C. ACS at concentrations of 2.5 and 5% was able to prevent mold growth and browning and maintained red pericarp color after 15 days of storage at either 5 or 10 °C. Thus, even though storage temperature and duration had little influence on ACS effect on the patterns of pericarp color change, they made a big difference in the development of decay.

SSC and Sugar and Organic Acid Changes during Storage. SSC in litchi fruit is relatively high compared to most other fruits. Freshly harvested 'Brewster' litchi in our study had 22.8% of SSC (**Table 2**). However, SSC gradually declined during storage, particularly at higher temperatures. It decreased to 19.4−20.0% after 5 days of storage at 5 °C and to 18.6−19.9% at 10 °C. SSC further decreased to 18.6−18.9% after 15 days of storage at 5 °C and to 17.5−18.2% at 10 °C. ACS treatment did not seem to affect the SSC. Virtually no difference in SSC could be discerned among various treatments within each storage duration at the same temperature (**Table 2**).

Three major sugars were found in litchi fruit including fructose, glucose, and sucrose (**Table 2**). Litchi pulp contained comparable high amounts of all three sugars. Similar to SSC, the sugar levels decreased with time in storage. However, little difference was detected among different ACS treatments within the same storage temperature and same duration. Malic acid was found to be the predominant organic acid in litchi pulp (**Table 2**). Citric acid was also detected but only in minor amounts. The malic acid values in all treatments maintained at relatively high levels for the first five days of storage, but decreased substantially and simultaneously after 15 day, especially at 10 °C. This decline in acid values may have contributed to the rapid deterioration of flavor and taste. No differences were found among various treatments.

Changes in PPO and POD Activities As Affected by ACS. Both PPO and POD have been found to be associated with the litchi pericarp browning process (*32*). The oxidation of anthocyanins by PPO and POD was shown to be the primary mechanism of the surface color change. The enzymatic reaction causes rapid degradation of red anthocyanin pigment and formation of *o*-quinone and other brown-colored compounds. This browning process is accelerated by water loss and desiccation of the pericarp. In addition to SO_2 fumigation, several other techniques have been reported in an attempt to inhibit PPO and POD activities. These include hot water and oxalic acid dip, exposure to pure oxygen atmosphere, treatment with citric acid and chitosan or *N*-acetylcysteine and isoascorbic acid, and integrated application of 1-methylcyclopropene and modified atmosphere packaging. An almost complete inhibition of POD activities and a substantial retardation of PPO activities by 2.5 and 5% of ACS were found in our study (**Figure 1**). This inhibition persisted even after 15 days of storage at both 5 and 10 °C. These low enzyme activities corresponded to high a^* values and retention of red color (**Table 1**).

Antioxidant Enzyme Activities. Oxygen scavenging enzymes including CAT, AsA-POD, and GSH-POD and enzymes of the ascorbate−glutathione cycle (GR, MDAR, and DHAR) are

Table 2. Changes of Soluble Solids Content (SSC), Sugars, and Organic Acids in Litchi Pericarp Treated with Acidified Calcium Sulfate during Storage at 5 and 10 °C[a]

				sugars (mg/g of fresh weight)			organic acids (mg/g of fresh weight)	
days in storage	storage temp (°C)	concn (%)	SSC (%)	fructose	glucose	sucrose	malic	citric
0		initial	22.8 ± 0.07	64.7 ± 4.8	72,6 ± 5.3	68.2 ± 4.2	7.6 ± 0.5	0.8 ± 0.04
5	5	control	20.0 ± 0.07	55.6 ± 3.2	63.2 ± 4.2	57.6 ± 3.3	4.7 ± 0.2	0.4 ± 0.02
		0.625	19.4 ± 0.07	52.5 ± 3.6	60.1 ± 4.7	53.7 ± 4.5	4.4 ± 0.3	0.5 ± 0.03
		1.25	19.6 ± 0.07	55.1 ± 2.6	58.3 ± 3.8	52.2 ± 5.2	4.1 ± 0.7	0.3 ± 0.04
		2.5	19.5 ± 0.07	53.0 ± 2.3	65.3 ± 5.2	54.7 ± 2.5	4.8 ± 0.3	0.5 ± 0.04
		5.0	19.4 ± 0.07	52.7 ± 3.9	62.9 ± 5.6	53.2 ± 3.8	4.4 ± 0.2	0.5 ± 0.03
	10	control	19.9 ± 0.07	50.5 ± 3.4	60.3 ± 4.1	52.8 ± 4.1	4.2 ± 0.3	0.3 ± 0.04
		0.625	19.6 ± 0.07	48.4 ± 2.2	57.6 ± 3.5	51.6 ± 3.3	4.0 ± 0.1	0.3 ± 0.03
		1.25	19.5 ± 0.00	46.8 ± 3.8	55.2 ± 3.2	49.5 ± 2.4	3.9 ± 0.3	0.2 ± 0.02
		2.5	19.6 ± 0.07	51.2 ± 4.7	56.4 ± 4.3	52.7 ± 4.3	4.2 ± 0.4	0.4 ± 0.04
		5.0	18.6 ± 0.14	47.1 ± 4.5	59.1 ± 4.1	52.1 ± 3.9	4.3 ± 0.3	0.3 ± 0.01
15	5	control	18.9 ± 0.07	44.6 ± 3.5	52.5 ± 3.7	46.5 ± 4.3	3.6 ± 0.4	0.1 ± 0.02
		0.625	18.6 ± 0.07	40.7 ± 2.8	50.8 ± 2.8	43.9 ± 4.1	3.3 ± 0.2	0.2 ± 0.01
		1.25	18.9 ± 0.00	44.8 ± 3.4	51.1 ± 3.5	46.8 ± 3.3	3.6 ± 0.3	0.2 ± 0.02
		2.5	18.8 ± 0.07	43.1 ± 3.2	52.4 ± 4.3	47.3 ± 2.8	3.5 ± 0.2	0.3 ± 0.02
		5.0.	18.6 ± 0.14	42.2 ± 3.4	51.8 ± 3.2	48.3 ± 3.7	3.6 ± 0.4	0.3 ± 0.03
	10	control	17.7 ± 0.07	38.4 ± 3.1	48.4 ± 3.5	39.6 ± 4.3	3.4 ± 0.3	0.1 ± 0.01
		0.625	17.6 ± 0.07	36.8 ± 4.3	46.8 ± 4.1	37.8 ± 5.0	3.1 ± 0.1	0.1 ± 0.02
		1.25	17.5 ± 0.00	36.2 ± 4.1	45.2 ± 4.4	38.9 ± 3.2	3.2 ± 0.3	0.3 ± 0.02
		2.5	18.0 ± 0.14	38.8 ± 2.5	48.8 ± 3.7	41.5 ± 2.9	3.6 ± 0.4	0.2 ± 0.01
		5.0	18.2 ± 0.14	40.2 ± 3.1	51.2 ± 3.9	43.4 ± 4.8	3.4 ± 0.5	0.2 ± 0.02

[a] Data expressed as mean ± SD.

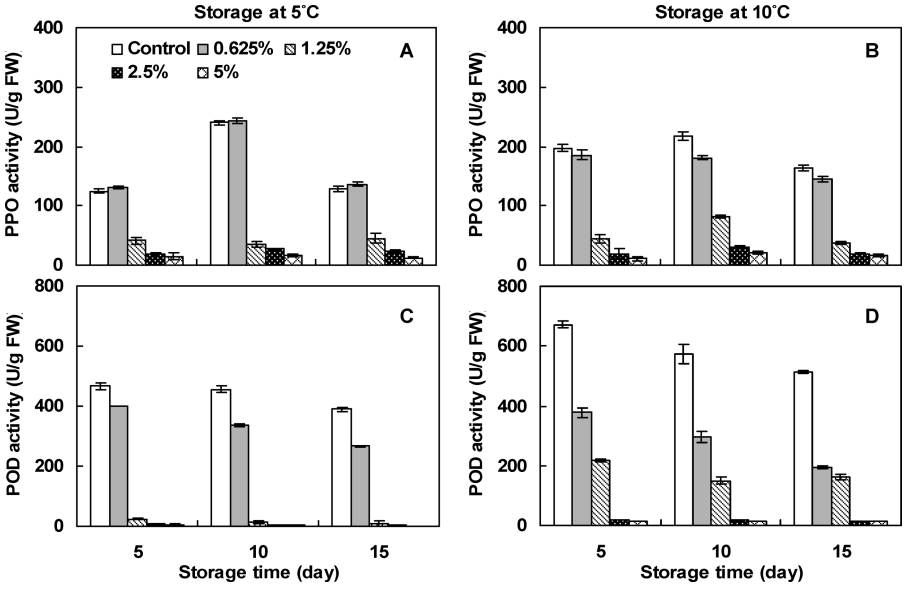

Figure 1. Changes of polyphenol oxidase (PPO) and peroxidase (POD) activities in litchi pericarp treated with acidified calcium sulfate during storage at 5 and 10 °C.

important because they are needed to keep the levels of free radicals and active oxygen species low, so that the plant tissues can maintain normal cell metabolisms. Free radicals can be produced during stress or even in normal conditions. They can be generated enzymatically through mitochondrial respiration, the cytochrome P450 system, by various soluble enzymes, and also from nonenzymatic reactions of oxygen with organic compounds (*33*). Increased activities of these free radicals could create oxidative stress, which leads to a variety of biochemical and physiological malfunctions often resulting in metabolic impairment. Fortunately, plants have this efficient enzymatic system for active oxygen detoxification. Therefore, maintaining proper levels of these oxygen-scavenging enzymes is essential to avoid creating any disorders. The activities of oxygen-scavenging enzymes, CAT, AsA-POD, and GSH-POD, in litchi pericarp declined substantially during storage at both 5 and 10 °C (**Figure 2**). The largest decrease occurred in GSH-POD, from 125 U/g of fresh weight after 5 days of storage to 62 U/g of fresh weight after 15 days of storage at 5 °C. Treatment with 1.25% or higher concentrations of ACS could significantly increase the activities

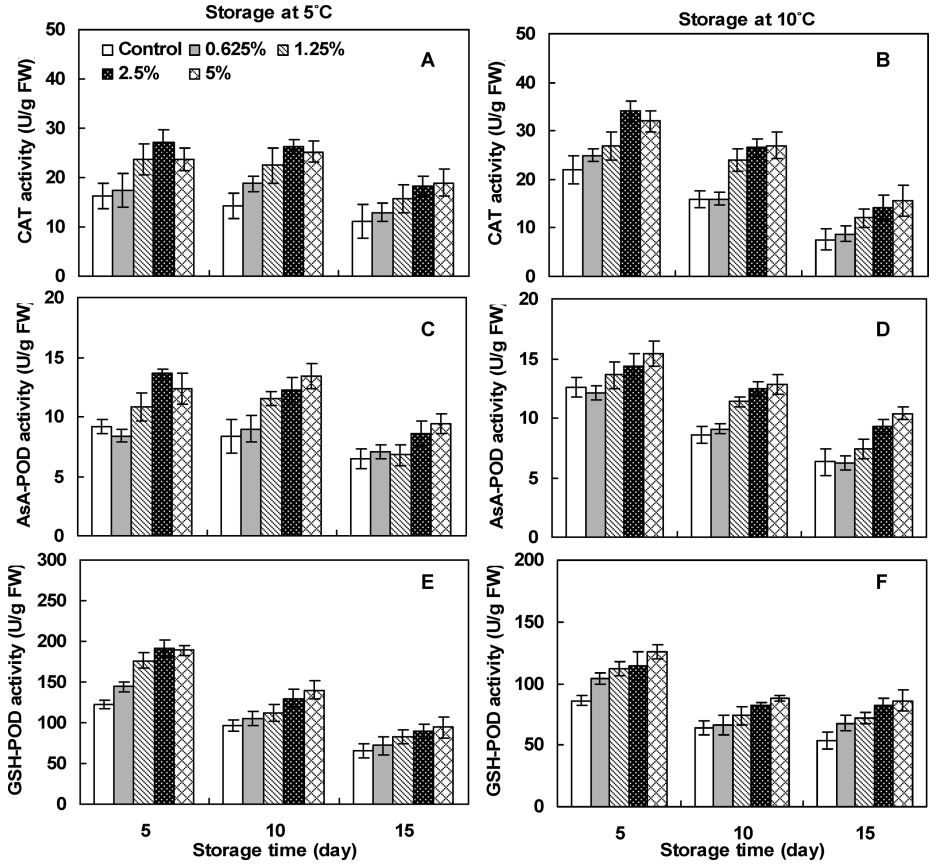

Figure 2. Changes of catalase (CAT), ascorbate peroxidase (AsA-POD), and glutathione peroxidase (GSH-POD) activities in litchi pericarp treated with acidified calcium sulfate during storage at 5 and 10 °C.

of CAT, AsA-POD, and GSH-POD (**Figure 2**). This was apparent only after 5 days of storage at 5 °C. The differences among various treatments, however, diminished with time in storage at both 5 and 10 °C for GSH-POD. Fruit treated with 2.5 or 5% ACS maintained higher AsA-POD activities throughout the 15 days of storage at both 5 and 10 °C. The activities of GR, MDAR, and DHAR in fruit treated with 2.5 or 5% ACS were also significantly enhanced after 5 days at 5 °C (**Figure 3**). These treated fruit consistently maintained higher activities of GR and MDAR throughout the 15 days of storage at both 5 and 10 °C. These higher activities of antioxidant enzymes should have also contributed in preventing the oxidation and browning of the pericarp.

Free Radical Scavenging Activities. The scavenging activities of litchi pericarp against $ROO^{\bullet}$, $DPPH^{\bullet}$, $^{\bullet}OH$, and $O_2^{\bullet-}$ are shown in **Table 3**. Fruit treated with 1.25% or higher concentrations of ACS had markedly higher radical scavenging activities for $ROO^{\bullet}$, $DPPH^{\bullet}$, $^{\bullet}OH$, and $O_2^{\bullet-}$ compared to untreated fruit during 5 and 15 days of storage at 5 and 10 °C. Our previous study showed that high scavenging activity was important in reducing reactive oxygen species and protecting cellular constituents from oxidative damage, thereby strengthening the defensive system in the tissues against microbial invasion and retarding the physiological deterioration and spoilage of fruit (*34*).

Total Phenol and Total Anthocyanin. The total phenolic and total anthocyanin contents in litchi pericarp are shown in **Figure 4**. On the basis of fresh weight, fruit treated with 2.5 and 5% ACS had significantly higher anthocyanin and phenolic contents than other treatments during 15 days of storage at both 5 and 10 °C. Previous research showed that a linear relationship existed between total anthocyanin or phenolic content and ORAC in various fruits (*35*, *36*). This suggests that the antioxidant activity of fruit is mainly derived from the contribution of both anthocyanin and phenolic compounds in fruits. The individual anthocyanin and phenolic compounds contributed to total antioxidant activities in litchi pericarp were separated and identified by using reversed-phase HPLC and are presented in the next section.

HPLC Analysis of Flavonoids in Litchi Pericarp. The HPLC analysis of litchi pericarp extracts revealed that major flavonoids were cyanidin-3-rutinoside, cyanidin-3-glucoside and quercetin-3-rutinoside (**Table 4**). The pericarp also contained pelargonidin-3,5-diglucoside, pelargonidin-3-rhamnoside, and quercetin-3-glucoside, with minor amounts of malvidin-3-arabinoside and malvidin-3-acetylglucoside. Therefore, the content of cyanidin-based flavonoids was higher than those of pelargonidin- or malvidin-based flavonols. The levels of flavonoids generally increased with increasing concentration of ACS used, but decreased with time in storage, particularly at higher temperature. With a few exceptions, 2.5 and 5% ACS treated samples retained higher flavonoids than other treatments in both storage temperatures and in all sampling times. This trend was particularly true in the three major litchi pericarp flavonoids, cyanidin-3-rutinoside, cyanidin-3-glucoside and quercetin-3-rutinoside (**Table 4**). Flavonoids are effective antioxidants. They are potent quenchers of $ROO^{\bullet}$, $O_2^{\bullet-}$, and $^1O_2$ free radicals. Therefore, increasing flavonoid content would tend to retard the deterioration of fruit tissue and maintain the quality. Anthocyanins are glycosides that release aglycon forms (anthocyanidins) by hydrolysis. In addition to providing red color, anthocyanins have been reported to help reduce damage caused by free radical activities, such as low-density lipoprotein oxidation (*37*). Retention of high anthocyanin content by ACS treatment is, therefore, beneficial to quality maintenance of litchi fruit.

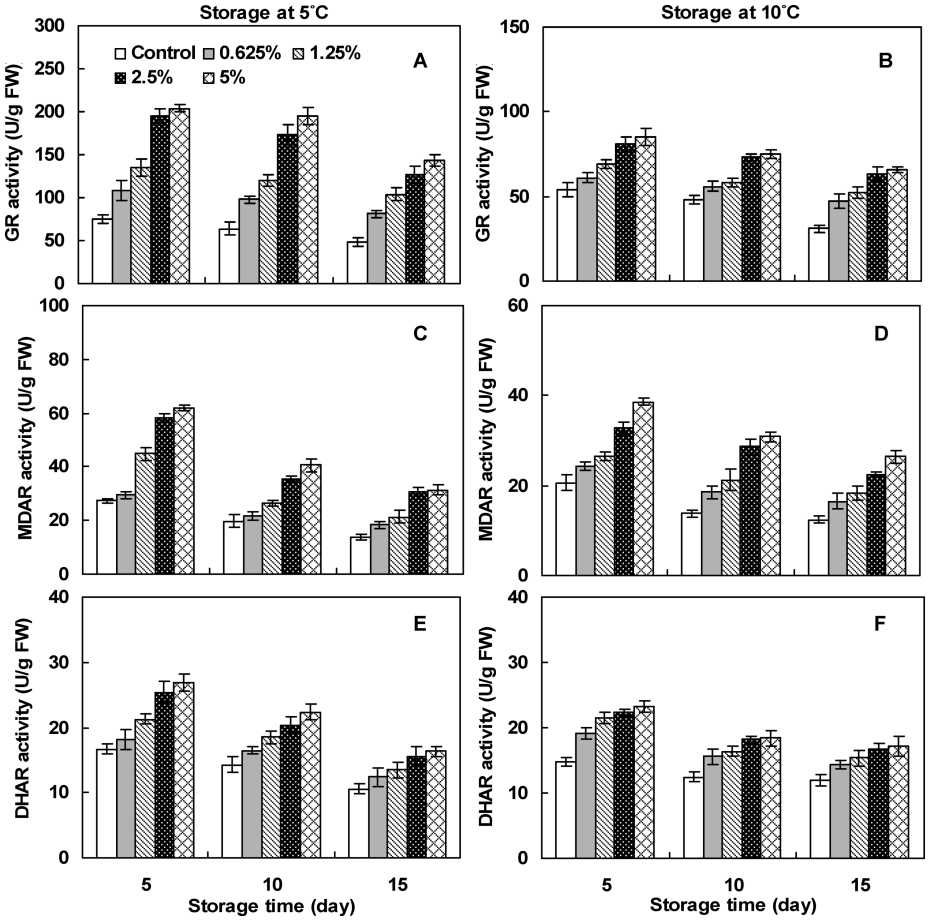

Figure 3. Changes of glutathione reductase (GR), monodehydroascorbate reductase (MDAR), and dehydroascorbate reductase (DHAR) activities in litchi pericarp treated with acidified calcium sulfate during storage at 5 and 10 °C.

Table 3. Changes of Free Radical Scavenging Activities in Litchi Pericarp Treated with Acidified Calcium Sulfate during Storage at 5 and 10 °C[a]

days in storage	storage temp (°C)	concn (%)	ORAC (μmol/g of fr wt)	DPPH (mg/g of fr wt)	HOSC (μmol of TE/g of fr wt)	superoxide radicals (mg of ascorbate/g of fr wt)
0		initial	331.3 ± 5.7	37.1 ± 0.3	348.7 ± 6.0	128.5 ± 8.4
5	5	control	283.8 ± 3.2	38.5 ± 0.1	236.9 ± 6.4	69.7 ± 4.5
		0.625	507.8 ± 5.5	40.2 ± 0.6	551.5 ± 7.4	199.3 ± 3.9
		1.25	506.1 ± 2.5	42.7 ± 0.4	693.7 ± 5.8	206.6 ± 2.6
		2.5	517.6 ± 2.4	44.5 ± 0.9	782.8 ± 8.2	259.7 ± 3.9
		5.0	585.3 ± 3.6	40.7 ± 0.2	736.2 ± 9.8	246.9 ± 3.9
	10	control	241.7 ± 6.1	35.8 ± 0.8	284.8 ± 3.8	94.6 ± 4.5
		0.625	437.4 ± 7.1	35.8 ± 1.0	465.0 ± 7.1	205.3 ± 7.1
		1.25	529.8 ± 7.9	39.4 ± 0.5	697.5 ± 6.5	216.7 ± 1.3
		2.5	573.1 ± 4.7	44.7 ± 1.1	844.6 ± 6.4	251.4 ± 1.3
		5.0.	550.2 ± 4.8	40.6 ± 1.0	727.5 ± 9.7	238.6 ± 2.6
15	5	control	303.3 ± 2.9	34.1 ± 1.1	310.5 ± 4.1	58.5 ± 3.9
		0.625	348.0 ± 2.8	37.7 ± 1.3	333.3 ± 4.0	210.3 ± 6.5
		1.25	490.1 ± 6.5	38.8 ± 0.8	660.9 ± 6.8	220.4 ± 3.9
		2.5	557.2 ± 5.6	42.6 ± 1.3	855.6 ± 8.3	262.9 ± 1.9
		5.0	543.4 ± 5.6	40.4 ± 0.9	734.2 ± 4.2	251.4 ± 1.3
	10	control	291.7 ± 4.6	33.5 ± 0.3	228.4 ± 3.2	122.2 ± 1.3
		0.625	245.8 ± 8.0	33.6 ± 0.7	180.1 ± 1.4	230.4 ± 2.6
		1.25	381.2 ± 8.5	36.1 ± 1.1	333.7 ± 4.2	275.7 ± 4.5
		2.5	499.0 ± 4.3	38.8 ± 0.8	608.5 ± 8.0	253.3 ± 7.8
		5.0	498.5 ± 6.6	37.4 ± 0.8	633.7 ± 3.7	262.4 ± 2.6

[a] Data expressed as mean ± SD.

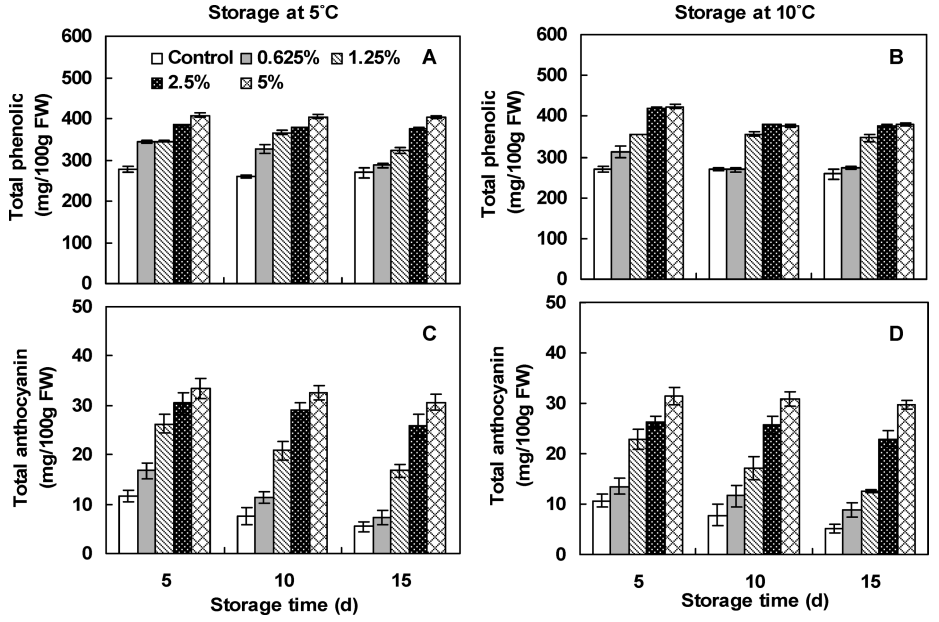

Figure 4. Changes of total phenolic and total anthocyanin content in litchi pericarp treated with acidified calcium sulfate during storage at 5 and 10 °C.

Table 4. Changes of Flavonoids in Litchi Pericarp Treated with Acidified Calcium Sulfate during Storage at 5 and 10 °C[a]

days in storage	storage temp (°C)	concn (%)	quercetin-3-rutinoside	quercetin-3-glucoside	pelargonidin-3-rhamnoside	pelargonidin-3,5-diglucoside	cyanidin-3-glucoside	cyanidin-3-rutinoside	malvidin-3-acetylglucoside	malvidin-3-arabinoside
0		initial	150.6 ± 0.8	29.9 ± 0.3	68.0 ± 1.2	86.8 ± 2.5	195.2 ± 4.4	284.9 ± 1.3	1.9 ± 0.0	1.8 ± 0.1
5	5	control	105.6 ± 0.2	26.2 ± 0.2	10.2 ± 0.3	29.7 ± 0.2	69.2 ± 0.1	124.9 ± 2.1	0 ± 0.0	0.3 ± 0.0
		0.625	110.5 ± 0.2	32.9 ± 0.2	36.7 ± 0.5	68.4 ± 2.2	165.6 ± 3.1	264.7 ± 1.9	0.9 ± 0.1	2.8 ± 0.2
		1.25	145.1 ± 2.6	37.3 ± 0.7	78.8 ± 1.2	130.5 ± 1.7	269.3 ± 10.1	430.7 ± 0.8	3.3 ± 0.0	4.1 ± 0.1
		2.5	131.0 ± 2.3	38.3 ± 0.2	65.7 ± 2.5	89.8 ± 1.2	243.6 ± 8.5	464.6 ± 0.7	4.1 ± 0.0	5.2 ± 0.3
		5.0	190.0 ± 0.9	62.9 ± 0.0	73.8 ± 0.8	109.4 ± 2.1	254.9 ± 2.9	487.4 ± 0.0	6.4 ± 0.2	7.8 ± 0.0
	10	control	144.5 ± 0.1	41.3 ± 0.1	2.8 ± 0.1	16.2 ± 0.3	33.1 ± 0.8	80.0 ± 0.1	0.0 ± 0.0	0.0 ± 0.0
		0.625	115.5 ± 2.2	29.6 ± 0.5	16.6 ± 1.1	29.6 ± 0.9	87.2 ± 0.9	156.6 ± 0.1	0.0 ± 0.0	1.2 ± 0.1
		1.25	151.1 ± 3.8	44.1 ± 0.2	59.5 ± 2.4	81.4 ± 2.0	230.5 ± 4.4	358.8 ± 3.8	3.0 ± 0.3	4.5 ± 0.1
		2.5	168.4 ± 2.4	44.1 ± 0.3	88.7 ± 1.3	141.0 ± 3.1	277.5 ± 7.1	458.1 ± 5.4	8.9 ± 0.2	9.5 ± 0.1
		5.0.	172.5 ± 0.5	53.4 ± 0.1	84.5 ± 2.0	115.6 ± 0.3	257.5 ± 0.7	434.9 ± 0.3	8.5 ± 0.5	9.2 ± 0.1
15	5	control	102.4 ± 0.5	17.5 ± 0.7	11.5 ± 0.3	30.1 ± 0.2	63.9 ± 0.3	138.6 ± 1.1	0.2 ± 0.0	0.9 ± 0.2
		0.625	138.7 ± 1.8	30.8 ± 0.7	13.9 ± 0.1	46.2 ± 1.0	85.8 ± 2.6	219.8 ± 2.2	0.3 ± 0.0	1.7 ± 0.0
		1.25	128.7 ± 0.8	21.1 ± 0.1	36.5 ± 3.3	80.2 ± 1.1	197.8 ± 8.7	345.0 ± 0.5	1.4 ± 0.1	3.6 ± 0.0
		2.5	178.5 ± 1.1	29.4 ± 0.0	76.5 ± 9.8	112.5 ± 0.5	260.2 ± 2.1	493.0 ± 0.6	5.1 ± 0.2	8.8 ± 0.0
		5.0.	135.0 ± 0.3	21.8 ± 0.2	35.3 ± 3.7	108.3 ± 2.6	229.3 ± 5.3	527.8 ± 2.6	5.1 ± 0.1	9.3 ± 0.0
	10	control	114.0 ± 0.1	18.4 ± 0.5	5.6 ± 0.3	31.8 ± 1.0	61.7 ± 1.7	147.1 ± 0.3	0.3 ± 0.1	1.7 ± 0.2
		0.625	47.8 ± 0.3	12.4 ± 0.1	0.8 ± 0.1	13.4 ± 0.1	40.2 ± 2.5	95.1 ± 0.7	0.1 ± 0.0	0.9 ± 0.0
		1.25	61.6 ± 0.1	16.0 ± 0.4	14.9 ± 5.0	35.8 ± 0.3	78.0 ± 1.0	193.4 ± 3.5	1.2 ± 0.0	3.9 ± 0.0
		2.5	183.8 ± 2.5	33.3 ± 0.0	57.5 ± 1.9	104.4 ± 0.4	187.0 ± 1.1	501.8 ± 6.9	10.0 ± 0.2	16.4 ± 0.4
		5.0	141.8 ± 1.1	22.2 ± 0.9	51.4 ± 12.8	101.0 ± 1.6	173.6 ± 0.8	392.1 ± 12.8	7.6 ± 0.4	12.7 ± 0.1

[a] Data expressed as mean ± SD (micrograms per gram of fresh weight).

Correlations. A negative correction was found between red color (a^* values) and browning enzyme activities, particularly PPO and POD. High negative correlations were shown for color and PPO after only 5 days at both 5 and 10 °C with $R^2 = 0.952$ and 0.986, respectively. Reverse correlations were also high for color and POD, but to a lesser extent, with $R^2 = 0.904$ and 0.808 for 5 and 10 °C, respectively, after 5 days. These results indicate that the browning of litchi pericarp is closely associated with both of these enzymes, particularly with PPO. There was a positive correlation between ORAC values with total phenol, total anthocyanin, and DPPH and OH radical scavenging activity. The correlation between ORAC and DPPH was relatively low ($R^2 = 0.6422$). Comparable high correlation values were found between ORAC with total phenol ($R^2 = 0.8406$) and ORAC with total anthocyanin ($R^2 = 0.8493$). Positive correlations between ORAC and total phenolic or anthocyanin content have also been reported previously in other fruits (*35*, *36*). However, the highest correlation was found between OH radical scavenging activity and ORAC values with a high R^2 of 0.9219. This indicates that the antioxidant capacity of litchi could be measured either by the ORAC or OH radical scavenging assay.

Our study demonstrates that ACS was effective in maintaining quality of litchi fruit after harvest including prevention of browning, retention of red color, and inhibition of microbial growth. The present study also shows that these effects on litchi pericarp occurred without adversely affecting SSC, sugars, and organic acids of the pulp. Further investigation is warranted to determine the effect of this compound on flavor, texture, and other sensory parameters of the fruit. The effect of ACS on other litchi cultivars also deserves to be evaluated.

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