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Sucrose delays senescence and preserves functional compounds in *Asparagus officinalis* L.

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ABSTRACT

The high metabolic rate of harvested asparagus spears (*Asparagus officinalis* L.) causes rapid deterioration. To extend shelf life, we investigated the effect of sucrose treatment on asparagus during storage. Asparagus spears were treated with 3%, 5%, and 10% sucrose and stored at 2 °C for 20 h. Cellular respiration decreased, but other processes were unaltered by exogenous sucrose. The overall appearance of asparagus treated with 3% sucrose and stored at 2 °C for 18 days was rated as good and excellent, unlike that of untreated spears. Asparagus treated with sucrose maintained firmness for 15 days, while untreated spears lost firmness and showed increased water-soluble pectin content during storage. Carbohydrate levels were also higher in sucrose-treated than in control samples. Transcript levels of cell-wall-related genes, including xyloglucan endotransglycosylase (XET)1, XET2, and peroxidase (prx)1, prx2, and prx3 were upregulated by sucrose. Cyanidin 3-*O*-rutinoside and rutin levels immediately increased upon addition of sucrose and remained high relative to the control during storage. Thus, sucrose modulates asparagus cell wall components and maintains the functionality of important compounds during storage, thus effectively prolonging shelf life.

1. Introduction

Asparagus (*Asparagus officinalis* L.) spears deteriorate rapidly post-harvest and have a shelf life of 3–5 days at ambient temperature [1]. The short shelf life is related to high metabolic rate and consequently high respiratory activity post-harvest. Symptoms of deterioration include bract opening, toughening, loss of soluble solids, and fungal decay [2]. Toughening occurs due to changes in the mechanical properties of cell wall components [3]. Levels of carbohydrates soluble in sucrose decline rapidly in asparagus spear tips, triggering deterioration in the whole spear [4]. The rapid growth of developing plant tissues can be limited by carbohydrate depletion; however, addition of exogenous carbohydrates can increase respiration rate and longevity [5]. Higher metabolism in asparagus spears results in rapid carbohydrate depletion.

Sucrose, the primary carbohydrate source in most plants, can prolong the post-harvest shelf life of asparagus spears; storage in a controlled atmosphere prevents sucrose loss and consequent deterioration [6]. Sucrose treatment also delays senescence in broccoli [5] and prolong the vase life of cut flowers by increasing the levels of respiratory components [7,8]. Pre-culturing on sugar-rich medium increased freezing tolerance of excised asparagus shoot tips, possibly by stimulating sugar uptake and inducing dehydration [9]. A previous study investigated whether the shelf life of green asparagus could be extended by wrapping in a semi-permeable film with an adsorbent material immersed in ascorbic acid solution [10]; however, no studies have examined whether dipping in sucrose can prolong shelf life by stimulating soluble sugar uptake.

Texture is an important quality of asparagus spears. Toughening during cold storage decreased the extractability of water-soluble polysaccharides [11]. Changes in total soluble sugar content were highly correlated with concentrations of cell wall components and storage duration in white asparagus [12]. The activity of sucrose synthase modulates cellulose production and ultrastructure without deleterious effects on plant growth [13]. However, the effect of exogenous sugar on green asparagus cell wall components is unknown.

Asparagus contains many valuable bioactive compounds, including saponins, flavonoids, fructan, and amino acids [14]. The most abundant flavonoid in asparagus is rutin (quercetin-3-rhamnosyl glucoside), which accounts for 60%–80% of the total phenolic content of purple and green asparagus extracts and

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confers antioxidant properties to the plant [15]. Sucrose affects anthocyanin accumulation in plants [16]. The increasing importance of the health characteristics of foods in consumers' purchasing decisions necessitates investigating the effect of sucrose on constituent functional compounds.

We therefore examined changes in quality and concentrations of functional compounds of asparagus spears dipped in a sucrose solution and changes in carbohydrate composition and gene expression related to senescence during storage.

2. Materials and methods

2.1. Plant material and sucrose treatment

Freshly harvested green asparagus (*A. officinalis* L.) from a commercial farm were transported to the laboratory and sorted by size, based on diameter, into medium and large spears without defects before being randomly separated into ~200-g batches (~100 spears) all with a length of 22 cm. Each batch was loosely arranged in a plastic container (27 × 21 × 20 cm, 12 l) and soaked in a 0%, 3%, 5%, or 10% (w/v) sucrose solution. The container was sealed with polypropylene film and stored at 2 °C for 20 h. Samples were then placed in a covered styrofoam box and stored at 2 °C and a relative humidity of 90% in a cold storage room for 20 days.

2.2. Measurement of respiration and ethylene production

Fifteen spears were placed in a 2.1-l sealed container and stored at 2 °C for 4 h. A 1-ml volume of air inside the container was analyzed in duplicate by gas chromatography (450-GC; Bruker, Fremont, CA, USA). Respiration and ethylene production were measured with thermal conductivity and flame ionization detectors, respectively. The temperatures of the column, injector, and flame ionization detector were 70 °C, 110 °C, and 250 °C, respectively.

2.3. Quality evaluation

The rate of weight loss, determined by periodic weighing, is expressed as a percentage of the initial weight. Total soluble solid content (SSC; in °Brix) was measured with a digital refractometer (PAL–1; Atago Co., Tokyo, Japan). The color of each spear tip was determined using a colorimeter (Model CR-300; Minolta, Osaka, Japan) and texture was analyzed as previously described [17]. Asparagus spears 22 cm in length were marked at 7-cm intervals from the tip and sectioned at the markings into three cylindrical parts (apical, middle, and basal), and measured by the penetration test using a 2-mm diameter flat probe applied at 1 mm/s and recorded with a texture analyzer (TA Plus; Lloyd Instruments/Ametek, Leicester, UK). Sensory evaluation was carried out by assigning an overall visual quality score on a 4-point scale: 4 = excellent, 3 = good, 2 = poor, and 1 = very poor.

2.4. Carbohydrate content analysis

Carbohydrate content was analyzed as previously described [18]. Briefly, soluble sugars were extracted from 50-mg samples of freeze-dried tissue powder with 5 ml of 62.5% (v/v) methanol at 55 °C for 15 min, followed by precipitation of contaminating substances from the supernatant with centrifugation at 10,000 rpm for 25 min. The supernatant was then filtered through a C-18 Sep-Pak preparatory column (Waters, MA, USA) followed by a 0.2-μm filter before injection into a high-performance liquid chromatography (HPLC) system fitted with a refractive index detector (Youngin, Seoul, Korea) and a 6.5 × 300 mm sugar pack column (Waters). The mobile phase was 100% water (v/v; flow rate 0.5 ml min^{-1}). Fructose, glucose, and sucrose were quantified using 1 mg/ml external standards.

2.5. Analysis of cell wall components

Asparagus spears were ground in liquid nitrogen and ethanol-insoluble solids (EIS) were prepared using a previously described method [19], slightly modified. Briefly, 10 g of tissue was homogenized in 30 ml of 95% ethanol at full speed for 1 min (Ultra-Turrax T25; IKA-Labortechnik, Staufen, Germany), with each sample extracted three times. The homogenate was refluxed in a boiling water bath for 20 min and filtered through a Miracloth (Calbiochem, CA, USA). The residue was rinsed with 100 ml each of 80% ethanol and 100% acetone before drying at 37 °C. EIS were used to prepare protopectin and water-soluble pectin (WSP) according to a previously published protocol [20]. WSP was extracted from 50 mg of EIS with 25 ml water at 4 °C for 16 h followed by room temperature for 3 h. Solubilized pectin in the supernatant was collected by centrifugation at 5000 rpm for 20 min. The remaining insoluble material was re-extracted with 0.05 mol l^{-1} NaOH at 4 °C for 16 h followed by room temperature for 3 h. After centrifugation, the supernatant was filtered and protopectin and WSP content was determined as previously described [21].

2.6. Quantitative real-time (qRT)-PCR analysis of gene expression

Total RNA was extracted from asparagus spears using the RNeasy Plant Mini kit (Qiagen, Valencia, CA, USA), and 1 μg was used to synthesize cDNA with a QuantiTect Reverse Transcription kit (Qiagen), all according to the manufacturer's instructions. The cDNA (50 ng) was used as a template for PCR amplification with gene-specific primer sets (Table S1); qRT-PCR was performed on a Rotor-Gene Q system using the Rotor-gene SYBR Green RT-PCR kit (Qiagen). The thermal cycling conditions were 95 °C for 5 min, followed by 40 cycles of 95 °C for 5 s and 60 °C for 10 s. Data were normalized to actin expression levels in each sample. Transcript levels were calibrated relative to the expression level of each gene in harvested asparagus before treatment (set as 1). Table S1 lists GenBank accession numbers for genes used in this work.

2.7. Analysis of cyanidin 3-O-rutinoside and rutin

Freeze-dried powder of asparagus spears (0.1 g) was mixed with 2 ml of extraction solvent [water:formic acid, 95:5 (v/v)] and vortexed. The mixture was sonicated for 20 min and centrifuged at 12,000 rpm and 4 °C for 15 min to remove tissue particles. The supernatant was filtered through a 0.45-μm syringe filter (Whatman, NJ, USA). A 20-μl sample volume was injected into the HPLC system fitted with a binary pump (1525), an autosampler (717 Plus) and an ultraviolet detector (2487 Dual λ absorbance detector) (all from Waters). A Synergi 4u POLAR-RP 80A column (250 × 4.6 mm, 4 μm; Phenomenex, Torrance, CA, USA) with a guard cartridge was also used. HPLC conditions were as follows: 1.0 ml min^{-1} flow rate and 40 °C column temperature. Solvents A [water:formic acid, 95:5 (v/v)] and B [acetonitrile: formic acid, 95:5 (v/v)] were programmed as follows: 15%–45% solvent B for 12 min and 100% solvent B for the next 3 min; the column was then re-equilibrated at 15% solvent B for 5 min, from 12% to 30% solvent B for 12 min, then 12% solvent B at 14 min followed by re-equilibration of the column at 12% solvent B for 6 min. Cyanidin 3-*O*-rutinoside and rutin were detected at 530 and 350 nm, respectively, and their content was quantified based on retention time and peak areas and was calculated as a fraction of the standard compounds.

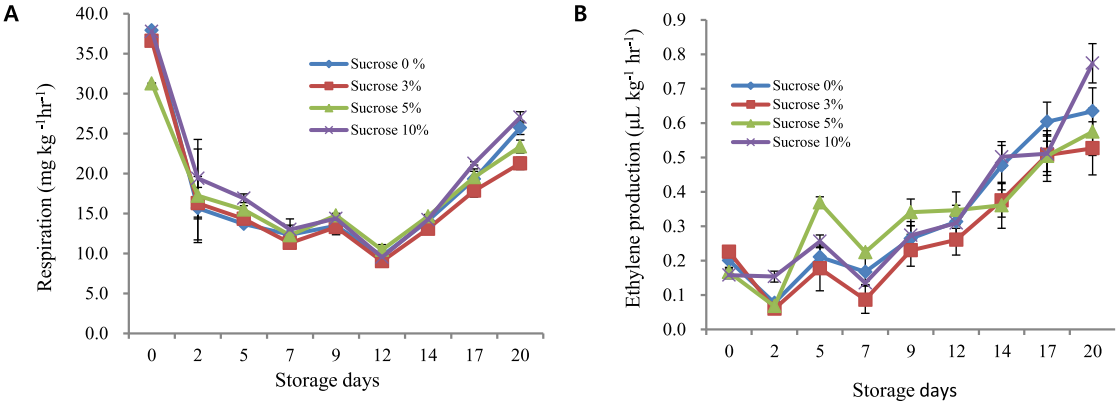

Fig. 1. Changes in respiration rate (A) and ethylene production (B) in asparagus spears treated with sucrose or left untreated for 20 days at 2 °C. Data represent the means of triplicate samples (15 samples/replicate), with vertical lines representing the standard error of the mean.

2.8. Statistical analysis

Values are the mean ± standard error. Differences between groups were evaluated by analysis of variance and means were compared with Duncan's multiple range test or the *t*-test: significance was assumed at $P < 0.05$. Data were analyzed using SAS 9.2 software (SAS Institute, Cary, NC, USA) and Microsoft Excel v.2010 (Seattle, Washington).

3. Results and discussion

3.1. Respiration and ethylene production

The respiration rate in asparagus initially increased but then decreased by 75% after 2 days. Respiration rate after storage at 2 °C for 20 days did not differ between sucrose-treated and untreated control groups (Fig. 1A).

The respiration rate in asparagus is about 60 mg CO_2/kg/h at 5 °C, which causes rapid maturation and induces senescence [22]. Ethylene production increased with longer storage times, although it remained below 0.8 ml/kg/h. Asparagus treated with 3% and 5% sucrose produced less ethylene than untreated controls after 12 days' storage, during which senescence commenced (Figs. 1B and 2). Quantitative RT-PCR analysis revealed *1-aminocyclopropane-1-carboxylate* (*ACC*) synthase and oxidase gene expression levels were unaltered by sucrose treatment at the beginning of the storage period (Fig. 4). Cold storage might have slowed respiration and minimized any difference between sucrose-treated and untreated groups. Respiration rates in asparagus vary with temperature and time post-harvest [1]. At high temperatures, controlled atmospheric conditions suppress respiration, leading to partial inhibition of energy for cell wall synthesis and changes in carbohydrate storage [23].

3.2. Quality evaluation

All samples lost weight over time (Table 1). The greatest decrease (2.3%) was observed in the control sample at the end of the storage period; the weight of 3%, 5%, and 10% sucrose-treated samples decreased by 2.1%, 2.1%, and 2.5%, respectively. The lower degree of weight loss in sucrose-treated samples preserved their quality during cold storage, except for 10% sucrose-treated samples. SSC was higher at high sucrose concentrations.

The firmness in all parts of each sample decreased over time. However, spears treated with sucrose maintained better texture than other samples throughout the storage period, consistent with observed weight loss trends. The firmness of control samples was considerably lower than that of sucrose-treated samples after 12 days' storage; however, all sample weights declined after 18 days. Thus, sucrose treatment might be a source of a cell wall component maintaining firmness. Asparagus texture is extremely sensitive to temperature and delayed cooling [24]. Asparagus spears treated

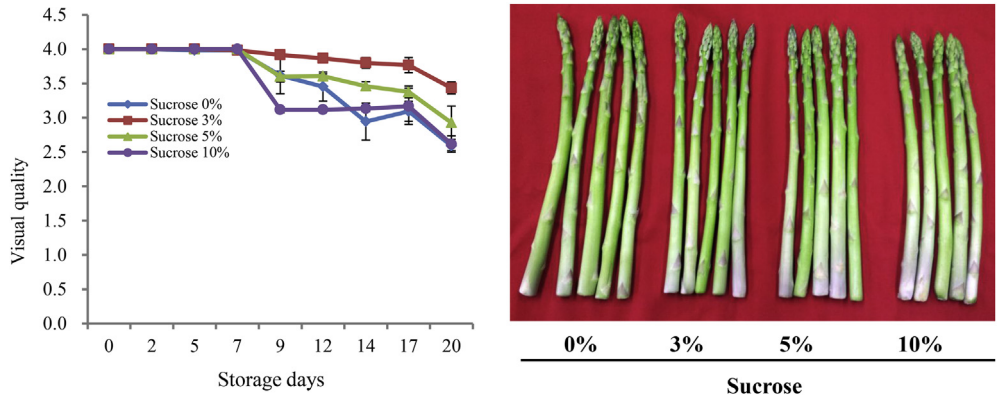

Fig. 2. Changes in visual quality of asparagus spears treated with sucrose or untreated for 20 days at 2 °C. Data represents the means of three replicates (15 samples/replicate), with vertical lines representing the standard error of the mean. Images were acquired on day 20 of storage. Visual quality was rated as follows: 4 = excellent, 3 = good, 2 = poor, and 1 = very poor.

Table 1
Changes in weight loss, soluble solid content (SSC), color (hue value), and firmness of asparagus spears treated with sucrose or untreated for 20 days at 2 °C.

Sucrose	Storage period (days)							
(%)	0[z]	3	5	7	10	12	15	18	20
Weight loss (%)									
0	0.00	0.33 ± 0.13eA[y]	0.49 ± 0.12deA	0.70 ± 0.19deA	1.10 ± 0.29cdeA	1.33 ± 0.30bcdA	1.86 ± 0.34abcA	1.99 ± 0.30abA	2.31 ± 0.36aA
3		0.23 ± 0.11eA	0.55 ± 0.21deA	0.79 ± 0.18cdeA	0.99 ± 0.22bcdeA	1.28 ± 0.24abcdA	1.60 ± 0.23abcA	1.79 ± 0.36abA	2.09 ± 0.38aA
5		0.19 ± 0.10gA	0.48 ± 0.13fgA	0.70 ± 0.17efA	0.88 ± 0.13deA	1.21 ± 0.14cdA	1.52 ± 0.14bcA	1.81 ± 0.05abA	2.09 ± 0.09aA
10		0.40 ± 0.14fA	0.79 ± 0.13efA	1.14 ± 0.21deA	1.38 ± 0.21cdeA	1.65 ± 0.25bcdA	2.02 ± 0.29abcA	2.17 ± 0.23abA	2.50 ± 0.26aA
SSC (Brix)									
0	5.07 ± 0.08abA	4.87 ± 0.05abC	4.90 ± 0.09abD	4.97 ± 0.05abD	4.67 ± 0.05abB	4.57 ± 0.04bC	5.00 ± 0.17abA	5.17 ± 0.05aAB	4.93 ± 0.13abA
3	5.30 ± 0.13abA	5.70 ± 0.07aB	5.40 ± 0.06abC	5.30 ± 0.06abC	5.30 ± 0.09abA	4.77 ± 0.02cBC	5.27 ± 0.13abA	5.00 ± 0.03bcB	5.07 ± 0.07bcA
5	5.30 ± 0.03cdA	6.30 ± 0.07aA	5.77 ± 0.04bB	5.93 ± 0.02bB	5.47 ± 0.02cA	4.97 ± 0.04eB	5.20 ± 0.06cdeA	5.23 ± 0.05cdeAB	5.13 ± 0.12deA
10	5.13 ± 0.10cA	6.53 ± 0.04aA	6.40 ± 0.06aA	6.43 ± 0.05aA	5.70 ± 0.10bA	5.63 ± 0.08bA	5.63 ± 0.07bA	5.33 ± 0.08bcA	5.57 ± 0.01bA
Hue value									
0	107.72 ± 1.13aA	85.07 ± 5.71dB	98.60 ± 3.86abcB	108.10 ± 0.88aA	108.32 ± 0.73aA	101.33 ± 2.54abA	97.46 ± 2.97bcA	94.17 ± 1.58bcdBC	90.61 ± 8.28cdB
3	85.73 ± 6.79cB	104.13 ± 1.57aA	106.26 ± 1.04aA	101.65 ± 4.48aA	95.81 ± 5.08abcB	98.15 ± 2.19abA	96.75 ± 3.29abcA	88.12 ± 2.34bcC	94.62 ± 4.20abcAB
5	94.94 ± 2.9dAB	98.15 ± 2.03cdA	108.05 ± 2.20aA	99.92 ± 3.89bcdAB	106.85 ± 2.54abA	103.90 ± 1.46abcA	93.54 ± 2.13dA	104.99 ± 1.95abcA	97.99 ± 4.45cdAB
10	87.17 ± 5.66cB	95.12 ± 4.83abcAB	92.99 ± 2.46abcB	90.92 ± 3.01bcB	103.05 ± 2.99aAB	101.24 ± 1.97abA	100.82 ± 2.07abA	98.63 ± 3.37abAB	101.02 ± 4.42abA
Firmness (N)									
0	8.21 ± 0.78 abA	7.23 ± 0.37abA	7.62 ± 0.36abA	8.46 ± 0.68aA	8.21 ± 0.34abA	6.75 ± 0.16abA	6.68 ± 0.43bB	6.49 ± 0.22bA	6.34 ± 0.18bA
3	8.93 ± 0.95aA	8.74 ± 0.91abA	7.87 ± 0.37abcA	8.19 ± 0.54abAc	6.44 ± 0.41abcA	7.44 ± 0.35abcA	7.91 ± 0.91abcA	6.01 ± 0.24cA	6.51 ± 0.39bcA
5	7.97 ± 0.81aA	7.48 ± 0.46aA	8.02 ± 0.49aA	6.99 ± 0.54aA	7.60 ± 0.47aA	7.13 ± 0.41aA	7.12 ± 0.63aA	6.20 ± 0.27aA	6.84 ± 0.39aA
10	8.22 ± 0.58aA	8.05 ± 0.89aA	8.06 ± 0.40aA	7.43 ± 0.42aA	7.13 ± 0.29aA	7.43 ± 0.24aA	7.99 ± 0.67aA	6.46 ± 0.33aA	6.53 ± 0.46aA

[z]Day 0 samples were measured immediately after sucrose treatment and before storage.
[y]Data represent mean and standard error of three replicates. Means with the same uppercase letter in a column or same lowercase letter in a row were not significantly different at $P < 0.05$ with Duncan's multiple range test.

with 3% sucrose maintained a good marketable visual quality for up to 18 days, unlike the untreated control (12 days) (Fig. 2). These results indicate that 3% sucrose treatment can extend asparagus shelf life by ~6 days.

3.3. Carbohydrate analysis

Carbohydrate content increased immediately with sucrose testament at 0 day. Fructose (142.07 ± 92.75 mg g^{-1} dw) was the predominant sugar, followed by glucose (61.87 ± 3.45–139.46 ± 1.29 mg g^{-1} dw) and then sucrose (29.18 ± 4.24–54.19 ± 0.10 mg g^{-1} dw). Sucrose content gradually decreased during storage and was highest in asparagus treated with 10% sucrose (Table 2). Glucose and fructose content in asparagus treated with 3% or 5% sucrose decreased less than did the control at later stages of storage, and remained higher than control levels. The dramatic loss of sugars in non-treated asparagus occurred early during storage and led to change a quality. These results are consistent with previous studies reporting early and rapid loss of glucose and fructose in asparagus spears [4], as well as increases in glucose and xylose and reductions in arabinose and galactose content during cold storage of asparagus [11,25]. In the present study, sucrose treatment maintained carbohydrate levels during storage and delayed changes in quality.

3.4. Analysis of cell wall components

Sugar content is correlated with cell wall component expression in white asparagus [26] We analyzed WSP and protopectin contents of asparagus spears treated with sucrose or left untreated. WSP content increased in all groups during the storage period (Fig. 3), but was significantly lower in sucrose-treated than in untreated spears. In contrast, protopectin content decreased in all groups for up to 6 days of storage, although sucrose levels were higher in sucrose-treated spears. Water-soluble proteins and proto-proteins are important cell wall components that influence the firmness and tenderness of asparagus spears [20]. During senescence, protopectin gradually decomposes, leading to tissue tenderness being lost [27]. Sucrose treatment can preserve the tenderness of asparagus spears by slowing the decomposition rate of protopectin and inhibiting the increasing WSP content. It is unclear whether secondary cell wall thickening is stimulated by asparagus cell wall polysaccharide turnover [11] or by stored soluble sugar consumption [27]. In the present study, sucrose was mainly used for synthesis of cell wall components and not for respiration, and hence suppressed symptoms of senescence such as bract opening, shrinkage and toughening and maintained firmness (Fig. 2 and Table 1).

3.5. Gene expression analysis

Low sugar concentrations induce the expression of senescence-associated genes [28]. Molecular changes after harvesting contribute to postharvest deterioration. To identify the responsible factors, we examined early changes in ethylene production and *ACC synthase* (*(pAS-ACO)* and *ACC oxidase (pAS-ACS)* expression as well as S-adenosyl methionine (SAM) level in whole spears. We found that all these factors were higher in asparagus treated with 3% sucrose than in untreated controls at the beginning of the storage period until 144 h (Fig. 4). This was unexpected, since ethylene production was relatively low in asparagus treated with 3% sucrose (Fig. 1B) and other studies have shown that sucrose treatment reduced ACC oxidase and ACC levels in carnation exposed to ethylene [7] and *ACC synthase 1* levels in broccoli [29]. In *Arabidopsis*, sucrose altered ethylene metabolism

Table 2
Effect of sucrose treatment on carbohydrate content of asparagus during storage for 20 days at 2 °C.

Sucrose	Storage period (days)					
(%)	0[z]	3	7	12	17	20
Sucrose (mg g^{-1} dry weight)						
0	41.23 ± 0.75aA[y]	33.83 ± 0.91abA	35.83 ± 0.34abA	33.84 ± 3.42abC	31.47 ± 1.96bB	29.18 ± 4.24bB
3	52.79 ± 6.68aA	36.84 ± 3.53aA	37.18 ± 7.41aA	46.17 ± 1.47aAB	44.24 ± 1.44aA	41.52 ± 3.21aA
5	48.65 ± 2.62aA	43.07 ± 3.37abA	41.55 ± 1.18abA	36.85 ± 3.70bcBC	29.62 ± 3.87cB	39.63 ± 1.03abcAB
10	50.79 ± 5.23aA	46.85 ± 18.49aA	48.97 ± 3.34aA	54.19 ± 0.10aA	39.70 ± 2.68aAB	34.96 ± 2.02aAB
Glucose (mg g^{-1} dry weight)						
0	102.45 ± 5.07aD	108.10 ± 9.69aAB	78.93 ± 5.47bcA	92.57 ± 2.61abA	61.87 ± 3.45cB	65.57 ± 5.51cA
3	126.75 ± 0.15aB	95.85 ± 2.23bB	73.56 ± 1.57cA	98.06 ± 7.34bA	102.78 ± 1.48bA	82.40 ± 3.66cA
5	139.46 ± 1.29aA	127.53 ± 5.00aA	92.25 ± 7.10bA	113.26 ± 7.78abA	93.42 ± 3.91bA	88.79 ± 13.41bA
10	114.30 ± 1.95aC	112.82 ± 1.76abAB	87.01 ± 5.09cA	110.03 ± 5.21abA	101.28 ± 1.13abcA	96.96 ± 8.42bcA
Fructose (mg g^{-1} dry weight)						
0	164.58 ± 5.40aA	164.46 ± 14.92aAB	146.46 ± 13.14aA	142.70 ± 12.34aB	147.14 ± 13.91aA	142.53 ± 9.75aA
3	187.03 ± 3.16aA	157.16 ± 8.15bcB	143.07 ± 2.79cA	175.16 ± 10.01abAB	154.78 ± 10.13bcA	142.07 ± 11.27cA
5	183.13 ± 8.88aA	175.42 ± 3.16aAB	159.52 ± 11.02aA	177.60 ± 10.31aAB	166.31 ± 8.55aA	164.08 ± 16.18aA
10	171.05 ± 5.92aA	192.75 ± 0.91aA	182.07 ± 21.77aA	183.73 ± 2.07aA	185.96 ± 5.46aA	185.22 ± 3.95aA

[z]Day 0 samples were measured immediately after sucrose treatment and before storage.
[y]Data represent mean and standard error of three replicates. Means with the same uppercase letter in a column or same lowercase letter in a row were not significantly different at P < 0.05 with Duncan's multiple range test.

[30]. ACC synthase catalyzes what is generally regarded as the first, rate-limiting step of ethylene biosynthesis [31], and is regulated via both positive and negative feedback by ethylene itself. We found that low-temperature (2 °C) storage inhibited ethylene synthesis in asparagus; exogenous sucrose may thus reduce ethylene production during later stages of postharvest storage, as evidenced by downregulation of genes involved in carbohydrate transport or metabolism.

We investigated the expression of cell wall components following sucrose treatment. *Xyloglucan endotrans glycosylase* (*AoXET*)*1* and *AoXET2* levels were immediately upregulated by sucrose. After 144 h of storage, *AoXET1* expression decreased whereas that of *AoXET2* increased. *AoXET* transcript was previously found to be upregulated after harvest in all parts of the asparagus spear, although this was not associated with an increase in XET activity, which is likely related to the time of harvest; later induction of *AoXET1* and *AoXET2* expression has been linked to the development of lignified secondary cell walls [32]. In the present work, *AoXET1* and *AoXET2* were found to modulate lignification in green asparagus during storage. Another study found that the toughening of asparagus is primarily related to the degree of lignification of pericyclic fibers [33], which is enhanced by enzymes such as phenylalanine ammonia lyase (PAL), peroxidase (prx), and isopeoxidase [34]. Transcript levels of *Aoprx1*, *Aoprx2,* and *Aoprx3* in sucrose-treated asparagus were similar to or lower than those in untreated controls, but expression was upregulated after 72 h of storage (Fig. 4). Pectin lyase-like superfamily genes showed similar expression patterns as *prx* genes, although the levels were below the initial values. Together, our results suggest that the marketability of asparagus spears can be preserved by sucrose treatment, which alters cell wall components via modulation of *XET* and *prx* genes without inhibiting those involved in ethylene synthesis during cold storage.

3.6. Changes in asparagus anthocyanin and rutin content during storage

Green asparagus spears are enriched in quercetin-3-rhamnosyl glucoside (also known as rutin; 1.51–7.29 mg/g dry weight) [14] as well as anthocyanin, which has high antioxidant activity in

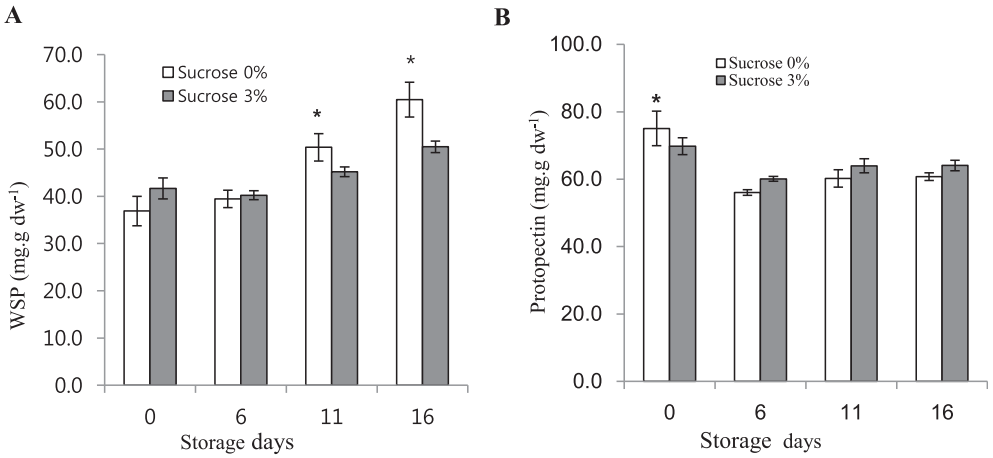

Fig. 3. Changes in water soluble protein (WSP) (A) and protopectin (B) levels in asparagus spears treated with sucrose or left untreated for 20 days at 2 °C. Data represent mean ± standard error (n = 6). Asterisks indicate significant difference between 0%- and 3%-sucrose-treated samples (*P < 0.05, **P < 0.01).

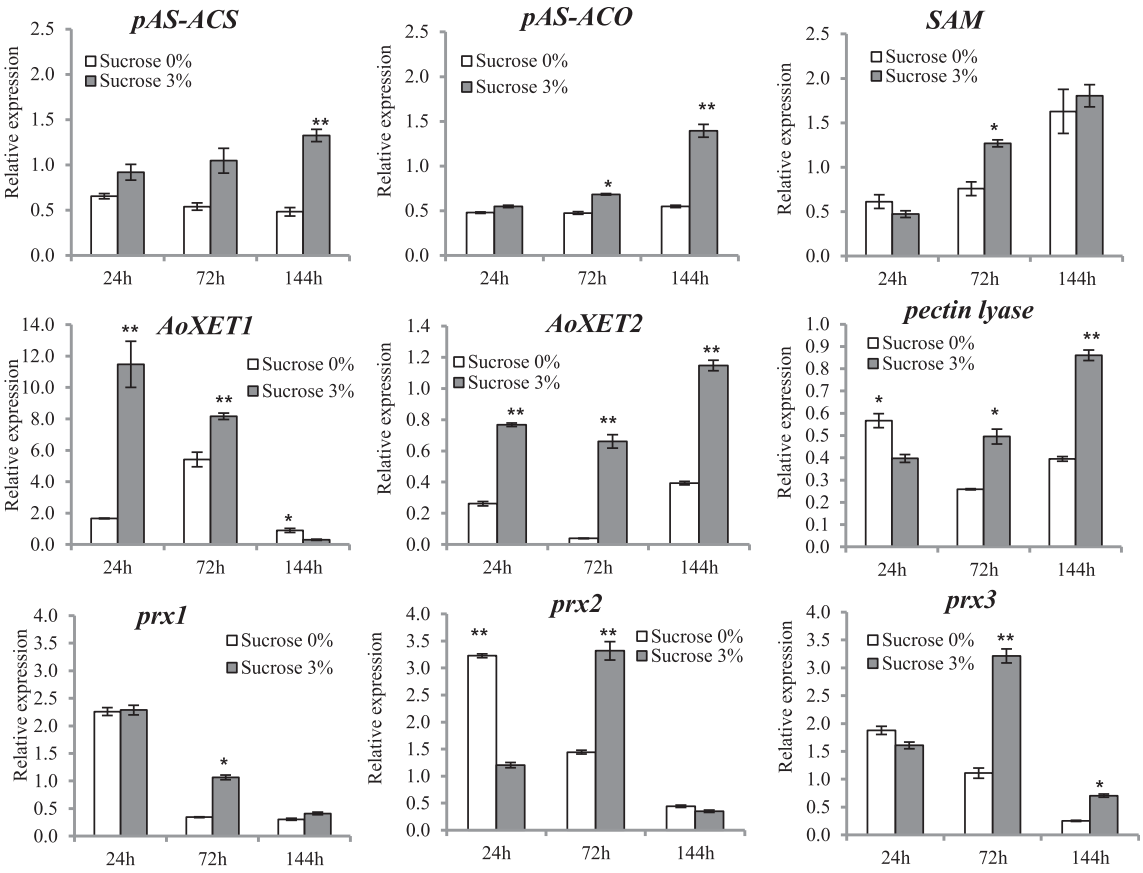

Fig. 4. Expression of genes related to ethylene synthesis and encoding cell wall components: *ACC synthase* (*pAS-ACS*); ACC oxidase (*pAS-ACO*); S-adenosyl-L-methionine-dependent methyltransferase (*SAM*); pectin lyase-like superfamily (*pectin lyase*): *xyloglucan endotransglysosylase* (*AoXET1*, *AoXET2*); peroxidase (*Aoprx1*, *Aoprx2*, and *Aoprx3*). Gene transcript levels were assessed by qRT-PCR and calibrated relative to the expression level of each gene in harvest asparagus before treatment (set as 1), with actin serving as an internal reference for each sample. Values represent mean ± SE of three replicates. Asterisks indicate significant difference between 0%- and 3%-sucrose-treated samples (*$P < 0.05$, **$P < 0.01$).

asparagus [35]. We investigated the effect of sucrose on the content of anthocyanin, cyanidin 3-*O*-rutinoside, and rutin by HPLC analysis using established standards (Fig. S1). Cyanidin 3-*O*-rutinoside, levels increased with storage time in both untreated and treated groups (from 125.29 ± 0.8 to 200.18 ± 5.35 μg g^{-1} dw and from 131.76 ± 8.92 to 248.38 ± 2.31 μg g^{-1} dw, respectively. The cyanidin 3-*O*-rutinoside, content of asparagus spears treated with 3% and 10% (v/w) sucrose increased within 3 days and remained higher than that of the control during storage. However, in asparagus spears treated with 5% (v/w) sucrose, cyanidin 3-*O*-rutinoside, concentration was initially 132.65 ± 1.24 μg g^{-1} dw; this increased to 248.38 ± 2.31 μg g^{-1} dw after 20 days of storage showing highest content (Table 3).

Rutin content of both sucrose-treated and untreated asparagus tended to increase during the storage period (Table 3). A similar pattern in rutin concentration has been reported in two other

Table 3
Effect of sucrose treatment on the cyanidin 3-O-rutinoside and rutin contents of asparagus during storage for 20 days at 2 °C.

Sucrose	Storage period (days)					
(%)	0[z]	3	7	12	17	20
Cyanidin 3-O-rutinoside (μg g^{-1} dry weight)						
0	125.29 ± 0.8cA[y]	175.25 ± 4.85bB	175.47 ± 8.78bA	168.67 ± 3.88bA	175.59 ± 5.61bA	200.18 ± 5.35aB
3	155.05 ± 23.24bA	197.25 ± 7.32abA	168.86 ± 8.09abA	177.96 ± 19.16abA	184.68 ± 4.66abA	205.09 ± 3.51aB
5	132.65 ± 1.24dA	131.76 ± 8.92dC	140.48 ± 7.38dB	193.08 ± 5.91cA	214.79 ± 8.27bA	248.38 ± 2.31aA
10	140.98 ± 1.24cA	181.35 ± 3.32abcB	160.88 ± 2.60bcA	176.16 ± 2.64abcA	189.20 ± 31.13abA	205.28 ± 3.20aB
Rutin (mg g^{-1} dry weight)						
0	3.79 ± 0.38cD	5.21 ± 0.24aA	4.99 ± 0.50bA	5.25 ± 0.09abB	4.61 ± 0.17bAB	4.75 ± 0.17bB
3	5.11 ± 0.02abA	5.11 ± 0.07abA	4.69 ± 0.08cA	5.24 ± 0.30abB	5.30 ± 0.82abA	5.50 ± 0.33aA
5	4.64 ± 0.17bcA	4.72 ± 0.32cB	5.24 ± 0.25abA	5.79 ± 0.16aB	5.23 ± 0.61abA	5.73 ± 0.16aA
10	4.51 ± 0.20bcC	4.35 ± 0.04bB	4.51 ± 0.05bcA	6.30 ± 0.26aA	3.36 ± 0.78cB	4.65 ± 0.09bB

[z]Day 0 samples were measured immediately after sucrose treatment and before storage.
[y]Data represent mean and standard error of three replicates. Means with the same uppercase letter in a column or same lowercase letter in a row were not significantly different at $P < 0.05$ with Duncan's multiple range test.

asparagus cultivars [36]. We found that spears treated with 3% (v/w) sucrose had a rutin content of 5.11 ± 0.02 mg g^{-1} dw on day 0, which was 35% than higher than that of untreated controls (3.79 ± 0.38 mg g^{-1} dw). Spears treated with 5% sucrose had the highest rutin content at the end of the 20-day storage period. Overall, the trends in rutin content were similar to those observed for cyanidin 3-*O* rutinoside. Rutin levels were higher in spears treated with 3%–5% sucrose than in controls upon storage beyond 12 days. Sugar uptake in *Arabidopsis* is modulated by changes in endogenous Ca^{2+} levels, in turn regulated by anthocyanin accumulation [37]. Thus, exogenous sucrose (3%–5%) effectively preserves anthocyanin and rutin content and the quality of asparagus spears during low-temperature storage for up to 20 days.

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Appendix ASupplementary data

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Transparency document

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