



Article

Quality Improvement of Tomato Fruits by Preharvest Application of Chitosan Oligosaccharide

Jirong Zheng ¹, Hao Chen ², Tonglin Wang ¹, Ghazala Mustafa ^{2,3,4}, Lihong Liu ², Qiaomei Wang ^{2,*} and Zhiyong Shao ^{1,*}

¹ Institute of Vegetable, Hangzhou Academy of Agricultural Science, Hangzhou 310024, China

² State Agricultural Ministry Laboratory of Horticultural Crop growth and Development, Ministry of Agriculture, Department of Horticulture, Zhejiang University, Hangzhou 310058, China

³ Lishui Institute of Agriculture and Forestry Sciences, Lishui 323000, China

⁴ Department of Plant Sciences, Quaid-I-Azam University, Islamabad 45320, Pakistan

* Correspondence: qmwang@zju.edu.cn (Q.W.); shaozy@zju.edu.cn (Z.S.)

Abstract: Chitosan oligosaccharide (COS), a degradation product of chitosan, is easily accessible, highly bioactive, non-toxic, and well-soluble in water. The effects of COS on the qualitative attributes of tomato fruits were investigated in the current study. COS was administered to tomato plants (*Solanum lycopersicum* cv. Ruixinghongniu) by foliar spray and root irrigation in alternate cycles at concentrations of 0.5 g·L⁻¹ and 0.16 g·L⁻¹, respectively. The experimental outcomes revealed that COS treatment promoted the coloring and softening of tomato fruits. Lycopene, vitamin C, fructose, and glucose levels increased by 49.0%, 25.4%, 30.2%, and 33.4%, respectively, in COS-treated ripe fruits compared to controls. The volatile metabolome showed that COS application also increased the release of ten volatiles correlated with consumer preference (1-penten-3-one, (*E*)-2-pentenal, (*E*)-3-hexen-1-ol, (*E*)-2-heptenal, 2-isobutylthiazole, phenylacetaldehyde, 2-phenylethanol, 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, and β -ionone), contributing to an improved tomato flavor. Moreover, increased transcript levels of genes participating in ethylene biosynthesis, perception, and response along with enhanced ethylene production were observed in COS-treated fruits, suggesting that COS may regulate tomato fruit quality via the ethylene pathway. Taken together, our results indicated that the pre-harvest application of COS could improve tomato fruit quality attributes.

Keywords: tomato fruit; chitosan oligosaccharide; quality; volatile

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1. Introduction

Tomatoes (*Solanum lycopersicum* L.) are one of the most popular and nutritious vegetables in the world and in China, serving as a major source of carbohydrates, vitamins, carotenoids, and minerals. A high yield and disease resistance, as well as a long shelf life, have long been major goals in tomato breeding and production, but fruit quality has been neglected. Today, with the improvement in living standards, people's demand for high-quality tomato fruits has increased significantly. Tomato quality attributes include size, color, firmness, nutritional content, and flavor [1,2]. Among these, fruit color is an important characteristic that determines the consumer's first impression of the tomato [3]. Firmness affects the taste of the fruit, and despite being better-suited for long-distance transportation and storage, there is growing dissatisfaction regarding the toughness of modern tomato cultivars [4]. Tomatoes provide health-promoting nutrients such as carotenoids. The most-studied carotenoids in tomato fruit are lycopene, β -carotene, and lutein, which are responsible for tomatoes' characteristic color and associated with reducing the risk of certain cancers and cardiovascular and eye diseases [5]. Tomato flavor involves a mix of tastes (a balance of sugars and organic acids) and a complex blend of volatiles [6]. The sugars in tomato fruit (mainly fructose and glucose) provide sweetness, while the

organic acids (mainly malic and citric acid) provide sourness [7]. Crushing and chewing the fruit releases volatiles that contribute to tomato flavor [6,8].

Fruit ripening, a highly coordinated, genetically controlled process that involves tissue softening; color changes; and an increase in the accumulation of sugars, organic acids, and volatile compounds, occurs in synchronization with tomato fruit quality formation [9,10]. As a climacteric fruit, tomatoes exhibit a burst of ethylene production at the onset of fruit ripening and need ethylene to complete the full ripening process [11]. Therefore, ethylene biosynthesis and signal transduction are crucial processes for fruit ripening. The biosynthesis of ethylene comprises two successive steps catalyzed by 1-aminocyclopropane-1-carboxylic acid (ACC) synthase (ACS) and ACC oxidase (ACO), respectively [9,11]. Ethylene binds to membrane-located ethylene receptors (ETRs), and its signal is transduced via constitutive triple response 1 (CTR1), ethylene insensitive 2 (EIN2), ethylene insensitive 3 (EIN3), and ethylene response factors (ERFs), leading to the expression of ripening-related genes [9,11].

Fruit quality can be enhanced by cultivar development, environment/crop management, and postharvest handling. The preharvest application of plant bio-stimulants is increasingly used in fruit production [12]. Chitosan oligosaccharide (COS) is one of natural bio-stimulants obtained from the degradation of chitin or chitosan. COS has many advantages, such as easy accessibility, high water solubility, low viscosity, non-toxicity, biocompatibility, biodegradability, and high bioactivity [13]. Because of this, COS has attracted much attention in the agricultural sector, particularly for organic agricultural practices that do not harm the environment. Numerous studies have demonstrated the crucial role of COS in controlling plant growth [14–17], seed germination [18], photosynthetic components [19], secondary metabolism [20,21], and tolerance to abiotic and biotic stress [22–24]. Previous research on tomatoes has focused on the impact of COS on plant growth, resistance, and yield [25–28], but the consequences of COS on the whole range of fruit quality attributes is still limited. Therefore, the current study focused on the impacts of COS on tomato color, firmness, nutritional content, and flavor. Additionally, the effects of COS on ethylene biosynthesis and signaling were investigated.

2. Materials and Methods

2.1. Plant Materials and Growth Conditions

The tomato cultivar '*Solanum lycopersicum* cv. Ruixinghongniu' with large red fruits was selected for the experiment. The experiment was conducted in a greenhouse located in Longgang (lat. N27°31', long. E120°30', Zhejiang Province, China) from winter to spring 2020. The average temperature from October 2020 to May 2021 was 15.4 °C. The soil conditions of the experimental field were as follows: pH 4.8, organic matter 36.1 g·kg⁻¹, total nitrogen 2.2 g·kg⁻¹, available nitrogen 203.9 mg·kg⁻¹, available phosphorus 401.2 mg·kg⁻¹, available potassium 668.4 mg·kg⁻¹. On 5 October 2020, one-month-old tomato seedlings were transplanted with a row distance of 1.6 m and an in-row spacing of 40 cm. The fruit load was set at five fruits per truss. Tomato fruits were harvested from February to May in the following year.

2.2. Treatments and Sampling

COS (95% deacetylated, molecular weight ≤ 3000 Da, agricultural-grade) was purchased from Golden-Shell Pharmaceutical (Yuhuan, China). For COS treatments, COS was formulated at 0.5 g·L⁻¹ (dissolved in water) for foliar application in tomato plants or 0.16 g·L⁻¹ (dissolved in nutrient solution) for drip irrigation in tomato plants based on the results of the preliminary experiment. Foliar spray and root irrigation were alternately applied at an interval of 7 days from the flowering stage until the mature green (MG) stage of the second truss of fruits was achieved. Tomato plants treated with an equal amount of water or nutrient solution were used as controls. The recommended dose of COS for each foliar spraying and root irrigation application was 1200 L·ha⁻¹ and 13500 L·ha⁻¹,

respectively. Apart from the different treatments, all tomato plants in both treatment groups were kept under the same intercultural management conditions such as pruning, irrigation, and staking. A randomized complete block design (RCBD) was executed with three replicates per treatment and thirty plants in each replicate.

At the onset of anthesis, flowers of the second truss were tagged and harvested at four different stages of maturity: (1) mature green stage (MG)—fully expanded, with the surface of the tomato completely green in color; (2) breaker stage (B)—yellow or pink <10%; (3) pink stage (P)—30–60% pink or red skin; (4) red ripened stage (RR)—100% red skin. Thirty fruits were selected randomly from the COS-treated and control groups and divided into three groups (each group representing one replicate). In each group, two fruits were used to measure ethylene production; two were used to determine firmness; and the remaining six (whole fruits) were cut into pieces, quick-frozen in liquid nitrogen, ground into powder, and stored at $-80\text{ }^{\circ}\text{C}$ for further chemical determination and gene expression analysis.

2.3. Fruit Quality Index Determination

Tomato fruit firmness was measured at two opposite points in the equatorial region of fruits using a TA-XT2i Plus texture analyzer (Stable Micro Systems, Godalming, UK) with a 7.5 mm cylindrical probe [29].

Carotenoid and vitamin C contents were determined by a Shimadzu HPLC instrument (Kyoto, Japan) coupled with a C18 column ($4.6 \times 250\text{ mm}$, $5\text{ }\mu\text{m}$ particle size; Elite Analytical Instruments, Dalian, China) and an SPD-M20A detector (Shimadzu) following the method of Zhang et al. [30]. Glucose, fructose, malic acid, and citric acid contents were measured by an Agilent 6890N gas chromatograph (Palo Alto, CA, USA) coupled with an HP5 capillary column ($30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$, Agilent) according to the method of Zhang et al. [31]. The total contents of soluble sugars and organic acids were measured as described by Wang and Xing [32], and the sugar/acid ratio was calculated by the total soluble sugars content/total organic acid content.

2.4. Volatile Profiles Analysis

Volatile profiles were measured as described by Zhang et al. [31]. Frozen powder (5 g) was transferred to 20 mL glass vials and mixed well with 5 mL saturated NaCl solution. After adding 2-octanol (internal standard) into the matrix, the vials were sealed with Teflon-lined septa (Gerstel, Linthicum, MD). Volatiles were analyzed by a headspace solid-phase microextraction (HS-SPME) system coupled to a gas chromatography–mass spectrometry (GC-MS) instrument. After incubating the sample vials at $40\text{ }^{\circ}\text{C}$ for 30 min, an SPME fiber with a divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) ($50/30\text{ }\mu\text{m}$, Supelco, Bellefonte, PA, USA) coating was inserted in the headspace of the vial to extract the volatiles from the matrix, then immediately inserted into the GC injector port to desorb the extract for 5 min at $240\text{ }^{\circ}\text{C}$. Chromatographic analysis was performed using an Agilent 7890A gas chromatograph coupled with an Agilent 5975C mass spectrometer. The capillary column was an Agilent HP-5 column ($30\text{ m} \times 0.25\text{ mm} \times 0.25\text{ }\mu\text{m}$). The column oven was temperature-programmed as follows: starting at $40\text{ }^{\circ}\text{C}$, $4\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ increase to $230\text{ }^{\circ}\text{C}$, $100\text{ }^{\circ}\text{C}\cdot\text{min}^{-1}$ increase to $260\text{ }^{\circ}\text{C}$, and held for 11.7 min. Helium (purity 99.99%) was used as a carrier gas at a constant flow of $1\text{ mL}\cdot\text{min}^{-1}$. The mass spectrometer was operated using the following conditions. The temperature of the inlet, transfer line, and ion source were set at $250\text{ }^{\circ}\text{C}$, $230\text{ }^{\circ}\text{C}$, and $280\text{ }^{\circ}\text{C}$, respectively; mass units were monitored in electron-impact (EI) mode; and the ionization energy was 70 eV. Individual volatile compounds were identified by comparing the mass spectra data with those from the NIST/EPA/NIH Mass Spectral Library (NIST 08, National Institute of Standards and Technology, Gaithersburg, MA, USA) or analyzing the standard under the same experimental conditions. Quantification was conducted using the peak area of the internal standard as a reference based on the total ion chromatogram (TIC). Data are expressed as $\mu\text{g}\cdot\text{kg}^{-1}$ fresh weight.

2.5. Ethylene Production Analysis

Two tomato fruits were sealed at 22 °C for 2 h in a 2.0 L air-tight container. After that, 1 mL of headspace gas was drawn and injected into a gas chromatograph (Agilent 6890N, Palo Alto, CA, USA) fitted with a flame ionization detector. The temperature of the injector was held at 140 °C, the oven temperature was 230 °C, and the detector was set at 100 °C.

2.6. Gene Expression Analysis

The extraction of total RNA was performed using RNAiso plus (Takara, Otsu, Japan). The extracted RNA was reverse-transcribed into cDNA with a PrimeScript™ RT reagent kit (Takara). Real-time quantitative PCR (RT-qPCR) was performed according to the method of Shao et al. [33]. The primers used are listed in Table S1.

2.7. Statistics

Data were analyzed with the SPSS 19.0 software package (Chicago, IL, USA). Pair-wise comparisons were computed using Student's *t*-test at a significance level of 0.05. The values are presented as mean with standard deviation (SD).

3. Results

3.1. Effect of COS Treatment on Fruit Coloring and Firmness

As shown in Figure 1a and Table S2, COS treatment did not significantly affect fruit size. However, COS-treated fruits showed stronger pigmentation compared to control from the MG to the RR stage. The firmness of the tomato fruits decreased rapidly with the progression of ripening in both the control and COS-treated fruits; however, COS application resulted in significantly lower firmness from the B stage to the RR stage of maturity (Figure 1b).

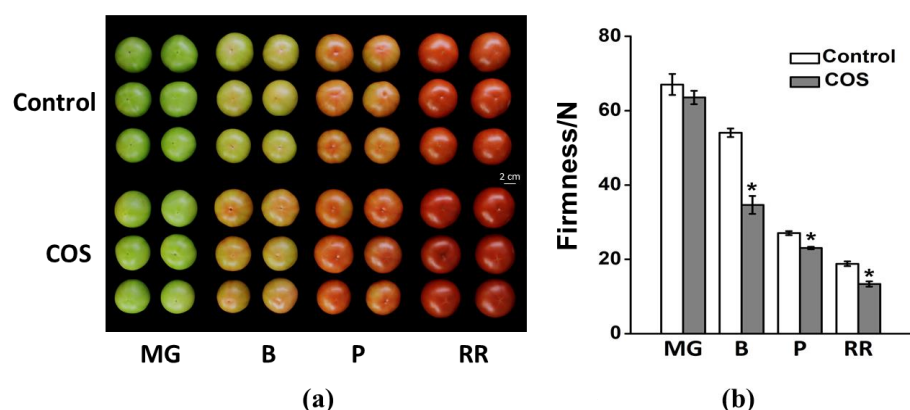


Figure 1. Effect of COS on the coloring (a) and firmness (b) of tomato fruits. In (b), data represent the means \pm SD of six fruits. The asterisk represents a significant difference compared with the control based on Student's *t*-test (* $p < 0.05$). MG: mature green; B: breaker; P: pink; RR: red ripened. FW: fresh weight.

3.2. Effect of COS Treatment on Sugar and Organic Acid Content

The effects of COS treatment on the concentration of sugars and organic acids were investigated by the GC-MS analysis of the primary sugars (fructose and glucose) and organic acids (malic acid and citric acid) in the tomato fruit. As shown in Figure 2, the contents of fructose and glucose were significantly increased by COS treatment at the MG, P, and RR stages. In particular, at the RR stage, the concentrations of fructose and glucose in the COS-treated fruits were increased by 30.2% and 33.4%, respectively, compared to controls (Figure 2). Among the organic acids, citric acid was determined to be dominant at all stages, and the mature green tomato fruit also contained significant amounts of malic acid. The malic acid contents continuously decreased during ripening in both the control and

COS-treated fruits. However, COS treatment significantly decreased the malic acid levels from the MG to the P stage (Figure 2). The concentration of citric acid remained relatively constant in both the control and COS-treated fruits during fruit ripening. No difference in citric acid levels was observed at the P and RR stages in the COS-treated and control fruits (Figure 2). Fruit flavor requires a balanced level of sugars and organic acids; therefore, we further analyzed the ratio of sugars and organic acids in tomato fruits. Increased levels of fructose and glucose and decreased levels of malic acid led to a significantly increased ratio of sugars to acids in the COS-treated fruits at the B, P, and RR stages compared to control (Figure 2). At the RR stage, the sugar/ acid ratio in the COS-treated fruits increased by 35.2% compared to control (Figure 2), which potentially made the tomato fruits sweeter and tastier.

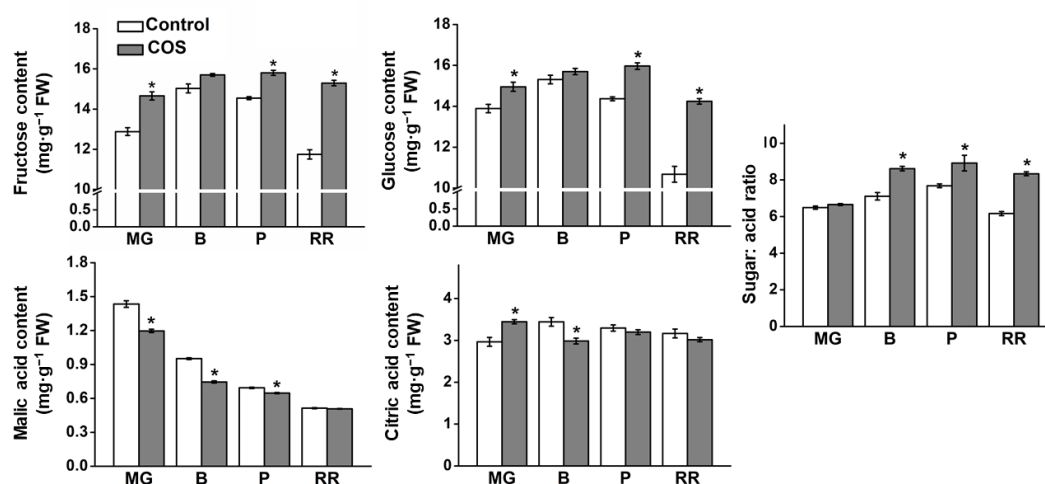


Figure 2. Effect of COS on the contents of sugars and organic acids, as well as the sugar/acid ratio, in tomato fruits. Data represent the means \pm SD of three biological replicates. The asterisk represents a significant difference compared with the control based on Student's *t*-test (* $p < 0.05$). MG: mature green; B: breaker; P: pink; RR: red ripened. FW: fresh weight.

3.3. Effect of COS Treatment on Carotenoid Composition and Content

The primary colored carotenoids (lycopene, β -carotene, and lutein) were analyzed at different stages of tomato fruit development in the COS-treated and the control groups. As expected, the most abundant carotenoid was identified as lycopene, followed by β -carotene and trace amounts of lutein (Figure 3a). The lycopene content increased dramatically with the ripening progress of the tomato fruits and accounted for 70.6% and 81.5% of the total colored carotenoids in the control and COS-treated ripe fruits, respectively (Figure 3a). However, the COS-treated fruits were characterized by significantly higher lycopene levels at each stage compared to the control (Figure 3a). Notably, the COS-treated fruits at the RR stage (the edible stage) presented a 49.0% increase in lycopene content as compared to the control. The concentration of β -carotene displayed a dramatic increase from the MG to the P stage and slightly decreased at the RR stage in the control fruits. The β -carotene levels followed a different pattern in the COS-treated fruits, peaking at the B stage and then slowly decreasing. Significantly higher concentrations of β -carotene were observed in the COS-treated fruits compared to the control at the MG and B stages (Figure 3a). The lutein levels did not change significantly from the MG to the P stage, while they decreased at the RR stage in both the control and COS-treated fruits; however, COS treatment resulted in a 40.8% reduction in the lutein concentration in RR fruits (Figure 3a). The concentration of total carotenoids followed a similar pattern to that of lycopene and was significantly higher in COS-treated fruits compared to controls at each stage (Figure 3a). At the RR stage, a 29.0% increase in the total carotenoid content was observed in COS-treated fruits compared to controls.

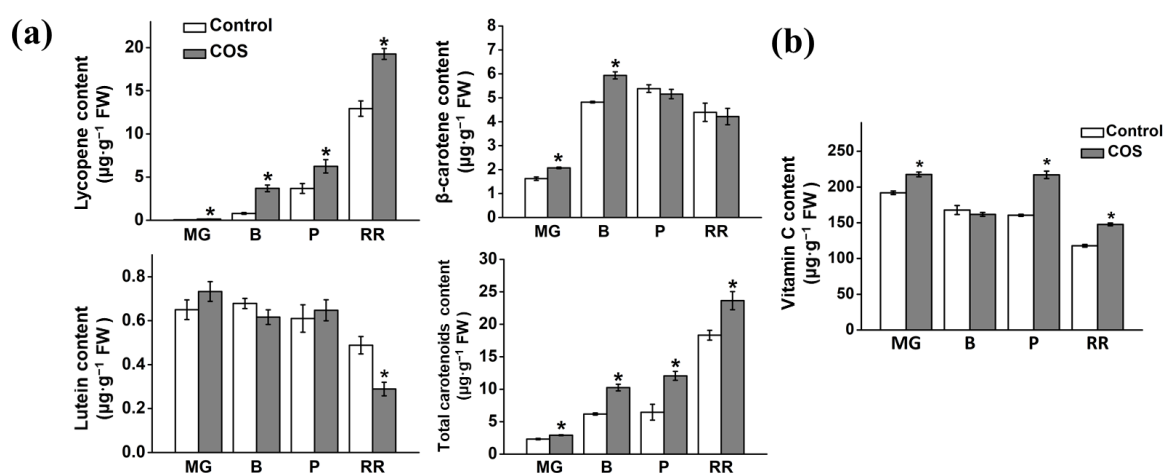


Figure 3. Effect of COS on the health-promoting compounds in tomato fruits. (a) The effect of COS on carotenoid composition and content. (b) The effect of COS on vitamin C content. Data represent the means \pm SD of three biological replicates. The asterisk represents a significant difference compared with the control based on Student's *t*-test (* $p < 0.05$). MG: mature green; B: breaker; P: pink; RR: red ripened. FW: fresh weight.

3.4. Effect of COS Treatment on Vitamin C Content

The content of vitamin C declined slightly from the MG to the P stage, while it dropped significantly at the RR stage in control fruits (Figure 3b). In COS-treated fruits, the concentration of vitamin C fluctuated during fruit ripening, and the lowest level was at the RR stage. The COS-treated fruits were characterized by a significantly higher level of vitamin C compared to the controls (Figure 3b), with 13.3%, 35.2%, and 25.4% increases at the MG, P, and RR stages, respectively.

3.5. Effect of COS Treatment on Volatile Profiles

Tomato flavor is associated with sugars and organic acids and closely related to the composition and content of volatiles. Depending on the precursor, tomato volatiles are classified into three categories, i.e., lipid-derived, amino-acid-derived, and carotenoid-derived [34]. In this study, a total of 22 volatiles were identified and quantified in tomato fruits at the RR stage. These included 13 lipid-derived, 5 amino-acid-derived (including branched-chain and phenolic volatiles), and 4 carotenoid-derived volatiles. COS-treatment significantly promoted the emission of volatiles. As shown in Table 1 and Figure S1, the total amount of detected volatiles increased by 52.0%, with the highest increase observed in carotenoid-derived volatiles (61.9% increase), followed by lipid-derived (53.4% increase) and amino acid-derived volatiles (27.3% increase) in COS-treated fruits. The production of 18 volatiles was upregulated to varying degrees by COS treatment, 10 of which were correlated with overall liking, 13 of which were significantly related to flavor intensity, and 9 of which were associated with both overall liking and flavor intensity, as previously described [8] (Table 1). For instance, the content of 1-pentone-3-one, (*E*)-2-pentenal, (*E*)-3-hexen-1-ol, and (*E*)-2-heptenal, the lipid-derived volatiles highly correlated with both consumer preference and flavor intensity in fruits [8], increased by 11.8%, 158.3%, 20.9%, and 66.0%, respectively, in COS-treated fruits (Table 1). Likewise, higher levels of 2-isobutylthiazole, a branched-chain volatile unique to tomato [35], as well as several phenolic volatiles (2-phenylacetaldehyde and 2-phenylethanol), were observed in COS-treated fruits (Table 1). The latter are important contributors to tomato aroma, imparting a “fruity” note [6]. Carotenoid-derived volatiles including geranylacetone, 6-methyl-5-hepten-2-one, 6-methyl-5-hepten-2-ol, and β -ionone are characterized as fruity or floral and have a broad impact on the perception of the sweetness of tomato fruits [8]. COS treatment also promoted the emission of these substrates (Table 1).

In addition, COS-treated fruits exhibited the enhanced production of (*E*)-2-hexenal (Table 1), the most abundant volatile, which is not correlated with consumer liking but is related to flavor intensity [8].

Table 1. Effect of COS on volatile content in ripe tomato fruits.

Volatiles	Precursors	Content ($\mu\text{g}\cdot\text{kg}^{-1}$ FW)		Overall Liking	Overall Flavor Intensity
		Control	COS Treatment		
1-penten-3-one	Lipid	53.1 \pm 1.1	59.4 \pm 1.4 *	+	+
(<i>E</i>)-2-pentenal	Lipid	11.7 \pm 0.6	30.3 \pm 0.5 *	+	+
Hexanal	Lipid	382.6 \pm 11.2	787.6 \pm 16.2 *		
(<i>Z</i>)-3-hexenal	Lipid	136.9 \pm 4.1	110.3 \pm 3.7 *		
(<i>E</i>)-2-hexenal	Lipid	1069.8 \pm 59.8	1734.8 \pm 28.2 *		+
(<i>E</i>)-3-hexen-1-ol	Lipid	3.6 \pm 0.1	4.3 \pm 0.2 *	+	+
Hexanol	Lipid	37.6 \pm 5.7	77.1 \pm 3.9 *		
Heptanal	Lipid	15.2 \pm 0.4	19.1 \pm 0.5 *		
(<i>E</i>)-2-heptenal	Lipid	60.0 \pm 1.5	99.6 \pm 3.3 *	+	+
2-octanone	Lipid	323.4 \pm 2.5	301.9 \pm 6.2 *		
1-octen-3-one	Lipid	26.5 \pm 1.8	34.5 \pm 0.8 *		+
1-nonanal	Lipid	26.3 \pm 2.0	26.1 \pm 1.6		
(<i>E, E</i>)-2,4-decadienal	Lipid	U.d	7.3 \pm 0.4 *		+
2-isobutylthiazole	Branched-chain amino acid	288.1 \pm 7.5	350.4 \pm 6.1 *	+	+
Isovaleric acid	Branched-chain amino acid	15.9 \pm 0.3	15.4 \pm 0.4	+	+
Phenylacetaldehyde	Phenylalanine	2.4 \pm 0.1	7.0 \pm 0.1 *	+	+
Benzyl alcohol	Phenylalanine	2.7 \pm 0.1	5.0 \pm 0.2 *		+
2-phenylethanol	Phenylalanine	5.7 \pm 0.2	23.1 \pm 0.6 *	+	+
Geranylacetone	Carotenoid	142.1 \pm 3.4	253.9 \pm 8.6 *		
6-methyl-5-hepten-2-one	Carotenoid	307.1 \pm 12.8	496.8 \pm 6.1 *	+	
6-methyl-5-hepten-2-ol	Carotenoid	42.8 \pm 0.1	46.7 \pm 0.9 *	+	+
β -ionone	Carotenoid	11.3 \pm 0.5	17.1 \pm 0.7 *	+	+
Total volatiles		2964.9 \pm 116.7	4507.5 \pm 90.4 *		

Data are expressed as means \pm SD of three biological replicates. U.d: under detected limit. Asterisks denote statistically significant differences compared with the control (* $p < 0.05$). “+” indicates a significant correlation with overall liking or overall flavor intensity as suggested by Tieman et al. [8].

3.6. Effect of COS on Ethylene Production and Signaling in Tomato Fruits

As shown in Figure 4a, the ethylene production increased rapidly from the MG stage, reached a maximum at the P stage, and then decreased at the RR stage in both control and COS-treated fruits. However, COS-treated fruits produced a significantly higher level of ethylene than the control at all four stages of maturity (Figure 4a). In tomatoes, it is well-established that *SlACS2*, *SlACS4*, and *SlACO1* are highly expressed during fruit ripening and participate in the autocatalytic biosynthesis of ethylene [36,37]. Consistent with the enhanced emission of ethylene, the transcript levels of the aforementioned genes significantly increased in COS-treated fruits at the MG and B stages (Figure 4b). Ethylene is perceived by ethylene receptors (ETRs), among which *SlETR3* is highly expressed in tomato fruits and regulated by ethylene [38]. The expression level of *SlETR3* was also upregulated upon COS treatment at the MG and B stages (Figure 4b). An ethylene-responsive gene encoding polygalacturonase (*SIPG2a*), involved in cell wall softening [39], showed significantly higher expression in COS-treated fruit during ripening (Figure 4b). Similarly, phytoene synthase 1 (*PSY1*), a rate-limiting enzyme that regulates the flux of carotenoids [5,40], showed increased transcript levels in COS-treated fruit at the onset of fruit ripening (Figure 4b). These results suggest that COS treatment could enhance ethylene biosynthesis and signaling.

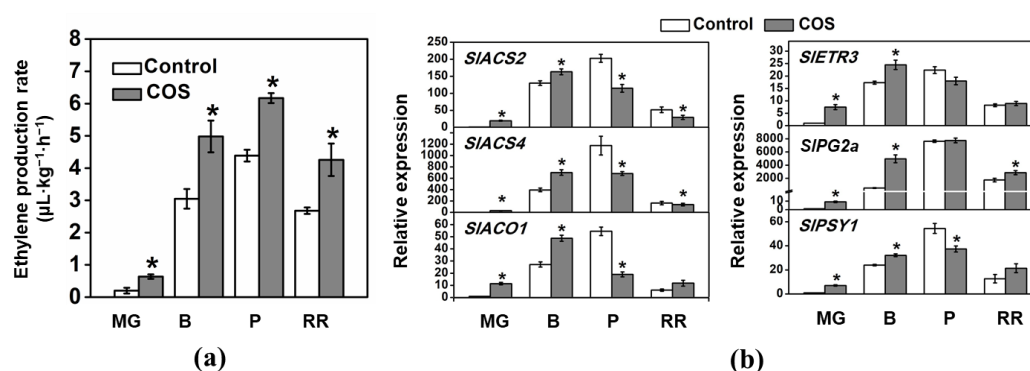


Figure 4. Effect of COS on ethylene production and signaling in tomato fruits. **(a)** Ethylene production in COS-treated and control fruits. **(b)** Expression levels of genes involved in ethylene biosynthesis, perception, and response in COS-treated and control fruits. Data represent the means \pm SD of three biological replicates. Asterisks represent a significant difference compared with the control based on Student's *t*-test (* $p < 0.05$). MG: mature green; B: breaker; P: pink; RR: red ripened.

4. Discussion

Fruit color is an important trait of tomato fruit that affects its marketability and consumer preference. Color formation during fruit ripening is related to dynamic changes in pigments such as chlorophyll, carotenoids, flavonoids, and anthocyanin. Generally, fruit color formation in red tomatoes is accompanied by the degradation of chlorophyll and the accumulation of colored carotenoids, particularly lycopene [41]. In the current study, we found that preharvest treatment with COS was significantly correlated to a dark red skin color with a higher content of lycopene and total carotenoids in tomato fruits (Figures 1 and 3a). Carotenoid biosynthesis is catalyzed by many carotenoid genes, the most important of which is *SIPSY1*, which regulates carotenoid flux [5,40]. Here, we found that the expression level of *SIPSY1* in COS-treated tomato fruits was significantly higher than in the controls at the onset of fruit ripening (Figure 4b). This may have been responsible for the increased lycopene content in the COS-treated fruits (Figure 3a).

Vitamin C is a water-soluble micronutrient with pleiotropic health benefits due to its antioxidant activity [42]. The application of COS to reduce vitamin C loss during postharvest storage has been extensively used in several horticultural crops such as strawberry [43], apricot [44], citrus [45], jujube [46], and tomato [33]. In the present study, the preharvest application of COS also promoted the accumulation of vitamin C in tomato fruits (Figure 3b). Our results were consistent with previous studies, which revealed that the foliar application of COS in appropriate concentrations could increase the vitamin C level in tomatoes [27,28].

Sugars and organic acids determine the balance of sweetness and acidity in tomato fruits [6]. Lei et al. suggested that the foliar spraying of COS significantly augmented the total soluble content while decreasing the titratable acid level in cherry tomatoes [27]. The present study further investigated the effect of COS on different individual sugars and organic acids. In agreement with previous studies, we found that the preharvest application of COS enhanced the accumulation of both glucose and fructose, two main sugars in tomato fruits (Figure 2). On the contrary, COS treatment led to a reduced level of malic acid in tomato fruits during the ripening process, although no significant change in organic acid content was observed in ripe tomato fruits (Figure 2). Fructose and glucose are hydrolysis products of sucrose, which is transported through the phloem from the leaf source to the non-photosynthetic fruit sink [47]. Hence, there is a close link between the photosynthetic output of leaves and the sugar accumulation of tomato fruits. Numerous studies have proven the effectiveness of COS in improving the photosynthetic rate by regulating the photosynthetic pigment contents [15,22,26], primary phytochemistry [21,24], and carbon and nitrogen metabolism [14,19,48] in various plants. Therefore, we speculated

that COS application might contribute to better photosynthetic performance, leading to the elevated production and partitioning of photosynthates to the fruits, which would ultimately result in the increased accumulation of soluble sugars. Further investigations are needed to elucidate the underlying mechanism of COS's role in soluble sugar metabolism.

Sugars and organic acids are essential for a delicious taste, but it is the volatiles that give tomatoes their unique flavor [9]. More than 400 volatiles are present in tomato fruits, but only a set of 20–30 volatiles positively contribute to tomato flavor [9,34]. Tieman et al. [8] claimed that a total of 33 chemicals are responsible for consumer liking and 37 chemicals contribute to flavor intensity, 28 of which are correlated with both overall liking and flavor intensity. In the present study, we identified 18 volatile chemicals that significantly increased with COS treatment, and most of them were correlated with overall liking, flavor intensity, or both (Table 1). Moreover, we found that nine flavor-associated volatiles that are diluted in modern tomato varieties, leading to the poor flavor of tomato fruits (as previously reported [8]), were dramatically increased in COS-treated tomato fruits (Table 1). Consequently, COS treatment could make tomato fruits tastier.

The phytohormone ethylene is a well-known regulator of tomato ripening that coordinates the genes that participate in multiple biological processes, such as color conversion, fruit softening, nutrient accumulation, and flavor formation [49]. In this study, we reported that COS promoted the production of ethylene at each stage of fruit ripening, with increased transcript levels of ethylene-biosynthesis- and signaling-related genes (Figure 4). Several ethylene-responsive genes regulating fruit ripening and quality were significantly induced by COS treatment (Figure 4b). For example, the transcript level of *SIPG2a*, which regulates cell-wall softening, was significantly higher in COS-treated fruit than in controls (Figure 4b), which may have accounted for the decreased firmness of COS-treated fruits (Figure 1b). The results indicated that COS could regulate fruit quality through the ethylene pathway.

5. Conclusions

The current study focused on the effects of COS on the comprehensive quality attributes of tomato fruit and revealed that COS promoted fruit coloring and softening and increased the contents of health-promoting compounds (lycopene and vitamin C). It also enhanced the accumulation of soluble sugars (fructose and glucose) and flavor-associated volatiles that improve taste. The analysis of ethylene production and signaling pathways suggested that COS could improve fruit quality by augmenting ethylene biosynthesis, perception, and response. These findings help us to better understand the role of COS in fruit ripening and quality improvement and provide theoretical support for its practical application in sustainable tomato production.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae9030300/s1>, Figure S1: Changes in volatiles derived from various pathways in response to chitosan oligosaccharide treatment in ripe tomato fruits, Table S1: Primers used for RT-qPCR, Table S2: Effect of chitosan oligosaccharide on tomato fruit size.

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