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Recent Advancements in Postharvest Fruit Quality and Physiological Mechanism

Edited by
Yuduan Ding, Xiaolin Ren and Yanrong Lv

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Editors

Yuduan Ding
Xiaolin Ren
Yanrong Lv

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Editors

Yuduan Ding
College of Horticulture
South Campus of Northwest
A&F University
Yangling
China

Xiaolin Ren
College of Horticulture
South Campus of Northwest
A&F University
Yangling
China

Yanrong Lv
College of Horticulture
South Campus of Northwest
A&F University
Yangling
China

Editorial Office
MDPI AG
Grosspeteranlage 5
4052 Basel, Switzerland

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Yuduan Ding

Yuduan Ding, associate professor at College of Horticulture in Northwest A&F University. The main research is based on big data of horticultural products, conducting transcriptome analysis, metabolic network, protein interaction network construction, and completing physiological and molecular biology research related to postharvest senescence physiology and quality regulation of horticultural products.

Xiaolin Ren

Xiaolin Ren, professor at College of Horticulture in Northwest A&F University. The research field is postharvest biology and storage preservation of fruits, with the main research content of postharvest quality changes; fruit storage and preservation technology, mainly includes fruits such as apple, pear, kiwifruit, date, and apricot.

Yanrong Lv

Yanrong Lv, lecturer at College of Horticulture in Northwest A&F University. The main research is engaged in physiological and molecular mechanism research on postharvest storage, preservation, and quality control of fruits. The specific research content includes fruit cold injury control technology, secondary metabolite synthesis and metabolism regulation mechanism, etc.

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Editorial

Recent Advancements in Postharvest Fruit Quality and Physiological Mechanism

Yanrong Lv, Xiaolin Ren and Yuduan Ding *

College of Horticulture, Northwest A & F University, Yangling, Xianyang 712100, China; lvyanrong618@163.com (Y.L.); renxl@nwsuaf.edu.cn (X.R.)
* Correspondence: dingyuduan@nwafu.edu.cn

1. Introduction

Fresh fruits can provide people with various nutrients, including carbohydrates, vitamins, minerals, phenols, and other bioactive compounds, and they are an indispensable component of the human diet. Fruit development and ripening is a genetically programmed series of coordinated processes, with various physiological changes occurring, such as a progressive loss of firmness, the release of volatile compounds, and the accumulation of soluble sugars and a wide range of secondary metabolites [1,2]. These physiological and biochemical changes make horticultural fruit more palatable and desirable to consumers. Appropriate postharvest commercialization techniques are particularly crucial for maintaining or improving fruit quality. This Special Issue concerns the biological and technological postharvest research of horticultural fruit crops and aims to enhance readers' in-depth understanding of the advanced technologies and regulatory mechanisms for maintaining postharvest fruit quality. A total of twelve articles (two review papers and ten research papers) are rigorously reviewed and included in this Special Issue, which determines and discusses the quality maintenance and physiological regulation mechanisms of postharvest fruit and fresh-cut products, as well as different fruit quality testing technologies.

2. Special Issue Overview

2.1. Preharvest Treatments Affect Postharvest Fruit Quality

The effects of preharvest applications on postharvest behavior are determinative in terms of fruit quality and storability [3]. The preharvest application of chemical elicitors, plant growth regulators, or controlled environmental factors can maintain postharvest quality and extend the storage period, especially for fruits with poor storability and a short shelf life [4,5]. Retamal-Salgado et al. (contribution 1) evaluated the effect of three preharvest applications of oxalic acid (OA) and salicylic acid (SA) on fruit firmness and phenolic compounds in blueberry ('Stella Blue' and 'Kirra'). The results showed that preharvest applications of OA and SA can improve fruit firmness by up to 20% at different harvest times. In addition, 2 mM SA generated a 100% increase in polyphenolic content and antioxidant capacity in 'Stella Blue', while 4 mM OA increased total anthocyanin and antioxidant capacity by 100% and 20%, respectively. The preharvest application of gibberellic acid (GA_3) on plum also led to a decrease in weight loss, with a corresponding increase in fruit firmness, total soluble solids (TSSs), and titratable acidity (TA); however, the total phenolic content values during storage did not exhibit any significant changes. According to these results, producers can be advised to apply 50 ppm GA_3 in the preharvest stage of plum [3]. Taking into consideration the potential side effects of preharvest applications on ecological conditions and cultural practice, it is advised that tests are repeated for different locations and/or fruit varieties to determine the site- and variety-specific responses before large-scale promotion and application.

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2.2. Postharvest Treatments Affect Fresh Fruit Quality

Sufficient evidence suggests that cell wall disassembly is responsible for a major portion of fruit softening, and this is initiated by a complex interplay between hormonal cues, epigenome changes, and the tightly regulated expression of numerous transcription factors (TFs) and downstream genes [6]. The postharvest handling of horticultural fruit is an effective means of maintaining or improving product quality and extending storage and shelf life, thus increasing the value of fruit after harvesting. 1-Methylcyclopropene (1-MCP) is an inhibitor of ethylene perception that is widely used to maintain the quality of climacteric fruits during storage [7]. Rivera-Ponce et al. (contribution 2) evaluated the diversity of postharvest characteristics in 10 chayote groups of varieties that allow them to be used in different ways including as a fresh fruit, in agroindustrial transformation, or in mixing with other vegetables. Moreover, 600 nL L^{-1} 1-MCP sealed treatment reduced the chilling injury, weight loss, and evident dehydration for five chayote varieties during cold storage. Shu et al. (contribution 3) found that a chitosan coating (2%, w/v, 310–375 kDa) containing 0.2% (v/v) carvacrol maintained the postharvest quality of guava compared to chitosan alone, with higher firmness, TSS, TA, and total phenol content and lower weight loss and pericarp browning. Mi et al. (contribution 4) showed that LED white light treatment (LWT) preserved the postharvest quality of 'Zaosu' pear fruit by inhibiting respiration and ethylene production, reducing weight loss and ascorbic acid degradation, and promoting the ratio of sugar and organic acid. In addition, LWT retarded the decrease in chlorophyll content of pear fruit by increasing the activities of chlorophyll synthase-associated enzymes, and suppressing the chlorophyll degradation-related enzymes and their gene expressions in pear peel. Zhao et al. (contribution 5) demonstrated that low-concentration NAA treatment on peach reduced the transcription level of *PpPG*, *PpbGAL*, and *PpACS1* genes. Furthermore, an interaction between the auxin receptor PpTIR1 (Transport Inhibitor Response 1) and PpIAA1/3/5/9/27 proteins was unveiled, and the results show that the PpTIR1-Aux/IAA module has a possible regulatory effect on fruit ripening and softening. Zhang et al. (contribution 6) reviewed the important role of gibberellins (GAs) in the physiological regulation of postharvest fruits, which includes improving the intrinsic and extrinsic quality, enhancing postharvest biotic and abiotic stress resistance, and effectively controlling some postharvest fruit diseases. The authors also suggested that GAs have important application prospects in postharvest fruits.

2.3. Testing Technology for Fruit Internal Quality

In recent years, the development of non-destructive sensing techniques for measuring fruit internal quality has been a challenge. The rapid identification of defective fruits is beneficial for reducing unnecessary storage and avoiding quality degradation [8]. Tang et al. (contribution 7) built internal quality prediction models for Korla fragrant pears. After determination, it showed that the model based on partial least-squares regression (PLSR) and using the dielectric constant as a variable predicted hardness the most accurately, while the model based on PLSR using the dielectric loss factor as a variable was the best for predicting SSC. Therefore, the study provides a new method for the non-destructive online testing of the internal quality of pear. Ni et al. (contribution 8) indicated that there would continue to be active research and publications on non-destructive testing technology for fruit quality. China and the USA are the major contributors to research in this field. The detection technologies mainly include electronic nose (E-nose) technology, machine vision technology, and spectral detection technology. In the future, technological developments in artificial intelligence and deep learning will further promote the maturation and application of non-destructive testing technologies for fruit quality. Firmness is a crucial feature that significantly influences the postharvest preservation of fruit and its acceptability by customers. The methods for measuring fruit firmness are mostly destructive, using a texture analyzer or firmness tester. Karageorgiadou et al. (contribution 9) studied the impact of fruit physiology under various and fixed distances for the firmness evaluation of sweet cherry fruit. The results suggested that a fixed distance of 0.16 mm and a minimal 1%

deformation force possess the potential to be employed and implemented for monitoring the firmness of sweet cherries using the texture analyzer during postharvest preservation.

2.4. Quality Maintenance and Regulation of Fresh-Cut Fruit Products

Fresh-cut fruit products are in considerable demand owing to the convenience of buying and cooking. However, the shelf-life of fresh-cut fruit products is short due to their physiological changes and maturation [9]. Yuan et al. (contribution 10) found that 0.4 g/L of nisin reduced the weight loss rate and whitening rate, inhibited the respiration rate, and maintained the firmness of fresh-cut pumpkins. Meanwhile, 0.4 g/L of nisin increased antioxidant-metabolism-related enzyme activities and prevented the rapid increase in reactive oxygen species (ROS), as well as maintaining higher contents of ascorbate and glutathione. Guan et al. (contribution 11) explored the effect of cutting methods on the quality of fresh-cut cucumbers, and found that the vitamin C content was gradually reduced in the sliced, pieced, and stripped cucumbers, while the glutathione content increased significantly compared with whole cucumbers. Furthermore, the fresh-cutting operation enhanced the total phenol content, but also induced the production of ROS ($O_2^{-\cdot}$ and H_2O_2). In general, the degree of quality indexes was sliced > pieced > stripped. This revealed that JA biosynthesis was activated by mechanical damage, and the up-regulation of phenylalanine metabolism and phenylalanine, tyrosine, and tryptophan metabolism affected phenylpropanoid biosynthesis, which may promote lignin synthesis. The lignin produced during secondary metabolism lignifies fresh-cut cucumber, which seriously affects the taste and appearance. Through transcriptome analysis, Wang et al. (contribution 12) demonstrated that pathways of amino acid metabolism, lipid metabolism, and secondary metabolism were affected by mechanical damage. Furthermore, the up-regulation of phenylalanine metabolism and phenylalanine, tyrosine, and tryptophan metabolism affected phenylpropanoid biosynthesis, which may promote lignin synthesis.

3. Conclusions

The twelve articles in this Special Issue were selected from a large number of submissions through rigorous evaluation, and they are of a high standard. This Special Issue aims to enable researchers to understand the cutting-edge technologies and methods related to maintaining postharvest fruit quality, while also providing new thoughts for in-depth research on the regulatory mechanisms related to postharvest fruit quality control. At present, consumers have increasingly high requirements for fruit quality, so it is necessary to continuously develop new technologies to maintain the postharvest quality of fruits. In addition, researchers need to strengthen their study on the mechanisms involved in the maintenance of fruit quality.

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List of Contributions:

1. Retamal-Salgado, J.; Adaos, G.; Cedeño-García, G.; Ospino Olivella, S.C.; Vergara-Retamales, R.; Lopéz, M.D.; Olivares, R.; Hirzel, J.; Olivares-Soto, H.; Betancur, M. Preharvest Applications of Oxalic Acid and Salicylic Acid Increase Fruit Firmness and Polyphenolic Content in Blueberry (*Vaccinium corymbosum* L.). *Horticulturae* **2023**, *9*, 639. https://doi.org/10.3390/horticulturae9060639.
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Article

Characteristics and Potential Use of Fruits from Different Varietal Groups of *Sechium edule* (Jacq.) Sw

Edgar Adrián Rivera-Ponce [1], Ma. de Lourdes Arévalo-Galarza [1,*], Jorge Cadena-Iñiguez [2], Marcos Soto-Hernández [1], Yeimy Ramírez-Rodas [3] and Cecilia García-Osorio [1]

[1] Colegio de Postgraduados, Campus Montecillo, Km. 36.5 Carretera México-Texcoco, Montecillo, Texcoco 56230, Mexico; rivera.edgar@colpos.mx (E.A.R.-P.); msoto@colpos.mx (M.S.-H.); gcecilia@colpos.mx (C.G.-O.)
[2] Colegio de Postgraduados, Campus San Luis Potosí, San Iturbide No. 73, Salinas de Hidalgo, San Luis Potosí 78600, Mexico; jocadena@colpos.mx
[3] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias. C.E. Valles Centrales, C. Melchor Ocampo 7, Sto. Domingo Barrio Bajo, Etla, Oaxaca 68200, Mexico; ramirez.yeimy@inifap.gob.mx
* Correspondence: larevalo@colpos.mx; Tel.: +52-595-9520200 (ext. 1571)

Abstract: (1) Background: Chayote [*Sechium edule* Jacq. (Sw.)] is a non-traditional export product; recently, demand has increased due to its nutritional and functional properties. There is a wide diversity of varietal groups (VGs) within this species. Despite this, only *virens levis* and *nigrum spinosum* varieties are commercialized on a large scale, while the rest are underutilized and poorly studied, so the genetic pool of this species is at risk. (2) Methods: The following variables were evaluated in the fruits of 10 chayote groups of varieties: shape, size, weight, stomatal frequency (SF), stoma size, stomatal index (SI), color index (CO*), pigments, titratable acidity (TA), total soluble solids (TSS), total sugars and moisture content. In addition, the postharvest behavior of the ten VGs stored at room temperature and the effect of 1-MCP on fruit quality during cold storage were evaluated. (3) Results: The groups *a. minor* and *n. minor* showed rapid weight loss, the *albus* varieties showed high epidermis oxidation, while *v. levis*, *n. maximum*, *n. spinosum* and *n. xalapensis* were susceptible to viviparity, blisters and fungal incidence. 1-MCP prevented chilling injury (CI) and weight loss. (4) Conclusions: The diversity of postharvest characteristics allows the use of VGs for different uses such as a fresh fruit, agroindustrial transformation or mixing with other vegetables.

Keywords: varietal groups; postharvest characterization; commercial quality; 1-MCP; cold storage

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1. Introduction

Chayote [*Sechium edule* Jacq. (Sw.)] is native to Mexico and Central America but is now cultivated in many tropical and subtropical regions of the world [1]. There is a wide varietal diversity within the *S. edule* species with fruits of different color, size, presence of spines, shape and phytochemical composition, which influences the taste of the fruits [2]. Currently, twelve varietal groups are recognized within the species; those of commercial importance are *virens levis* and *nigrum spinosum*. The high demand for these two varieties has meant that the other varietal groups have been ignored or are only traded in local markets, which puts the genetic richness at risk. The fruit is non-climacteric and is harvested at horticultural maturity [1].

Recently, the consumption of chayote has increased due to its nutritional properties; the fruit is low in calories (19–31 Kcal/100 g) and is a good source of fiber (0.40–7.53%) and minerals such as potassium and calcium. It also contains essential amino acids such as valine, leucine and phenylalanine and vitamins C, E and B9 [3,4]. Chayote fruits also are rich in compounds with functional properties and significant amounts of phenolic acids, flavonoids and different types of cucurbitacins (Cus), which have an antiproliferative activity against cancer cells such as HeLa, P-388 and L-929 [5]. They also have antifungal

properties, limiting the germination *of Botrytis cinerea* conidia [6] and antioxidant activity [7]. For example, the fruits of *nigrum xalapensis* contain 13.44, 5.60 and 0.62 mg g^{-1} of Cu types D, I and B, respectively, while the *v. levis* variety has 0.23, 0.08, 1.09 and 0.11 mg g^{-1} of rutin, florizidine, myricetin and floretin, respectively. Yellow varieties such as *a. levis* contain Cu D (4.77 mg g^{-1}), Cu I (3.52 mg g^{-1}), Cu B (0.46 mg g^{-1}) and Cu E (1.73 mg g^{-1}) [2]. Other cucurbits such as cucumber (*Cucumis sativus* L.) only contain 0.69 to 0.89 mg g^{-1} of Cu E [8], and in watermelon (*Citrullus colocynthis*), only Cu E (0.21–0.3 mg g^{-1} FW) can be detected [9].

Visual appearance, ripeness, weight, size, shape, characteristic color (from ivory to dark green) and no defects and diseases, as well as internal parameters such as flavor, aroma, texture and nutritional composition, are the important quality traits for the commercial acceptability of the fruits (Figure 1). In the case of chayote, size, uniform weight, the presence or absence of thorns and the color of the exocarp are important quality characteristics for the market [10].

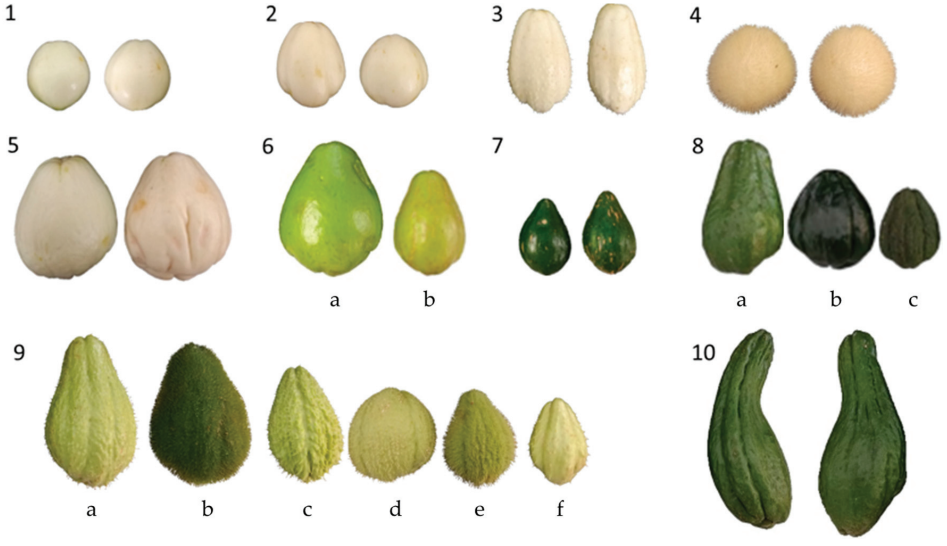

Figure 1. Morphological characteristics of 10 varietal groups of chayote [*Sechium edule* Jacq. (Sw.)]: (**1**) *a. minor*; (**2**) *a. levis*; (**3**) *a. dulcis*; (**4**) *a. spinosum*; (**5**) *a. levis gigante*; (**6**) *v. levis*; (**7**) *n. minor*; (**8**) *n. xalapensis*; (**9**) *n. spinosum*; (**10**) *n. maxima*. The letters (a, b, c, d, e, f) represent different morphotypes within the same varietal group. Bar size 3 cm.

The shelf life of chayote is short, and at room temperature, commercial quality can only be maintained for up to seven days [11]. The main problems are rapid weight loss, fungal incidence, blistering and early seed germination (viviparity) [12]. In this respect, refrigeration is an effective method of maintaining quality; however, temperatures below 7 °C can cause chilling injury (CI), presented as brown spots and depressions on the fruit epidermis which generally appear once the fruits are returned to room temperature [12,13].

1-methylcyclopropane (1-MCP) is a good option to prolong the shelf life of non-climacteric fruits. In cherries (*Prunus avium* L.) stored at 1 °C for 30 days, treatment with 1-MCP (1 µL L^{-1} for 24 h) reduced physical injury and the incidence of physiological disorders and maintained fruit firmness [14]. In zucchini (*Cucurbita pepo*), the application of 2.4 µL L^{-1} of 1-MCP for 48 h reduced the respiration rate and ethylene production by half, a significant reduction in weight loss and cold damage during storage for 14 days at 4 °C, although these effects depended on the variety [15]. Likewise, the application of

625–650 nL L^{-1} of 1-MCP reduced weight loss, softening and color changes in melon fruits (*Cucumis melo* L.), prolonging shelf life by 10 days [16].

Therefore, the aim of this work was to determine the commercial potential of 10 variety groups of *S. edule* as a strategy to preserve these genetic resources and promote their commercial cultivation. For this, the morphological and biochemical characterization of chayote fruits was carried out, and their postharvest behavior and the effect of 1-MCP during cold storage were evaluated.

2. Materials and Methods

2.1. Morphological and Physicochemical Characterization of the Fruits

Fruits were harvested from the National Germplasm Bank of *Sechium edule* in Mexico (BANGESe). Harvesting was carried out at horticultural maturity (18 ± 2 d after anthesis) [17], choosing fruit without any external defects or disease occurrence. The fruits of the 10 chayote varietal groups assessed were *albus minor*, *albus levis*, *albus dulcis*, *albus spinosum*, *albus levis gigante*, *virens levis*, *nigrum minor*, *nigrum xalapensis*, *nigrum spinosum* and *nigrum maxima* (Figure 1). At least 50 fruits per varietal group were selected in order to realize the characterization.

The shape of chayote fruit was classified as pyriform, elongated pyriform, obovoid and round [18]. The length (cm) was measured from the slit to the apex using a digital vernier. The weight (g) was determined with a digital balance (Model SI-2000S, Setra Systems, Inc., Boxborough, MA, USA) with an accuracy of 0.01 g.

Stomata size, stomata frequency (SF) and stomata index (SI) were reported as µm, stomata/mm^2 and percentage (%), respectively. For this, the epidermis printing method was used [19]. For this purpose, a thin layer of nail polish was placed on 1 cm^2 of the equatorial epidermis of the fruits and allowed to dry at room temperature. The print was then peeled off and placed on a slide. Photographs of these preparations were obtained with ImageJ® 1.45 software adapted to an optical microscope (model B-510PH, Optika S.L.R., Ponteranica, Italy) and a digital camera (Canon, model EOS Rebel T7, Japan). Photographs for SF were taken at 10×, representing 1.552 mm^2 each, while epidermal cell counts and stomata size were taken at 40×, representing 0.094 mm^2 each. The SI was calculated considering S = the number of stomata per unit area and E = the number of epidermal cells per unit area, according to the equation [20]:

$$\text{SI}\ (\%) = \frac{\text{S}}{\text{E} + \text{S}} \times 100$$

Color was measured with a colorimeter (3NH TECNOLOGY Co., Ltd., model NR20XE, Shenzhen, China). L, a* and b values were taken, and the color index (CO*) was calculated [21], where L = lightness, a* = red-green and b* = yellow-blue coordinates:

$$\text{CO}* = \frac{\text{a}^* \times 1000}{\text{L} \times \text{b}^*}$$

For total chlorophyll (C_{a+b}) and total carotenoids (C_{x+c}), 2 g of the epidermis was taken, 10 mL of 80% (*v*/*v*) acetone was added and stored in the dark at room temperature for 24 h. Afterward, absorbance (A) was measured at 470, 646 and 663 nm with a UV spectrophotometer (GENESYS™ 10UV, Thermo Scientific, Madison, WI, USA); the content was expressed as mg g^{-1} fresh weight and calculated according to that proposed by Lichtenthaler [22]:

$$C_{a+b} = 7.15A_{663} + 18.71A_{646}$$

$$C_{x+c} = \frac{1000A_{663} - 1.82C_a - 85.2C_b}{198}$$

For the calculation of titratable acidity (TA), 3 g of pulp was macerated with 30 mL of distilled water, the mixture was titrated with NaOH 0.1 N and phenolphthalein was used

as indicated, reporting the values as % citric acid. TSS (°Bx) was measured with a digital refractometer (PAL-1, Atago®, Saitama, Japan). For the measurement of total sugars (%), a flask was boiled with 5 g of pulp and 60 mL of 80% (v/v) ethanol until approximately 10 mL was reduced and the supernatant recovered. Then, 0.3 mL of supernatant–distilled water (1:100), 0.3 mL of 5% phenol (v/v) and 1.5 mL of 95% H_2SO_4 (v/v) were mixed. Subsequently, absorbance was measured at 490 nm; 95% (w/v) glucose was used as the standard [23].

For moisture content (%), 1 cm thick slices were taken from the center of the fruit without seed and skin and placed in a mechanical convection oven (Thermo Scientific, model Imperial V, Greenville, NC, USA) at 50 °C until a constant dry weight was obtained and calculated with the following equation:

$$\text{Moisture content } (\%) = \frac{\text{Wet weight } - \text{ dry weight}}{\text{Wet weight}} \times 100$$

2.2. Postharvest Characterization and 1-MCP Application

Postharvest characterization: The fruits (at least 50 per varietal group) were washed with a 1% sodium hypochlorite solution and dried at room temperature. Postharvest characterization of all groups was carried out under room conditions (21 °C and 70% RH).

Phytopathogenic fungi were isolated in the fruits with obvious symptoms such as mycelial growth or rot; isolation techniques and taxonomic keys were used [24]. Fruits were considered to lose commercial quality when viviparism (early seed germination) appeared and evident dehydration showed. The following scales were developed for this purpose: Viviparism: Level 0 = no seed present, 1 = seed visible and basal opening and 2 = seed fully exposed; Dehydration: Level 0 = none, 1 = mild, 2 = moderate and 3 = severe; Chilling injury: Level 0 = none, 1 = mild, 2 = moderate (light brown spots) and 3 = severe (brown and soaked spots); Blisters: Level 0 = any blister, 1 = 1 to 5 blisters, 2 = 6 to 15 blisters, 3 = 16 to 25 blisters and 4 = 26 to 50 blisters. The scales are according to Ramirez-Rodas [12].

The α-amylase activity was determined in those varieties with a high incidence of viviparity such as *v. levis*, *n. maxima*, *n. xalapensis* and *n. spinosum*. The Alpha-amylase SD kit (Megazyme, UK, Wicklow, Ireland) was used, following the manufacturer's recommended instructions. Six fruits per variety were evaluated, taking tissue from the basal part of the fruit and the terminal part of the seed, which in previous studies showed the highest enzyme activity. The tissue was freeze-dried (model Freezone 4.5, Labconco, Co., Kansas City, MO, USA) and then finely ground for analysis; 250 mg of tissue was used for the test, and the results were presented as amylase units SD g^{-1} of dry tissue.

The weight loss (%) was determined with a digital balance (Model SI-2000S, Setra Systems, Inc., Boxborough, MA, USA) with an accuracy of 0.01 g. For the postharvest characterization, fifteen fruits per variety were used; the measures were taken every day until fruit decay. For the second experiment (1-MCP), 15 fruits per variety were also used and measured at harvest (day 0) and every day after cold storage. One fruit was considered as a replicate. The following equation was used:

$$\text{Weight loss } (\%) = \frac{\text{initial weight } - \text{ final weight}}{\text{initial weight}} \times 100$$

1-MCP application: The effect of 1-MCP on the cold storage of chayote was only investigated in the fruit of some variety groups. For this purpose, the application of 1-MCP (600 nL L^{-1}) was performed at room temperature by placing the fruit in a hermetically sealed acrylic box (0.125 m^3) and leaving a vial containing the 1-MCP concentration (SmartFresh®; 14%, Rohm and Haas, Philadelphia, PA, USA). The exposure time was 24 h at 24 ± 1 °C and 60% RH. After application, fruits treated with 1-MCP and their controls were stored at 8.7 °C and 95% relative humidity. Storage period was 2 weeks for the varieties of *a. minor*, *n. minor* and *v. levis* (B) and 3 weeks for *a. dulcis*, *a. levis* and *v. levis* (A).

2.3. Statistical Analysis

Morphological and biochemical characterization data were expressed as the mean ± standard error. A principal component analysis (PCA) and dendrogram were performed with the data, using the ade4 and cluster.datasets packages, respectively. The hierarchical map of postharvest problems was drawn up using the Pheatmap package of R software (version 4.0.2). The physicochemical characteristics and postharvest variables were analyzed using Kruskall–Wallis ($\alpha = 0.05$) due to the measurements not meeting normality.

3. Results and Discussion

3.1. Morphological and Phytochemical Characterization of the Fruits

Phenotypic and chemical variations within and between populations of the same species can occur in the flowers, clusters, leaves and fruits [25]. *S. edule* varietal complexes have diversified over time [26], and this diversity can be explained by processes of microevolution, where the combination of genetic plasticity, phenotypic drift and environmental factors result in phenotypes with specific morphological and chemical characteristics [27]. However, diversity is also associated with the process of domestication through natural and artificial selection of a species, as producers focus on the selection of fruits with certain characteristics such as plant productivity, fruit size, quality, flesh content and flesh color [1].

According to Table 1, the most common shape of chayote fruits is pyriform; however, some varieties of the *albus* group are round, and *n. maxima* is elongated pyriform. On average, we can classify chayote varieties as very large (*n. maxima*), medium to large (*v. levis*, *a. levis gigante*, *n. spinosum*, *n. xalapensis*), medium (*a. spinosum*, *a. dulcis*), small (*a. levis*) and very small (*a. minor*, *n. minor*). Fruit weights ranged from 23.44 g for *a. minor* to 511.32 g for *n. maxima*. Currently, the most traded variety is *v. levis*, which is exported with an approximate weight of 310 g [12].

Stomata play a major role in water loss through transpiration and are the gateway for infections caused by plant pathogens [28]. In cherry, apple, pomegranate or litchi, the frequency and size of stomata is related to physiological problems such as fruit cracking [29,30]. Likewise, the agglomeration of pathogenic bacteria such as *Salmonella* occurs close to the stomatal opening of the fruit [31]. Stomatal density decreases as the fruit expands, even losing functionality as it becomes covered with cuticular waxes [32]. Stomatic size, aperture and stomatal index are influenced by external factors such as CO_2 concentration and temperature [33] and by endogenous signaling via the likes of phytohormones and proteases, which are fundamental for the physiological adaptations of plants to biotic and abiotic stresses [28,34].

The presence of stomata in the fruit epidermis suggests an important role in gas exchange, with the potential to assimilate CO_2 and optimize photosynthesis [35]. In this regard, Sui et al. [36] showed that cucumber fruit can contribute 9.4% of carbon fixation, although the stomatal frequency of the fruit is only 1.58 and 0.91% compared to the adaxial and abaxial parts of the leaves, respectively. In this study, the stomatal frequency of the VGs varied between 5.73 and 39.26 stomata/mm^2; other authors confirmed that there are less than 40 stomata/mm^2 in the fruits and leaves [13,37].

The highest stomata index (SI) was found in *n. xalapensis* with 0.66%, and the lowest was 0.14% for *a. spinosum*, although some morphotypes of *n. xalapensis* and *n. spinosum* can reach values higher than 0.90%. Fruit stomata measure on average 28.76 μm, being very similar between varieties, but *n. spinosum* (35.24 μm) has significantly larger stomata and *v. levis* (22.14 μm) the smallest. The size is quite similar for *n. xalapensis* and *n. spinosum* at 30.79 and 34.45 μm, respectively [12]. While there are differences in SI, SF and stomatal size between varieties, these values are known to be specific to a species grown in a given environment. For these reasons, it is very difficult to use the SI as an identification parameter [38]. The presence of chlorophylls and stomata in chayote suggests that, like other fruits, there is a photosynthetic activity, which may be differential depending on the stage of development.

Table 1. Phytochemical and morphological characterization of 10 varietal groups of chayote [*Sechium edule* Jacq. (Sw.)].

Varietal Group	Shape	Length (cm)	Weight (g)	SF (Stomata/mm^2)	Stomatal Size (µm)	SI (%)	CO*	C_{a+b} (µg g^{-1})	C_{x+c} (µg g^{-1})	TA (%)	TSS (°Bx)	Total Sugars (%)	Moisture (%)
a. minor	round	3.05–4.27	23.44 ± 0.64	11.30 ^{b} ± 1.37	28.70 ^{bc} ± 0.73	0.504	−0.184 ± 0.099	1.0 ^{a} ± 0.0	Nd	0.14 ^{bc} ± 0.004	5.42 ^{bc} ± 0.106	2.38 ^{cd} ± 0.12	90.97 ^{bc} ± 0.48
a. levis	pyriform	5.77–8.50	114.37 ± 11.30	6.89 ^{ab} ± 0.50	29.48 ^{bc} ± 0.81	0.190	−1.832 ± 0.165	Nd	Nd	0.11 ^{ab} ± 0.004	5.26 ^{abc} ± 0.104	2.39 ^{cd} ± 0.20	91.99 ^{bcd} ± 0.18
a. dulcis	pyriform	7.74–9.56	87.20 ± 1.74	19.52 ^{cd} ± 1.03	28.80 ^{bc} ± 0.79	0.444	−1.973 ± 0.081	Nd	1.0 ^{a} ± 0.0	0.18 ^{e} ± 0.007	5.56 ^{c} ± 0.160	2.55 ^{d} ± 0.28	81.23 ^{a} ± 0.78
a. spinosum	pyriform	7.30–7.90	214.08 ± 6.58	5.73 ^{a} ± 0.54	31.44 ^{cd} ± 0.92	0.142	0.341 ± 0.034	1.0 ^{a} ± 0.0	1.0 ^{a} ± 0.0	0.14 ^{bc} ± 0.004	5.02 ^{abc} ± 0.30	2.51 ^{d} ± 0.10	90.82 ^{ab} ± 0.19
a. levis gigante	pyriform	10.14–13.16	415.32 ± 21.13	17.35 ^{cd} ± 0.54	24.01 ^{a} ± 0.67	0.248	−1.071 ± 0.181	1.0 ^{a} ± 0.0	Nd	0.11 ^{ab} ± 0.005	5.18 ^{abc} ± 0.237	1.51 ^{a} ± 0.05	93.02 ^{cde} ± 0.19
v. levis	pyriform	7.68–12.50	197.56 ± 21.28	16.71 ^{c} ± 1.55	22.14 ^{a} ± 0.98	0.380	−4.793 ± 0.173	33 ^{bc} ± 4.0	5 ^{bc} ± 1.0	0.13 ^{bc} ± 0.005	4.82 ^{a} ± 0.216	2.17 ^{bcd} ± 0.15	95.34 ^{ef} ± 0.025
n. minor	pyriform	5.02–7.18	36.15 ± 1.67	7.23 ^{ab} ± 0.60	28.03 ^{b} ± 0.85	0.272	−8.87 ± 0.440	227 ^{d} ± 16.0	44 ^{d} ± 2.0	0.16 ^{de} ± 0.004	5.44 ^{bc} ± 0.104	2.25 ^{cd} ± 0.15	90.58 ^{ab} ± 0.37
n. spinosum	round–pyriform	7.62–15.82	310.00 ± 33.59	24.66 ^{d} ± 1.93	35.24 ^{e} ± 0.76	0.579	−5.553 ± 0.538	68 ^{bc} ± 17.0	13 ^{bc} ± 3.0	0.12 ^{ab} ± 0.004	5.05 ^{abc} ± 0.283	2.34 ^{cd} ± 0.13	93.86 ^{def} ± 0.020
n. xalapensis	pyriform	7.27–10.75	252.16 ± 16.61	23.50 ^{cd} ± 1.88	32.47 ^{de} ± 0.76	0.666	−11.080 ± 0.416	166 ^{cd} ± 16.0	30 ^{cd} ± 3.0	0.14 ^{bc} ± 0.005	5.01 ^{ab} ± 0.276	1.76 ^{ab} ± 0.12	92.14 ^{cde} ± 1.31
n. maxima	elongated pyriform	12.35–15.99	511.32 ± 13.82	39.26 ^{e} ± 1.83	27.30 ^{b} ± 0.71	0.632	−14.322 ± 3.893	149 ^{cd} ± 9.0	23 ^{cd} ± 3.0	0.10 ^{a} ± 0.004	4.78 ^{a} ± 0.11	2.20 ^{bcd} ± 0.09	96.25 ^{f} ± 0.18
Mean	-	-	227.47	17.21	28.76	0.406	-	0.065	0.012	0.13	5.11	2.21	91.62

Fruit length (range); weight (mean ± SE, n = 20); stomatal frequency (SF) and stomatal size (mean ± SE, n = 30); stomatal index (SI) (mean, n = 20); color index (CO*) (mean ± SE, n = 20); total chlorophyll (C_{a+b}); total carotenoids (C_{x+c}); titratable acidity (TA); total soluble solids (TSS); total sugars and moisture (mean ± SE, n = 9). Different letters in the same column indicate significant differences (Kruskall–Wallis, $\alpha = 0.05$).

The color of the epidermis is one of the most important quality characteristics. The yellow *albus* varietal groups had CO* values between −1.071 and 0.744, the light green *virens levis* varietal group was −4.793 and the dark green *nigrum* varieties values were between −14.322 and −8.87 (Table 1). Total chlorophyll and carotenoid content have a strong correlation with fruit color [39]. In this study, the content of total chlorophyll and carotenoids in the epidermis of chayote had an R^2 correlation of 0.92 and 0.86 with the color index, respectively. According to Fu et al. [40], the transcription of genes such as *HCAR* and *CHL1* is involved in chlorophyll synthesis, while *CHY2* influences carotenoid content.

Chlorophylls *a* and *b* are the main photosynthetic pigments; however, the role of carotenoids in photosynthesis is also important because they can absorb light at wavelengths where chlorophylls cannot (400–550 nm) and provide photoprotection to tissues under conditions of high light intensity [41]. The highest amount of chlorophylls and total carotenoids was found in *n. minor* with 0.227 and 0.044 mg g^{-1}, respectively, while the *albus* groups had at most 0.001 mg g^{-1} of both. Cadena-Iñiguez et al. [17] mentioned that the varieties *a. minor*, *a. dulcis* and *a. levis* have up to 10 times less chlorophyll than the varieties *nigrum* and *levis*. The C_a content was higher than that of C_b, indicating that the fruits grew under conditions of higher light intensity [42]. C_b is synthesized from the oxidation of C_a by the action of chlorophyllide oxidase [43].

Low acidity is a characteristic of cucurbits, for example, melon (0.11% acidity) [44], cucumber (0.098%) and watermelon (0.096%) [45]. In all the chayote varieties, the acidity was around 0.13%. The variety *a. dulcis* had the highest acidity with 0.18% and *n. maxima* the lowest with 0.10%. Riviello-Flores et al. [7] determined an acidity of 0.085 and 0.10% for *v. levis* and *n. spinosum* juices, respectively, slightly lower than those obtained in this study.

A high TSS content is associated with a longer shelf life because these reserves allow for the maintenance of respiration intensity. In cucumber, a 5.4% increase in TSS increased the probability of marketability by 1.8 times, i.e., fruits had less chilling damage, wilting, color loss and disease incidence. On average, chayote contains 5.11 °Bx, slightly higher than cucumber with 3.0 to 4.0 °Bx [46] and zucchini (*Cucurbita pepo* L.) with 3.76 °Bx [47]. In this study, the variety *a. dulcis* had the highest amount with 5.56 °Bx and *n. maxima* the lowest with 4.78 °Bx.

Fructose and glucose are the major components of total sugar in chayote [13]. Verma et al. [48] characterized 74 chayote accessions, which contained 1.09 to 2.94% total sugars, and these values are similar to those obtained in this study, which ranged from 1.51 to 2.55%. Other cucurbits are reported to have higher sugar content, such as melon with 3.85–8.5% [49], pumpkin 9.39–10.49% [50] and watermelon 7.27–11.38% [51], but cucumbers are similar to chayote with 2.87–4.72% [52].

Fresh fruits and vegetables are highly perishable due to their high water content and active metabolism after harvest [53]. Tissue turgidity and a high rate of transpiration make them susceptible to cuticle damage and conidia germination causing disease [54]. On average, chayote had moisture contents above 90%; the highest content was found in *n. maxima* with 96.25%, *v. levis* with 95.34% and some morphotypes of *n. spinosum* and *n. xalapensis* with amounts above 95.0%. The lowest content was found in *a. dulcis* with 81.23%. The humidity of chayote is very close to that of other cucurbits such as cucumber, which contains 95.10 to 96.26% [47] and watermelon with a content of 90.35 to 92.41% [55].

According to the dendrogram (Figure 2), there were two main groups for chayote fruits: one group was formed by the light–dark green varieties and the other by yellow *albus* varieties. Also, there were four distinguished groups. In group I, there is only the varietal group *n. maxima* of very large size and very elongated shape, with the lowest color, acidity and TSS and the highest moisture content. Group II consists of the varieties *v. levis*, *n. minor*, *n. spinosum* and *n. xalapensis*, generally pyriform in shape, light and dark green in color, higher pigment content and medium-high moisture content. In group III,

a. dulcis can be observed, which differs from the other *albus* varietal groups because it has the lowest moisture, slightly elongated pyriform shape and the highest TSS content and acidity. Finally, group IV includes the varieties *a. levis gigante*, *a. levis*, *a. spinosum* and *a. minor*, characterized by their yellow color and a color index close to 0, medium moisture content, very low chlorophyll and total carotenoids, generally higher TSS and total sugar content, and low stomatal index.

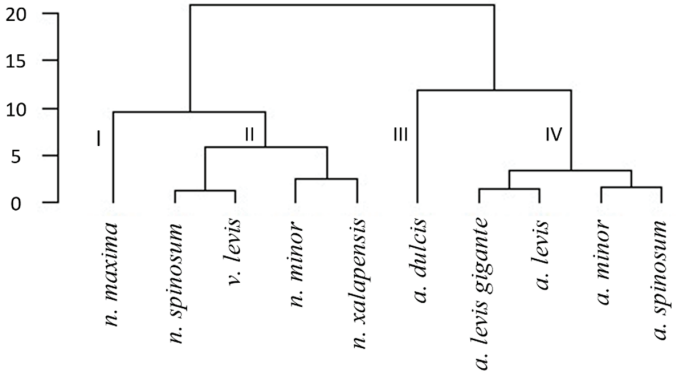

Figure 2. Cluster dendrogram of the physicochemical and morphological characteristics of 10 varietal groups of chayote [*Sechium edule* Jacq. (Sw.)]. Roman numerals I–IV indicate the groups similarity among chayote varieties.

3.2. Postharvest Characterization

Chayote is a non-climacteric fruit with no significant changes in organoleptic characteristics after harvest and during storage. In Figure 3, the varieties are grouped according to the main postharvest disorders; *a. minor* and *n. minor* are similar due to rapid weight loss and dehydration of the fruit. The varieties *a. levis*, *a. dulcis*, *a. levis gigante* and *a. spinosum* had a high disease incidence, which causes darkening of the exocarp, while *n. xalapensis* and *n. spinosum* were susceptible to viviparism and fungal incidence, and *v. levis* and *n. maxima* were highly susceptible to blistering and viviparism.

At room temperature, the shelf life of chayote was short, from 3 days for *a. minor* to 11 days for *v. levis*. Díaz-Perez et al. [56] determined that when cucumber loses between 5 and 6% of its weight, the probability of being marketed is reduced by 50%, and when it loses more than 10%, this probability is 0%. In this work, it was determined that a weight loss of more than 10% was critical because at this level the fruits showed signs of evident dehydration and oxidation of the epidermis, although in *a. minor* and *n. minor*, these affectations were evident with losses of 6%. Weight loss during the storage of chayote in general was very high and increased with time ($p < 0.001$), losing between 6.29 and 22.53% in 8 days and from 11.48 to 35.57% in 14 days with a daily loss ranging from 0.82 to 2.38% (Table 2). Lower weight losses were found for other non-climacteric fruits like mandarin (*Citrus reticulata Blanco*) [57] or prickly pear (*Opuntia Ficus-indica* (L.) Mill.) [58]. On the contrary, pumpkin, due to its hard epicarp, lost 2.33% after 42 d of storage at 23 °C and 45% HR [59].

Weight loss is caused by metabolic processes such as respiration and transpiration. Larger fruits lose less weight than smaller ones [60]; in this case, the fruits of *n. maxima* and *a. levis gigante* and *v. levis* lost significantly ($p < 0.01$) less weight than very small fruits such as *n. minor* and *a. minor*. The storage at 21 °C and 70% RH investigated in this study corresponds to the conditions under which the chayote fruits are marketed by local producers; changes in relative humidity can affect the quality of the fruits, due to water vapor pressure deficit. For example, pomegranate fruits (*Punica granatum*) stored at 20 °C and 65% RH lost more than 29% of their original weight, while fruits stored at 95% RH lost

only 5.79% because this reduces the water vapor pressure deficit between the fruit and the environment [61].

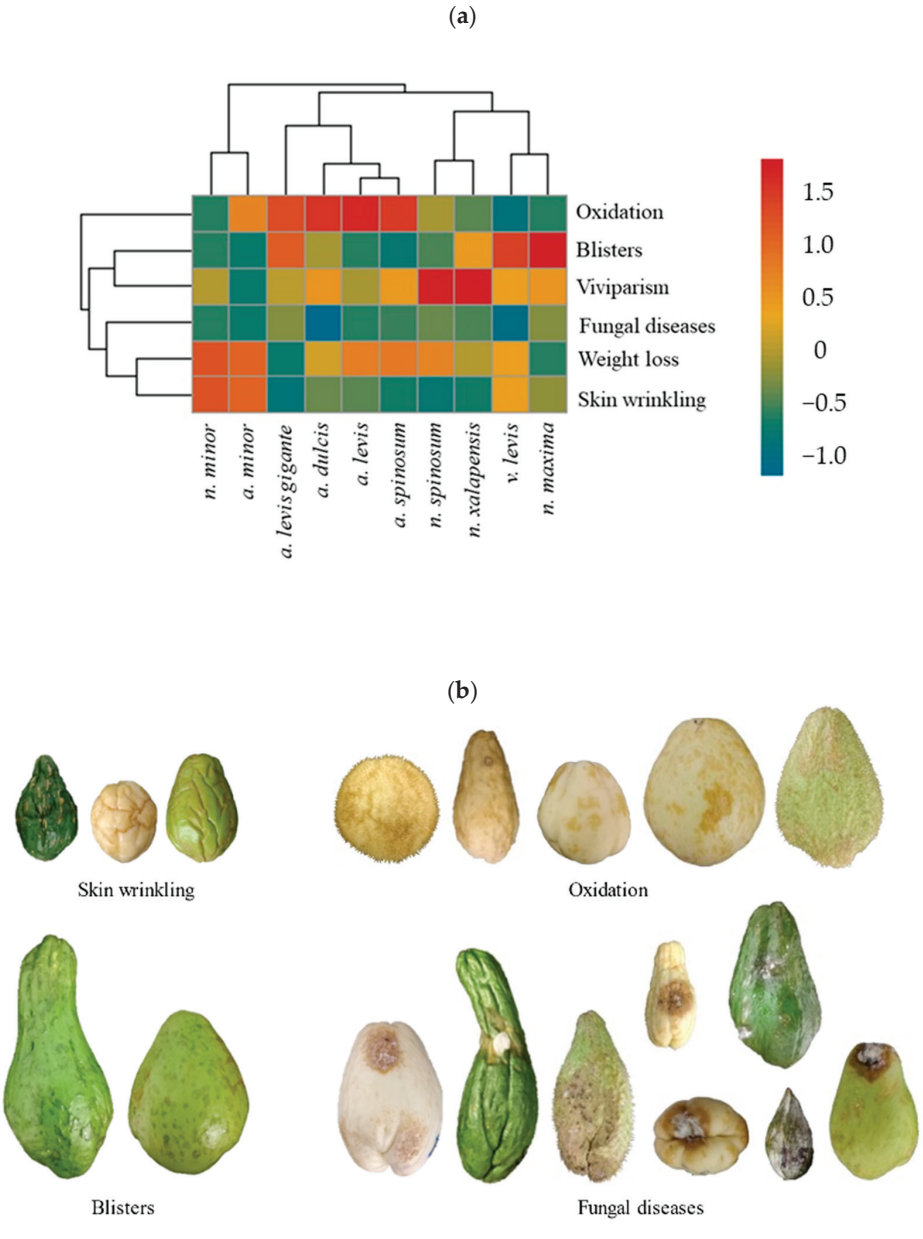

Figure 3. (**a**) Hierarchical map of postharvest disorders that initially affect the loss of quality of 10 varietal groups of chayote [*Sechium edule* Jacq. (Sw.)]; (**b**) Fruit chayote varieties with different symptoms and disorders that can affect their organoleptic quality.

Table 2. Weight loss (%) and shelf life (days) of 10 varietal groups of chayote [*Sechium edule* Jacq. (Sw.)] in storage at room temperature (21 °C and 70% RH) (n = 15 ± SE).

Varietal Group	Weight Loss		Shelf Life (Days)	Pathogen Isolated
	Daily (%)	Level		
n. minor	2.38 ± 0.13	High	4	*Colletotrichum* sp., *Fusarium* sp.
a. levis gigante	0.82 ± 0.03	Low	10	*Colletotrichum* sp., *Phoma* sp.
n. spinosum	1.62 ± 0.08	Medium	7	*Colletotrichum* sp., *Fusarium* sp., *Phoma* sp., *Alternaria* sp., *Aspergillus niger*
n. xalapensis	1.29 ± 0.06	Medium	9	*Phoma* sp., *Colletotrichum* sp., *Fusarium* sp., *Alternaria* sp., *Aspergillus niger*
a. levis	1.34 ± 0.08	Medium	7	*Phoma* sp.
a. dulcis	1.56 ± 0.09	Medium	7	*Phoma* sp., *Colletotrichum* sp.
n. maxima	1.07 ± 0.06	Low	9	*Phoma* sp., *Colletotrichum* sp.
a. minor	2.54 ± 0.15	High	3	No incidence
a. spinosum	1.45 ± 0.10	Medium	7	No incidence
v. levis	0.87 ± 0.3	Low	11	*Colletotrichum* sp., *Phoma* sp., *Alternaria* sp., *Epicoccum* sp.

High temperature and rainfall during chayote harvest season favors fungal attack and the presence of blisters, which affect the quality of the fruit. The infection can occur in the field or in the packinghouse; fungi such as *Colletotrichum*, *Fusarium*, *Geotrichum*, *Phytophthora*, *Didymella* and *Chaetomium* have been reported [62]. In this study, *Phoma* and *Alternaria* were also identified in most of the infected fruits (Table 2). In chayote fruits, it has been reported that brown rot is caused by *Fusarium citri* [63]. Blisters or bladders significantly affect the fruits of *v. levis*, *a. levis gigante*, *n. maxina* and *a. dulcis*; spines help to hide blisters in *spinosum* genotypes. It is generally accepted that blisters are caused by *Colletotrichum* sp. [62,64].

The premature germination of the seed inside the fruit is called viviparism, and in chayote, it is a major problem during storage and retail. It is especially important in *n. spinosum* and *n. xalapensis*, where around 50% of the fruits showed an exposed seed before day 10 after harvest and was higher than in *v. levis* (Figure 4). Previous studies showed that at harvest, the seed in *v. levis* is about 25% of its development and reaches physiological maturity on day 10 with the growth of the embryonic axis, leading to fruit opening [65]. During this stage, there may have been an increase in the activity of enzymes related to cell wall degradation metabolism such as α-galactosidases, β-1,3-glucanases, polygalacturonases and endotransglycosylases, which weaken the cellulose microfibrils and allow radicle protrusion. However, in this study, the germination of small fruits such as *a. minor* and *n. minor* did not appear during storage. Cucurbitaceae such as *Lagenaria siceraria* (Molina) also show viviparity at harvest, which increases to 97.84% during a season of heavy rainfall [66]. According to the above, as in fungal infections and blistering, viviparism also increases during the rainy season [63].

Increased α-amylase activity allows the hydrolysis of stored starch to form α-maltose and α-glucose, necessary to provide energy for germination [67]. During the first three days of storage, α-amylase activity remained constant in *n. maxima* and *v. levis*, decreased in *n. spinosum* and increased significantly in *n. xalapensis*. From day 3 onward, activity increases as viviparity increases. Interestingly, the activity in *n. xalapensis*, which showed the highest increase on day 3, was reduced on days 5 and 7 (Figure 5).

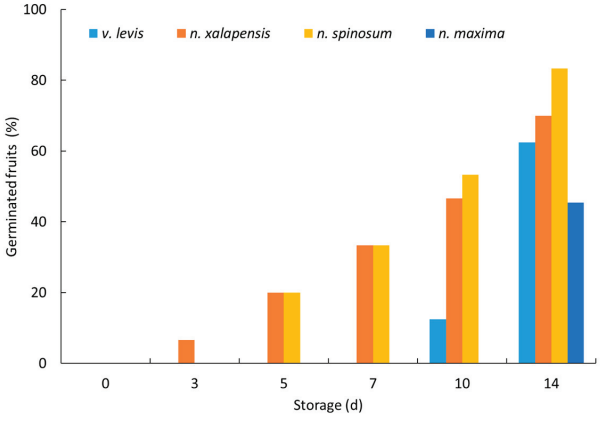

Figure 4. Percentage of germinated fruits of chayote [*Sechium edule* Jacq. (Sw.)] var. *v. levis*, *n. xalapensis*, *n. spinosum* and *n. maxima* at days 0, 3, 5, 7 and 14 in storage at room temperature (21 °C and 70% RH) (n = 15).

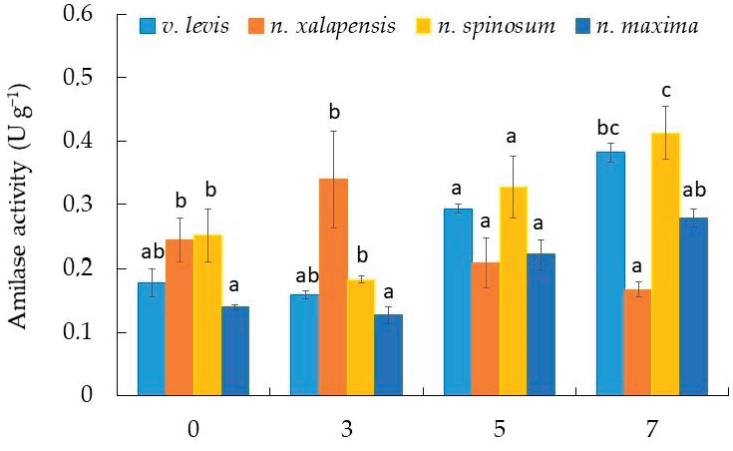

Figure 5. Activity of α-amylase of chayote [*Sechium edule* Jacq. (Sw.)] var. *v. levis*, *n. xalapensis*, *n. spinosum and n. maxima* at days 0, 3, 5, and 7 in storage at room temperature (21 °C and 70% RH). Different letters on the same day indicate significant differences between means according to Kruskall–Wallis (α = 0.05) (n = 6 ± SE).

There is a complex hormonal relationship in the regulation of viviparity, abscisic acid (ABA) being a negative regulator and gibberellins a positive regulator. The expression of α-amylase-related genes is activated in the presence of gibberellic acid and negatively regulated by the presence of sugars in the embryo [68]. Similarly, the increase in α-amylase activity is related to an increase in ethylene production. In banana, transcription factors involved in the ethylene signaling pathway, such as *MaEIL2*, induce the transcription of the *MaAMY3* and *MaISA2* genes coding for α-amylases and isoamylases, increasing their activity and thus starch degradation [69].

3.3. Effect of 1-MCP on Chayote Fruits

The variables of weight loss and chilling injury are shown in Table 3 and Figure 6. Temperatures less than 10 °C usually cause CI in tropical and subtropical crops. Cucumber fruits are susceptible to CI at 5 °C [70], while zucchini fruits show symptoms of chilling injury from day 3 at 1 °C [71]. In this study, storage at 8.7 °C and 95% RH for 2 weeks

caused CI in every varietal group. This agrees with previous studies that mention that chayote fruits *v. levis*, *n. spinosum* and *n. xalapensis* are susceptible to chilling injury when stored at low temperatures [12,13]; nevertheless, no reports are found for the other varieties.

Table 3. Effect of 1-MCP on weight loss (% WL) and CI index (0–3) after 2 weeks of cold storage (8.7 °C, 95% RH) for *n. minor*, *a. minor* and *v. levis* (B) and 3 weeks for *a. levis*, *a. dulcis* and *v. levis* (A).

Treatment		*n. minor*	*a. minor*	*v. levis* (*B*)	*a. levis*	*a. dulcis*	*v. levis* (**A**)
Control	%WL	17.20 ± 0.77 b *	18.99 ± 0.819 b	13.29 ± 0.55 b	14.34 ± 1.62 a	17.91 ± 1.324 b	10.22 ± 0.59 b
	CI	2.2	2.2	1.3	2.3	2.2	2.2
1-MCP	%WL	10.97 ± 0.76 a	13.52 ± 0.600 a	9.25 ± 0.50 a	11.36 ± 0.91 a	14.40 ± 0.598 a	6.62 ± 0.32 a
	CI	0.9	1.1	0.7	1.6	1.2	1

* Means ± SE with different letters in the same column for % WL are significantly different according to Kruskall–Wallis (α = 0.05) (n = 15 ± SE).

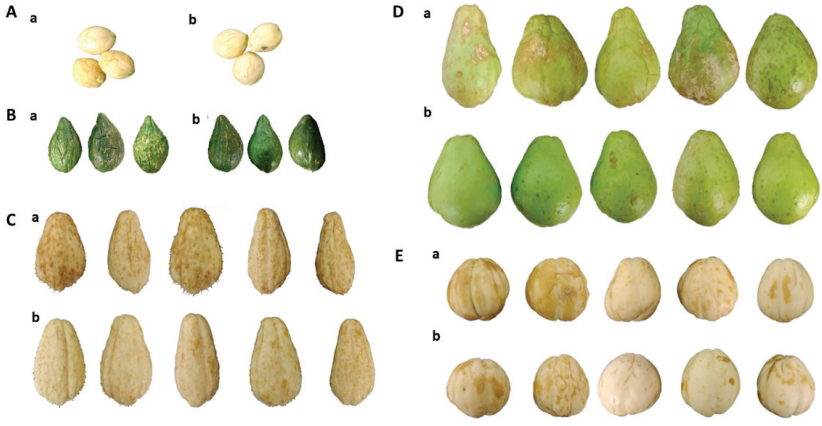

Figure 6. Effect of 1-MCP on the commercial quality of chayote fruits after refrigerated storage (8.7 °C, 95% RH). Letters show the treatments: (**a**) control and (**b**) 1-MCP treatment. The cold storage for 2 weeks are (**A**) *a. minor* and (**B**) *n. minor*. The storage for 3 weeks are (**C**) *a. dulcis*, (**D**) *v. levis* (**A**,**E**) *a. levis*.

1-MCP significantly reduced weight loss in all the chayote varieties except *a. levis* (Table 3). It has been proven that 1-MCP reduces fruit metabolism, such as respiration, transpiration and ethylene production reducing enzyme activity and starch degradation [72]. In tomato, 1 μL L^{-1} 1-MCP induced wax and cutin biosynthesis affecting the gene expression for cuticle formation, reducing fruit transpiration [73]. Recent research reports that the effectiveness of 1-MCP is based not only on the fact that it blocks the ethylene receptors but also affects biosynthesis due to its affinity to the active site of ACC oxidase [74].

The symptoms of CI in chayote fruit appear as dark brown spots and slight sinking and drying of the epidermis, which occur in the first week in *albus* varieties. In the fruits stored for 2 weeks, *v. levis* (B) had a lower CI than *n. minor* and *a. minor*, but after 3 weeks of storage, this disorder was similar within varieties. CI is associated with the accumulation of ROS (reactive oxygen species), which leads to lipid peroxidation, causing damage to the cell membrane and higher weight loss [75]. The composition of the plasma membrane of cells is related to cold tolerance, with saturated fatty acids solidifying faster than unsaturated fatty acids when exposed to low temperatures. Changes in membrane fluidity lead to biochemical disorders such as ion leakage, oxidative stress and energy imbalance [76]. The reduction in CI and weight loss by 1-MCP can be explained by the maintenance of cell wall integrity. Lin et al. [77] showed that applications of 1.2 μL L^{-1} of 1-MCP for 12 h delayed the softening of plums (*Prunus salicina* Lindl. cv. *Younai*), by decreasing the activity

of pectinesterase, polygalacturonase, cellulase and β-galactosidase, preventing the wall disassembly. In addition to cell wall strengthening, 1-MCP stimulated stress tolerance mechanisms by maintaining increased activity of the enzymatic antioxidant system, such as superoxide dismutase, catalase, ascorbate peroxidase and glutathione reductase, reducing chilling injury in nectarine [*Prunus persica* (L.) Batsch, var. *nectarine*] [78].

Due to the high content of chlorophylls, the *nigrum* chayote groups are a good option for markets that demand colorful fruits with health benefits. Larger VGs such as *n. maxima*, *a. levis gigante* and *v. levis* and morphotypes of *n. spinosum* can be used for agroindustrial process to obtain purees. On the other hand, the more sweet-tasting *albus* groups can be used as a minimally processed vegetable or desserts.

4. Conclusions

The postharvest behavior of the chayote varies depending on the cultivar. The smallest fruits like *a. minor* and *n. minor* showed high weight loss and exocarp wrinkling, while the *albus* chayotes like *a. levis*, *a. dulcis*, *a. levis gigante* and *a. spinosum* had a high oxidation index. On the other hand, in *n. xalapensis* and *n spinosum*, viviparity and fungal attack are the main problems, while *v. levis* and *n. maxima* are highly susceptible to blistering and viviparism. 1-MCP managed to reduce chilling injury, weight loss and evident dehydration during cold storage. This treatment can be practically used to preserve the commercial quality of *S. edule* fruit varieties.

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Non-Destructive Testing of the Internal Quality of Korla Fragrant Pears Based on Dielectric Properties

Yurong Tang [1], Hong Zhang [2], Qing Liang [1], Yifan Xia [1], Jikai Che [1] and Yang Liu [1,*]

[1] College of Mechanical Electrification Engineering, Tarim University, Alaer 843300, China; 120110010@taru.edu.cn (Y.T.); liangqing0406@163.com (Q.L.); tdxyf8@163.com (Y.X.); chejikai314159@163.com (J.C.)
[2] College of Water Resources and Architectural Engineering, Tarim University, Alaer 843300, China; 120230116@taru.edu.cn
* Correspondence: 120150012@taru.edu.cn; Tel.: +86-187-9990-1500

Abstract: This study provides a method for the rapid, non-destructive testing of the internal quality of Korla fragrant pears. The dielectric constant (ε') and dielectric loss factor (ε'') of pear samples were tested at 100 frequency points (range = 0.1–26.5 GHz) using a vector network analyzer and coaxial probe. The variations in the dielectric parameters of fragrant pears were analyzed. The linear relationships between the dielectric parameters and internal quality were explored. Internal quality prediction models for Korla fragrant pears were built using partial least squares regression (PLSR), support vector regression (SVR) and particle swarm optimization–least squares support vector regression (PSO-LSSVR). The optimal model was then determined. There was a weak correlation between the dielectric parameters and soluble solid content (SSC) under a single frequency. The model based on PLSR and using ε' as a variable predicted hardness the best, while the model based on PLSR using ε'' as a variable predicted SSC the best. Its R and MSE values were 0.77 and 0.073 in hardness prediction, respectively, and 0.91 and 0.087 in SSC prediction. This study provides a new method for the non-destructive online testing of the internal quality of Korla fragrant pears.

Keywords: Korla fragrant pears; non-destructive testing; dielectric properties; internal quality

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1. Introduction

Korla fragrant pears are in high demand for their thin pericarp, rich juice, sweet taste and crisp texture [1,2]. Korla fragrant pears were listed by China as a national product of geographical indication [3]. The hardness and soluble solid content (SSC) are the most important internal qualities of pears and are often used as indicators of the quality of Korla fragrant pears [4]. At present, the common apparatus used to measure the hardness and SSC of fragrant pears include fruit hardness testers and sugar detectors [5,6]. Although such methods can provide accurate results, they cause damage that cannot be repaired. Hence, it is difficult to use them for research and for developing online methods of detecting the internal quality and grade of fragrant pears, which significantly restricts their industrial development. Accordingly, developing a non-destructive and high-efficiency internal quality detection technique for fragrant pears is crucial in achieving the online detection of internal quality and for guiding sorting processes during and after harvest.

Dielectric property detection is an emerging technology that is rapid, sensitive, simple and non-destructive [7]. Based on internal physiological and chemical changes in response to an applied electric field, dielectric property technology has been applied to the quality detection of various agricultural products. For example, Guo tested the moisture content, hardness and SSC of apples, Nelson tested the moisture content and SSC of watermelons and Lan measured the SSC of fragrant pears [8–10]. However, there is a poor linear relationship between the dielectric properties and quality indicators when a single frequency is used for detection, resulting in difficulties in predicting fruit quality. Hence, Shang et al.

and Guo et al. constructed sugar content models of nectarines and apples based on a combination of dielectric constants under multiple frequencies and machine learning [11,12]. Both achieved good prediction results. Therefore, it is feasible to build models of fruit quality indicators based on a combination of dielectric properties under multiple frequencies and machine learning. In many machine learning methods, partial least squares regression (PLSR), support vector regression (SVR) and particle swarm optimization–least squares support vector regression (PSO-LSSVR) models have strong generalizability and predictive accuracy. They can handle large, complex datasets and identify patterns within them. They have been widely applied to the prediction of fruit quality, such as the hardness and SSC of Dangshansu pears, the SSC of apples, and the price of agricultural products [13–15]. Past research has provided a theoretical direction for the quality prediction of fragrant pears based on PLSR, SVR and PSO-LSSVR models. Nevertheless, there has been little research on predicting the hardness and SSC of fragrant pears based on a combination of dielectric properties under multiple frequencies and machine learning methods (PLSR, SVR and PSO-LSSVR).

This study tested the dielectric constant (ε') and dielectric loss factor (ε'') of Korla fragrant pear samples at 100 frequency points over a range of 0.1–26.5 GHz using a vector network analyzer and coaxial probe. The test records variations in these dielectric parameters with frequency. Moreover, the linear relationships between the dielectric parameters and internal quality were identified. Additionally, hardness and SSC models of fragrant pears were built using PLSR, SVR and PSO-LSSVR. The performance of these models was compared and the optimal model was verified. Finally, the internal quality of fragrant pears was predicted accurately.

2. Materials and Methods

2.1. Test Materials

Sampling of Korla Fragrant Pears

Korla fragrant pear samples were collected from a conventional pear orchard in Shilian, Shituan, Alear City, which is a high-quality fragrant pear production base in Southern Xinjiang, China. They were harvested on 1 and 8 October 2023. Only pears without implicit damage or pest/disease damage were used. The average weight was 115 g, with a deviation of $\pm$10 g. A total of 110 pears were collected on each date, making a total of 220. Among them, 110 were used for model construction and the rest were used for model verification. The pears were cleaned with water after harvest and then dried for the testing of the dielectric properties.

2.2. Measurement Methods

2.2.1. Measurement of Dielectric Parameters

The dielectric parameters (ε' and ε'') were measured using a vector network analyzer (3671D, Kesiyi Science and Technology Co., Ltd. of China Electric Equipment Group, Qingdao, China; Figure 1). Specifically, ε' refers to the dielectric media's capacity to store an electric field energy, and ε'' reflects the energy lost from the dielectric media in an alternating electric field [16,17]. Before the test, the vector network analyzer and coaxial probe were preheated for 1 h and connected through a coaxial cable. Later, they were calibrated with an open circuit, short circuit, and loading standard components. Finally, the measurement frequency range was set from 0.1 to 26.5 GHz. A total of 100 frequency points were chosen for measurement by using the method of equally spaced measurements in a logarithmic coordinate system.

The dielectric parameter test of fragrant pears was carried out in the Textile Engineering Laboratory of Xinjiang Tarim University at room temperature (mean = 15 °C). During measurement, the coaxial probe was kept static and the pears were placed horizontally on a lifting platform with an adjustable height. The pericarp was placed in contact with the probe. Three points were selected on the equator of each pear at intervals of about 120° to measure ε' and ε''. These three points were marked for the follow-up measurement of the

quality indicators. Each group of tests was repeated three times and the means of the test data were recorded.

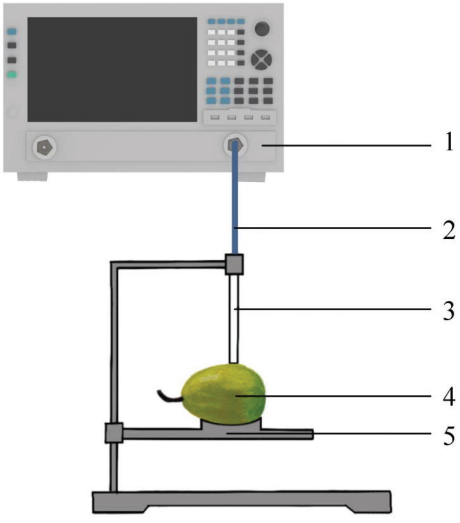

Figure 1. Dielectric property testbed for the fragrant pears. 1. Vector network analyzer; 2. connecting cable; 3. coaxial probe with an open end; 4. fragrant pears; 5. lifting platform.

2.2.2. Measurement of Hardness

The fruit hardness of fragrant pears was tested using a GY-1 fruit hardness meter. The pericarp at the dielectric measurement points was peeled off with a knife. The hardness meter was held perpendicular to the peeled surface, with its indenter pressed into the pear uniformly. The indenter stopped upon reaching a 10 mm depth and the hardness value was recorded. The reset button was rotated after each measurement to return the pointer to the initial scale line. The average hardness at three points was used (kg/cm^2).

2.2.3. Measurement of SSC

The SSC was tested using a B32T portable sugar refractometer. It was calibrated by making the bright–dark boundary of the viewfinder overlap with the zero scale line. Pulp with pericarp was collected near each of the three measurement points and squeezed manually to make juice drop onto the center of the mirrored surface of the refractometer for observation. The SSC value was read from the scale at the bright–dark boundary line. The refractometer was calibrated with distilled water after each measurement. The average SSC at the three points was used (%).

2.2.4. Data Assignment

The dielectric parameters, hardness and SSC of 220 pear samples were measured using the above method. The data of 110 pears were randomly selected for model construction, and the data for the other 110 pears were used for model verification.

2.3. Modelling Methods

Three modelling methods, PLSR, SVR and PSO-LSSVR, were applied to build predictive models of the pear quality indicators. The model input variables were ε' and ε'' and the output variables were the hardness and SSC.

2.3.1. PLSR Model

PLSR is a multivariate statistical method that combines multiple linear regression, principal component analysis and typical correlation analysis [18]. PLSR aims to solve

the multicollinearity problem between independent and dependent variables. It extracts information on the independent and dependent variables by establishing components, thus realizing the goal of regression modelling. PLSR recognizes the direction of the maximum covariance between the predicted and response variables by projecting them into a new space. Then, the predicted variables most highly correlated with the response variables are extracted. These components not only combine the predictive variable information but also have very strong correlations with the response variables [19]. The PLSR algorithm calculates the scores, weights and loads of the components through iteration, thus obtaining the regression coefficients.

2.3.2. SVR Model

SVR is a machine learning method that is used to solve regression problems [20]. It seeks a hyperplane to fit data by mapping the original data onto a high-dimensional characteristic space, aiming to minimize the distance from the data points to the hyperplane [21]. Hence, the function can give a prediction of the input data as precisely as possible. SVR models are composed of an input layer, hidden layer and output layer. The input layer is responsible for generating a sample set. The sample input is generated by nodes. The hidden layer is responsible for the inner product operation. The output layer is responsible for weighting the operation values, thus obtaining the decision-making function. To process noise and uncertainty in regression problems, SVR introduces an insensitive loss function (ε). In other words, when the gap between the predicted and actual values is lower than the threshold ε, the prediction is accurate and no loss is generated.

2.3.3. PSO-LSSVR Model

Particle swarm optimization (PSO) is an optimization algorithm based on swarm intelligence. In the PSO algorithm, each solution is viewed as a "particle" in the search space. Each particle has a fitness value, which is calculated by the optimization function. Particles move in the search space and search for better solutions by updating their speeds and positions continuously. The PSO algorithm has the characteristics of easy operation and fast convergence [22,23]. It has been extensively applied in various fields, such as function optimization, neural network training, pattern recognition, image processing, and so on.

The least squares support vector regression (LSSVR) is a regression method based on the support vector machine [24]. LSSVR is a deformation algorithm of SVR. It transforms the loss function from an error sum into a square sum of errors by introducing it into a least squares linear system. It also changes the solving algorithm from a convex quadratic optimization equation into a linear equation set. In addition, the number of solving variables decreases from $2n + 1$ to $n + 1$ (where n is the number of training samples). In this way, it decreases the calculation load significantly.

Therefore, the PSO and LSSVR were combined to determine the optimal parameter values of the LSSVR model. This can improve the learning and generalization capacities of the model and its predictive accuracy.

2.4. Model Evaluations

The predictive performance of the models was evaluated in terms of the mean square error (MSE) and correlation coefficient (R). Generally speaking, a high-precision model should have a low MSE and high R-value. The formulas for calculating R and MSE are as follows:

$$R = \frac{\sqrt{\sum_{j=1}^{N} \left(M_j - T_j\right)^2}}{\sqrt{\sum_{j=1}^{N} \left(M_j - \overline{T_j}\right)^2}} \quad (1)$$

$$MSE = \sum_{j=1}^{N} \frac{(M_j - T_j)^2}{N} \quad (2)$$

where M_j and T_j are the prediction and measured values of the data j, respectively; $\overline{T_j}$ is the mean value of the measured values of the data j; and N refers to the total amount of data.

3. Results and Analysis

3.1. Linear Correlation between Dielectric Parameters and the Internal Quality of Fragrant Pears

The means and standard deviations of the ε' and ε'' values of the 110 pears used for model construction and measured at 100 frequencies (0.1–26.5 GHz) are shown in Figure 2. The error bar was the mean ± standard deviation. It can be seen from Figure 2 that ε' declines gradually with increases in the frequency, with a greater decline in the low-frequency range. Within the frequency range of 0.1–2.33 GHz, ε'' declines rapidly with increases in the frequency up to 2.33 GHz, and then increases. There are some deviations in the electrical parameters of the 110 fragrant pears at each frequency. Pear fruits are living organisms that decompose respiratory substrates such as starches, sugars and organic acids into CO_2 and water through respiration. Changes in the composition of water and other substances affect the spatial charge distribution inside the fruit [7]. The distribution and intensity of the bioelectric field affect the dielectric properties of fruit on a macroscopic level. Therefore, there is an inevitable relationship between the internal quality of the fruit and the macroscopic dielectric properties. The variation in the internal quality of 110 fragrant pears leads to differences in the dielectric properties at every frequency.

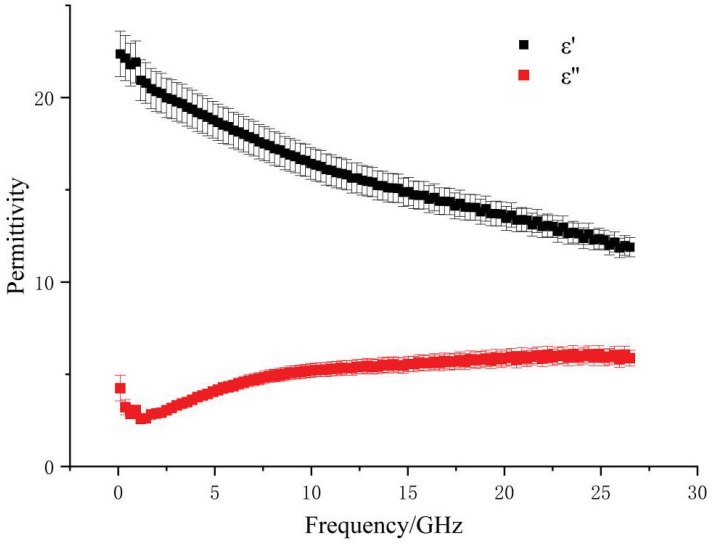

Figure 2. The standard deviation (mean ± standard deviation) of ε' and ε'' for the 110 pear samples was obtained at 100 frequencies from 0.1 MHz to 26.5 MHz.

The Pearson correlation analysis of ε' and ε'' with the hardness and SSC at different frequencies is shown in Figure 3. The Pearson correlation coefficient is a form of statistical data used to measure the degree of linear correlation between two variables, with values ranging from −1 to 1. When the value of the Pearson correlation coefficient is close to 1, it indicates a strong positive correlation between the two variables. When the value is close to −1, it shows a strong negative correlation, and when the value is 0, it indicates that no linear correlation exists between the two variables [25,26].

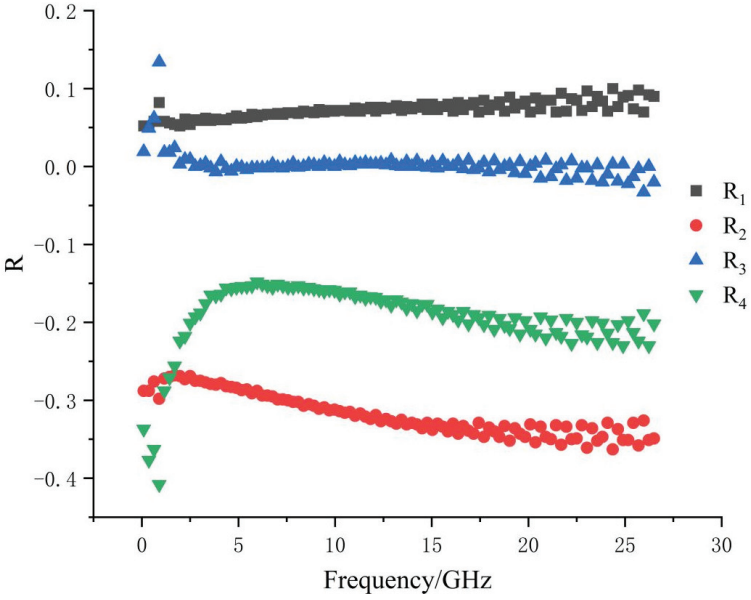

Figure 3. Linear relationships of ε' and ε'' with the hardness and SSC of fragrant pears at different frequencies. Note: R_1 represents the linear correlation coefficients of ε' with hardness. R_2 represents the linear correlation coefficients of ε' with SSC. R_3 represents the linear correlation coefficients of ε'' with hardness. R_4 represents the linear correlation coefficients of ε'' with SSC.

Specifically, R1 and R3 are the linear correlation coefficients of ε' and ε'' with hardness, respectively, and R2 and R4 are those with SSC. It can be seen from Figure 3 that ε' and ε'' are positively related to hardness but negatively related to SSC. The absolute values of all correlation coefficients are <0.4, indicating that the dielectric parameters have weak correlations with the hardness and SSC under a single frequency. Therefore, it is very difficult to predict the hardness and SSC of fragrant pears based on dielectric parameters under a single frequency. This is consistent with the research of Guo et al. [13]. When dielectric characterization techniques are used to detect fruit quality indicators, the ability of different measurement frequencies to penetrate the fruit varies. A single frequency cannot obtain the fruit's spatial information. Multi-frequency dielectric parameters are used to detect the quality of fruit to ensure the penetration performance of the fruit and to obtain sufficient spatial information [27]. Hence, the hardness and SSC should be predicted by dielectric parameters under multiple frequencies.

3.2. Sample Division

The 110 samples were divided randomly into a training set (77 pears) and test set (33 pears) at a ratio of 7:3. The training set was used to determine the intrinsic laws of the data to build the training model, and the test set was used to verify the prediction accuracy of the training model. To improve the training model's prediction ability, the training set sample interval is usually divided into the maximum range. The minimum value of the training set sample should be smaller than that of the test set and the maximum value of the training set sample should be larger than that of the test set [13]. This process indicates that the sample division is reasonable.

The minimum, maximum, mean and standard deviation of the hardness and SSC for the whole pear sample are listed in Table 1. The maximum and minimum hardness values are 7.00 kg/cm^2 and 4.06 kg/cm^2, respectively, a difference of 2.94 kg/cm^2. The maximum and minimum SSC are 14.80% and 10.97%, a difference of 3.83%. These results show that

the sample had relatively wide ranges of hardness and SSC. The minimum hardness and SSC of the training set were both lower than those of the test set, while the maximum values were higher. This reveals that the sample division was reasonable.

Table 1. Statistics of fragrant pear sample division.

Quality Indicators	Sample Set	Minimum	Maximum	Mean ± Standard Deviation
Hardness	Total sample	4.06	7	5.46 ± 0.50
	training set	4.06	7	5.44 ± 0.49
	test set	4.58	6.44	5.50 ± 0.52
SSC	Total sample	10.97	14.80	12.65 ± 0.65
	training set	10.97	14.80	12.62 ± 0.65
	test set	11.70	14.33	12.72 ± 0.65

3.3. Prediction of Hardness

The ε' and ε'' variables were used as model input parameters, with hardness used as the output variable. A total of 110 data points were randomly distributed into training and test sets at a ratio of 7:3 to evaluate the predictive performance of the trained models. The prediction results are listed in Table 2, which shows that in the prediction stage, ε' and ε'' were used as the model inputs. With the PSO-LSSVR and SVR models, the R-value of the training set is relatively high and the MSE value is relatively low, while the R-value of the test set is relatively low and the MSE value is relatively high, showing a poor goodness of fit. This indicates that the PSO-LSSVR and SVR models predict hardness poorly. With the PLSR model, the R and MSE values of the test set differ to some extent. When the input is ε', R = 0.77 and MSE = 0.073, and when the input is ε'', R = 0.64 and MSE = 0.148. The PLSR model using ε' and ε'' as model inputs can predict hardness well. The PLSR model using ε' as input achieves the maximum R value and minimum MSE value. This implies that the trained PLSR model using ε' as an input can predict hardness the best.

Table 2. Comparison of fragrant pear hardness prediction by different models.

Modeling Methods	Modeling Variables	Training Set		Test Set	
		R	MSE	R	MSE
PLSR	ε'	0.82	0.054	0.77	0.073
	ε''	0.81	0.055	0.64	0.148
SVR	ε'	0.74	0.112	0.39	0.233
	ε''	0.96	0.019	0.26	0.328
PSO-LSSVR	ε'	0.79	0.098	0.41	0.220
	ε''	0.89	0.046	0.46	0.300

3.4. Prediction of SSC

The variables ε' and ε'' were used as the model input parameters, while SSC was the output variable. A total of 110 data points were randomly distributed into a training set and a test set at a ratio of 7:3 and input into the trained prediction models. The predicted SSC values are shown in Table 3, which shows that in the prediction stage, ε' and ε'' are used as model inputs. With the PSO-LSSVR and SVR models, the R values of the training set are relatively high and the MSE values are relatively low, but the R-value of the test set is relatively low and the MSE value is relatively high, showing a poor goodness of fit. This shows that the PSO-LSSVR and SVR models poorly predict the SSC. In the PLSR model, the R and MSE values of the test set differ to some extent. When the input is ε', R = 0.84 and MSE = 0.090, and when the input is ε'', R = 0.91 and MSE = 0.087. The PLSR model that uses ε' and ε'' as model inputs can predict the SSC well. The PLSR model using ε'' as

the input achieves the maximum R value and minimum MSE. This implies that the PLSR model using ε'' as an input can predict the SSC the best.

Table 3. Comparison of fragrant pear SSC prediction by different models.

Modeling Methods	Modeling Variables	Training Set		Test Set	
		R	MSE	R	MSE
PLSR	ε'	0.91	0.055	0.84	0.090
	ε''	0.92	0.063	0.91	0.087
SVR	ε'	0.86	0.118	0.27	0.433
	ε''	0.97	0.04	0.15	0.378
PSO-LSSVR	ε'	0.95	0.055	0.45	0.347
	ε''	0.83	0.186	0.40	0.300

Based on the PLSR model, the prediction of hardness and the SSC of fragrant pears was better, while the prediction of the SVR and PSO-LSSVR models was worse. Additionally, the SVR and PSO-LSSVR models showed overfitting problems. To analyze this reason, compared with the PLSR model, the SVR and PSO-LSSVR models have more parameters and higher model complexity, which may not align with the experimental data samples, leading to the overfitting problem. Although the SVR and PSO-LSSVR models can predict the quality of other fruits and vegetables, they are not suitable for predicting the hardness and SSC of fragrant pear.

3.5. Model Verification

The PLSR model can predict hardness and SSC the best. However, the practical performance of PLSR models is still unclear. To verify the actual performance of the optimal predictive model, the dielectric properties and quality indicator data of the rest of the 110 fragrant pear samples were acquired. The dielectric parameters of the 110 fragrant pear samples were loaded as inputs into both trained models, and the predicted values of hardness and SSC were obtained, respectively. Through the linear fitting of the measured and predicted data (PLSR model), the performance of the optimal model was verified (Table 4).

Table 4. Performance verification of the optimal predictive model.

Modeling Methods	Modeling Variables	Quality Indicators	Model Accuracy	
			R	MSE
PLSR	ε'	Hardness	0.76	0.129
PLSR	ε''	SSC	0.85	0.095

The verification experiment shows that the R and MSE values of the PLSR model using ε' as inputs were 0.76 and 0.129 when it was used to predict hardness, showing a relatively high predictive accuracy. The R and MSE values of the PLSR model using ε'' as input were 0.85 and 0.095 when predicting the SSC, showing a relatively high predictive accuracy. In summary, the hardness and SSC can be obtained by inputting dielectric parameters into the trained optimal model. The PLSR model shows good actual performance.

4. Discussion

The results showed that the correlations between the dielectric parameters of the fragrant pear and the hardness and SSC at a single frequency were weak. The skin of the fragrant pear was thin and juicy, and there were many charged particles in it, forming biological electric fields. The distribution and intensity of the electric fields in different fragrant pears were different. During testing, dielectric parameters at a single frequency cannot fully reflect the internal electric field distribution of all fragrant pears [28], which has

significant limitations, resulting in weak correlation. The above problems can be avoided by using dielectric parameters at multiple frequencies. For example, Cao, Lin, Trabelsi et al. [29–31] conducted non-destructive testing of the fruit SSC and hardness. The dielectric parameters were tested at multiple frequencies and good results were obtained. Cavaco, Cruz et al. [32,33] outlined the application of near-infrared spectroscopy in fruit or plant detection. Near-infrared spectroscopy is a feasible method for detecting fruit quality, which has good accuracy and stability in the detection process. However, the hardness and SSC are non-uniformly distributed in the pear. Non-destructive testing techniques based on near-infrared spectroscopy do not have strong penetration capabilities and the required high sample uniformity. Furthermore, the acquisition is susceptible to the influence of the test area. Therefore, techniques with a high penetration depth are essential for acquiring sufficient information to comprehensively and accurately evaluate the internal quality of Korla fragrant pears. As a new high-penetration-depth technique, dielectric property detection has the advantages of simple operation, speed and sensitivity. When Cao et al. [27] used dielectric characteristics to detect the SSC of different varieties of pears, it was found that the effect of electrical detection was better than that of near-infrared spectrum detection. In this paper, the model established with ε' as the variable under the PLSR method has the best performance in predicting the pear SSC, and the model established with ε'' as the variable under the PLSR method has the best performance in predicting the pear SSC. In this case, the predicted hardness R and MSE were 0.77 and 0.073, respectively, and the predicted SSC R and MSE were 0.91 and 0.087, respectively. The results show that a combination of dielectric properties under multiple frequencies and machine learning can effectively predict the SSC and hardness of fragrant pear. In addition, they also reveal that the prediction effect of hardness is slightly worse than that of SSC, which is consistent with the prediction results of Fang et al. [34] when using dielectric parameters to predict the SSC and sugar content of pear fruit. The results of this study can provide a new method for the non-destructive online detection of the internal quality of Korla fragrant pear. Because all the samples used in the test were taken from the same pear garden, and the fragrant pear has a very strong growing region, different growing conditions or different fragrant pear varieties may cause large internal differences in fragrant pear. Whether this method is suitable for other varieties of fragrant pear or for different growing conditions still needs further verification. In the next step, fragrant pears in different growing environments can be collected to verify the model established, and the model can be further optimized to achieve the purpose of effectively predicting the quality of any fragrant pear.

5. Conclusions

With increases in frequency, ε' presents a declining trend while ε'' presents a V-shaped trend. The absolute values of the coefficients of the correlations between the dielectric parameters (under a single frequency) and hardness and SSC were <0.4, indicating weak correlations. The PLSR model using ε' as a variable showed the optimal performance in predicting hardness (R = 0.77, MSE = 0.073). The PLSR model using ε'' as a variable showed the optimal performance in predicting the SSC (R = 0.91, MSE = 0.087). According to the verification results, the PLSR model using ε' as a variable achieves good performance in predicting hardness (R = 0.76, MSE = 0.129). The PLSR model using ε'' as a variable achieves good performances in predicting the SSC (R = 0.85, MSE = 0.095). This study provides a new method for the non-destructive online detection of the internal quality of Korla fragrant pears.

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Sweet Cherry Fruit Firmness Evaluation Using Compression Distance Methods

Maria Karageorgiadou, Maria Rodovitou, Elpida Nasiopoulou, Vaia Styliani Titeli and Michail Michailidis *

Laboratory of Pomology, School of Agriculture, Aristotle University of Thessaloniki, 570 01 Thessaloniki-Thermi, Greece; karageom@agro.auth.gr (M.K.); marirodo@agro.auth.gr (M.R.); elpinasi@agro.auth.gr (E.N.); titelivg@agro.auth.gr (V.S.T.)
* Correspondence: msmichai@agro.auth.gr

Abstract: Flesh firmness in sweet cherries is determined using the measurement of normalized deformation force, i.e., determining the required force for a distance equal to 5 or 10% of the diameter of the cherries per millimeter. However, a firmness method involving a defined distance is quite simple and suitable for easy applications. Hence, our study focuses on the impact of fruit physiology under various and fixed distances. To assess the firmness evaluation, two sweet cherry cultivars (Canada Giant and Regina) were selected and subjected to three different levels of compression distance equal to 1%, 5%, 10% of the fruit's small thickness dimension along with a consistent compression distance of 0.16 mm. There was a strong correlation between panelists' preferences and the fruit that had been subjected to both a 1% deformation force and a fixed distance of 0.16 mm within each cultivar. Physiological traits, membrane integrity, and the metabolome of the fruit in these categories were mostly unaffected by the control (0%), or 1%, deformation force, as shown by clustering and PCA analysis. The control and 1% deformation force groups showed similar patterns, contrary to those of the 5% and 10% deformation force groups. Given these considerations, a fixed distance of 0.16 mm and a minimal 1% deformation force possess the potential to be employed and implemented for monitoring the firmness of sweet cherries during postharvest preservation.

Keywords: sweet cherry; firmness; primary metabolites; deformation force

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1. Introduction

Sweet cherry (*Prunus avium* L.) is greatly perishable at harvest and during postharvest storage but is also widely desirable for its attractive appearance and its nutritional value due to its high content of polyphenol compounds and vitamin C. Sweet cherry fruit displays a short harvest window, while several factors deteriorate its appearance, such as cracking and surface pitting [1–4]. Beyond the obvious physiological disorders, sweet cherry quality is significantly influenced by firmness and flavor, two of the main factors that determine consumers' acceptance. Consequently, breeders focus on improving these traits during the development of new cultivars [5]. In particular, cherry fruit firmness is a crucial feature that significantly influences the postharvest preservation of the fruit and its acceptability by customers [6–8]. It is noteworthy that consumers are willing to pay more for sweet cherries with an extra unit of sweetness and firmness [9]. Sweet cherry firmness naturally decreases during both on-tree fruit development and the subsequent postharvest period [10–12]. This loss of firmness happens due to the activation of enzymes related to pectin degradation, the main stabilizing factor of fruit's primary cell walls [13]. Nevertheless, it has been reported that sweet cherries with high firmness at harvest preserve higher firmness during cold storage compared to those with lower firmness at harvest [14]. Recently, it has been noted that sweet cherry softening is related to the activation of certain transcription factors. For example, *PavMADS7* is an important regulator factor of sweet cherry fruit ripening that induces the ABA-mediated signaling pathway and directly binds to the promoter of the *PaPG1* gene, which is involved in sweet cherry softening [15].

In recent years, firmness of the sweet cherry fruit has been evaluated either by applying a fruit size-dependent compression distance (i.e., 2, 3, 10, and 15%) [16–19] or by applying a fixed compression distance of 1 mm. The force required to deform fruits, is expressed as a normalized deformation force in N mm^{-1} [20]. In a recent review, it was suggested that high-speed functional equipment should be used for the firmness assessment of sweet cherries, especially in cases where quick and reproducible testing is necessary [21]. To date, several methods have been reported to determine the firmness of cherries such as penetration tests using instruments like the Effe-gi Tester, Magness-Taylor Device, Durofel, and Chatillon, etc. [22], puncture tests [23], Firmtech equipment [24], acoustic firmness sensors and compression tests applying pressure until a small deformation is reached, typically between 2% and 20% of fruit diameter [21,25,26]. In addition, a high positive correlation of the sweet cherry firmness between the trained panelists with the Firmtech2 device, which by applying 1 mm compression determines the maximum force required to achieve deformation, has been demonstrated [7,8]. Novel technologies for non-destructive measuring of sweet cherries' firmness using color sensors and near-infrared diffuse reflectance spectroscopy have been developed [27,28], but with controversial effectiveness on the prediction of firmness [29]. While the mechanical characteristics of several fruits have been examined [30], there is limited knowledge about the interaction between these traits and the physiological responses of the fruits. Currently, there is no established method for evaluating the firmness of sweet cherries at harvest and the postharvest period. In the present study, the main objective is to investigate the physiological impact of sweet cherries flesh firmness determination under different deformation distances and secondly, to uncover the deformation distance with the minimum effect on fruit physiology. For this purpose, experiments were carried out on two cherry cultivars (Canada Giant and Regina) to assess the firmness of sweet cherries. The tests involved applying various levels of compression dependent on the size of the fruit (1%, 5%, and 10%), as well as a fixed compression distance of 0.16 mm in the smallest thickness dimension of the fruit. Following the determination of textural properties, several fruit quality traits, sensory assessments of firmness, and primary metabolites of the fruits were evaluated.

2. Materials and Methods

2.1. Plant Material and Sampling Process

Fruit of two sweet cherry cultivars, namely Canada Giant (is a traditional cultivar, widely cultivated in Greece, [31]) and Regina (is a traditional, late harvest and cracking tolerant cultivar [3]) were collected with a soluble solid concentration of 16.3 $\pm$ 0.2 and 19.4 $\pm$ 0.3% Brix and a titratable acidity of 0.7 $\pm$ 0.1 and 0.6 $\pm$ 0.1% malic acid, respectively, in representative samples (5 replicates of 10 cherries per cultivar) at the commercial harvest stage. The experiment was conducted in a commercial sweet cherry orchard (Arnissa, Pellas's region, North Greece) during the 2023 growing season. The orchard consisted of 10-year-old trees planted at 3.5 $\times$ 1.25 m spacing between rows and along the row, grafted onto 'Gisela 5' rootstock, trained in tall spindle axe, and subjected to standard cultural practices. The experimental design was a completely randomized design (CRD). Fruit of each cultivar were picked and immediately transferred to the facilities of the Pomology Laboratory in the Farm of the Aristotle University of Thessaloniki. From a total of 900 fruit, 300 fruit were randomly divided into three 100-fruit sub-lots, and fruit firmness was assessed as described in detail below (Section 2.2). Ninety cherries were used for repeat measurements of firmness (see also Section 2.2). The assessment of firmness of 42 sweet cherry fruit was conducted by the panelists in each cultivar; further details are available in Section 2.3. The remaining 400 fruit were randomly divided into four equal groups, and deformation forces of 0% (control), 1, 5, and 10% were applied. Following these treatments, the flesh firmness and the quality traits of the fruit were evaluated.

2.2. Textural Properties

Fruit deformation forces, corresponding to 1, 5, and 10% of the fruit's small thickness dimension, were determined using a TA.XT.plusC Texture Analyzer (Stable Microsystems, Godalming, Surrey, UK), as previously described with slight modifications [17,19]. In particular, fruit firmness of 100 fruit of each condition (1, 5, and 10% fruit size and deformation forces) and cultivar was measured at harvest using a flat steel platen (75 mm diameter) fitted on the machine's branch; sweet cherries were placed with their small diameters on a stable steel surface. The required force in Newtons (N) for 1, 5, and 10% diameter compression (fruit size-dependent compression distance) or compression of 0.16 mm (fixed compression distance) on the small thickness dimension of fruit was recorded. The speed of the compression platen was set to 20 mm s^{-1}. The normalized deformation force of sweet cherries firmness, expressed in N mm^{-1}, was calculated as the ratio of the required force in order to achieve 1, 5, and 10% (fruit size-dependent compression distance) or 0.16 mm (fixed compression distance) deformation forces to the required distance of the platen in mm to achieve the deformation force during fruit compression in the small diameter of sweet cherries. Arithmetic data of 100 fruit for each treatment are provided in Table S1.

Also, to test if the repetition of the measurement processes affects the cherry fruit physiology, another 3 tests were performed for each condition (1, 5, and 10% fruit deformation forces) and cultivar using 10 cherries for each test (in total 90 sweet cherries per cultivar), as follows: (a) Calculation of the deformation force ratio after 5 successive compressions between the initial measurement in N mm^{-1} and the final measurement in N mm^{-1}, expressed as an increase in the required normalized force (percentage, %). (b) Calculation of the deformation force ratio at harvest and after 2 days at 20 °C between the final measurement in N mm^{-1} and the initial measurement in N mm^{-1}, expressed as a reduction in the required normalized force (percentage, %). (c) Calculation of the deformation force ratio at harvest and after 7 days at 4 °C between the final measurement in N mm^{-1} and the initial measurement in N mm^{-1}, expressed as a reduction in the required normalized force (percentage, %).

Fruit classification (described in detail in Section 2.3) of 7 individual sweet cherries was also determined (in N mm^{-1}) for each category (low and high fruit firmness based on panelist's classification), condition (1, 5 and 10% fruit deformation forces), and cultivar.

2.3. Firmness Assessment by Panelist

Every one of the seven panelists randomly selected 6 sweet cherries, which were classified into low and high flesh firmness (3 for each category), respectively, from a batch of at least 100 sweet cherries per cultivar. The selection and classification of 6 sweet cherries by the panelists were performed by squeezing them in their small thickness fruit dimension between their index finger and their thumb. Thereafter, one sweet cherry from each panelist and class was determined under the three conditions (1, 5 and 10% = varied distance or 0.16 mm = fixed distance, fruit deformation forces, 7 sweet cherries in each condition). The results were expressed as normalized deformation force in N mm^{-1} and the arithmetic data are provided in Table S1.

2.4. Evaluation of the Fruit's Cellular Damage

Membrane integrity of sweet cherries was tested as relative electrical conductivity (REC, %), as previously described [23] with slight modifications. Ten fruit per treatment, time-point, and cultivar were selected and were cut on both sides of their small thickness dimension (2 slices, 4 ± 0.2 g), and then they were submerged in 30 mL of deionized water for 30 min at 20 °C. The electrolyte content of the solution was determined by measuring the electrical conductivity with a conductivity meter in μS cm^{-1} (model HI 9033 and probe HI 7630, Hanna Instruments, Smithfield, RI, USA). Total electrolytes of the fruit slices were determined after boiling them for 15 min and then allowed to reach 20 °C, when electrical conductivity was recorded again. Relative electrical conductivity (REC, %) was calculated

as the percentage of a ratio of electrical conductivity just after 30 min to total electrolytes. Data are provided in Table S2.

2.5. Fruit Weight Loss and Respiration Rate

To determine fruit weight loss (%), weight of three batches of ten fruit from each treatment (0% (control), 1%, 5%, and 10% deformation forces) and cultivar were recorded at harvest and after 4, 24, and 48 h using an analytical balance (0.001 g). Furthermore, fruit respiration rate was measured by enclosing fruit in 2 L air-tight jars for 30 min at 20 °C and CO_2 production was determined in a 1 mL gas sample from the air of the jars by injecting it into a gas chromatograph (Shimadzu GC-2014, Kyoto, Japan), coupled with a thermal conductivity detector (TCD). To calculate CO_2 concentration, a correction was conducted by subtracting the ppm CO_2 of the laboratory air from that detected in the jars. The results were expressed as mL CO_2 kg^{-1} h^{-1}. Data are provided in Table S2.

2.6. Quantification of Primary Metabolites

Frozen (−80 °C) ground exo-and meso-carp tissues (0.5 g) of each treatment and cultivar in triplicate (biological replicates) in 48 h after treatments were transferred into 2 mL screw-cap tubes for primary polar metabolite extraction, as previously described with slight modifications [32]. In brief, 1.4 mL pure methanol and 0.1 mL of 1 mg mL^{-1} adonitol were added and incubated for 10 min at 70 °C. After centrifugation (10,000× *g*), the supernatant was collected, and 0.75 mL chloroform and 1.5 mL dH_2O were added. From the upper polar phase, 0.15 mL was dried at room temperature (RT) under vacuum. The residue was redissolved in 0.04 mL methoxyamine hydrochloride at a concentration of 20 mg mL^{-1}, and incubated for 120 min at 37 °C. Then it was derivatized with 0.07 mL N-methyl-N-(trimethylsilyl) tri-fluoroacetamide reagent (MSTFA) for 30 min at 37 °C. The GC–MS analysis was carried out with a Perkin Elmer Clarus™ SQ 8S (Waltham, MA, USA) as described in detail [33]. Compound peak identification was determined using standards or the NIST11 database and the GOLM metabolome database (GMD) in cases of unknown peaks [34]. The metabolites were presented based on the area after peak quantification compared to the area of adonitol (internal standard) and expressed as the relative abundance of adonitol. Data on metabolite description and relative abundance are provided in Table S3.

2.7. Statistical Analysis

Fruit textural properties, physiological traits, and polar primary metabolites were conducted using SPSS (SPSS v25.0., Chicago, IL, USA) by multivariate analysis of variance (MANOVA). Mean values were compared by the Student's *t*-test, the least significant difference (LSD), and the Duncan's multiple range test ($p \leq 0.05$). Furthermore, root-mean-square error (RMSE) was calculated using the R-package (ver. 3.6.2.). Metabolite visualization and clustering (hierarchical and PCA) were conducted using the Clustvis tool ver. 2.0 [35].

3. Results

3.1. Flesh Firmness Test in Sweet Cherries

In order to examine the firmness of sweet cherries, tests on two cultivars (Canada Giant and Regina) were conducted using different compression distances based on fruit size (1%, 5%, and 10%), as well as a fixed compression distance of 0.16 mm in the smallest thickness dimension of the fruit. The normalized deformation force (N mm^{-1}) of the fixed compression distance was measured at 1.8 and 2.1 for Canada Giant and Regina, respectively, indicating that Regina was firmer than Canada Giant (Figure 1a).

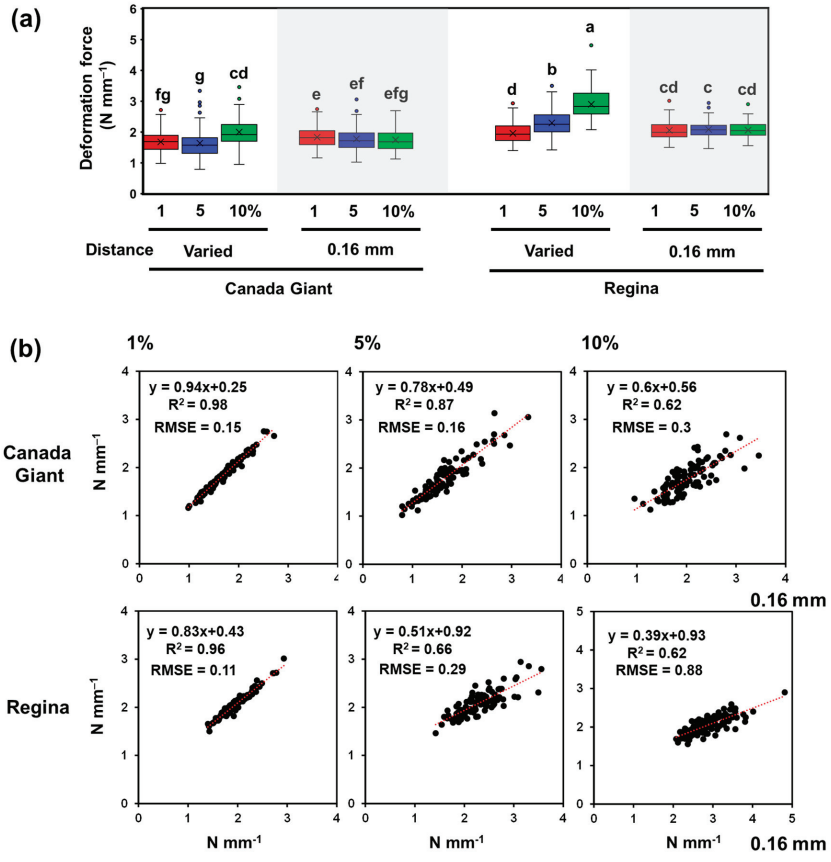

Figure 1. (**a**) Normalized deformation force (N mm^{-1}) of three different compression conditions (1, 5, 10%) and a fixed compression distance (0.16 mm) on the small thickness dimension of the fruit. (**b**) Scatter plot between the fixed (x axis) distance and the corresponding varied (y axis) distance. One hundred fruit for each condition and cultivar were used to create the box plot. Different letters indicate significant differences among treatments based on Duncan's multiple range test ($p \leq 0.05$). Best-fit linear regression, R squared, and the root-mean-square error (RMSE) were defined in each cultivar and in the pair of varied and fixed distance treatments. Data are provided in Table S1.

Furthermore, under the 1, 5, and 10% size-dependent compression distance conditions, flesh firmness of Canada Giant was 1.7, 1.6, and 2 N mm^{-1}, which are lower values than the corresponding of Regina 2, 2.3, and 2.9 N mm^{-1} (Table S1 and Figure 1a). Thereafter, a scatter plot was built between the deformation force for the fixed and the size-dependent compression distance to explore the linear regression match. The above-mentioned approach is employed to explore the fit of fixed and varied compression distances based on R2 and RMSE with a view to the possible replacement of the second one. Deformation force indicates a better fit between 1% of the size-dependent compression distance and the fixed distance (0.16 mm) in both cultivars compared to others (5 and 10%) with an R2 of 0.98 and 0.96 for Canada Giant and Regina, respectively (Figure 1b). On the other hand, the deformation forces of 5 and 10% and the fixed distance correlated with an R2 of 0.87 and 0.62, respectively, in Canada Giant, whereas for Regina, it was 0.66 and 0.62, respectively. Furthermore, the root-mean-square error (RMSE) was lower in the 1% deformation force and the fixed distance (0.15 in Canada Giant and 0.11 in Regina) compared to 5% (0.16 in Canada Giant and 0.29 in Regina) and 10% (0.3 in Canada Giant and 0.88 in Regina) in both

cultivars (Figure 1b). Subsequently, tests were carried out to measure the firmness of sweet cherries under different conditions, namely size-dependence and fixed distance. These tests involved subjecting the cherries to five consecutive compressions and then evaluating their firmness after either 2 days at 20 °C or 7 days at 4 °C.

The purpose of these tests was to assess the effectiveness of monitoring the firmness of cherries' flesh, as depicted in Figure 2a. Both cultivars exhibited a negative percentage increase in a fixed distance after undergoing five continuous compressions in 10% (Figure 2a). Moreover, in Canada Giant, the fixed distance after five continuous compressions in a 5% deformation force had the lowest increase of 2.7% compared to the others (various and fixed distance), and the elevation in firmness ranged from 16.5% to 21.6% after five continuous compressions (Table S1 and Figure 2a). In addition, Regina firmness increase ranged from 3.8% (fixed distance after five continuous compressions in 5%) to 17.4% (10% deformation force) following five continuous compressions (Table S1 and Figure 2a). After 2 days of shelf life at 20 °C, Canada Giant cherries exhibited a reduction in firmness, ranging from 24.1% (5% deformation force) to 32.5% (fixed distance after 5% deformation force and 2 days at 20 °C). In parallel, Regina cherries showed a lower reduction in the 5% deformation at a varied distance of 9% and a fixed distance of 12.9% than 1% and 10% deformation forces after 2 days at 20 °C, ranging from 26.4 to 39.5% (Table S1 and Figure 2a). An increase in firmness was recorded for a 5% deformation force in sweet cherries of Canada Giant after 7 days of cold storage (4 °C), while for the rest of the deformation conditions, the reduction ranged from 12% (fixed distance after 1% deformation force and 7 days at 4 °C) to 25.9% (fixed distance after 10% deformation force and 7 days at 4 °C). After 7 days at 4 °C, a decrease in firmness was observed in Regina fruit ranging from 27.9% (5% deformation force) to 46.7% in the fixed distance after 1% deformation force and 7 days at 4 °C (Table S1 and Figure 2a).

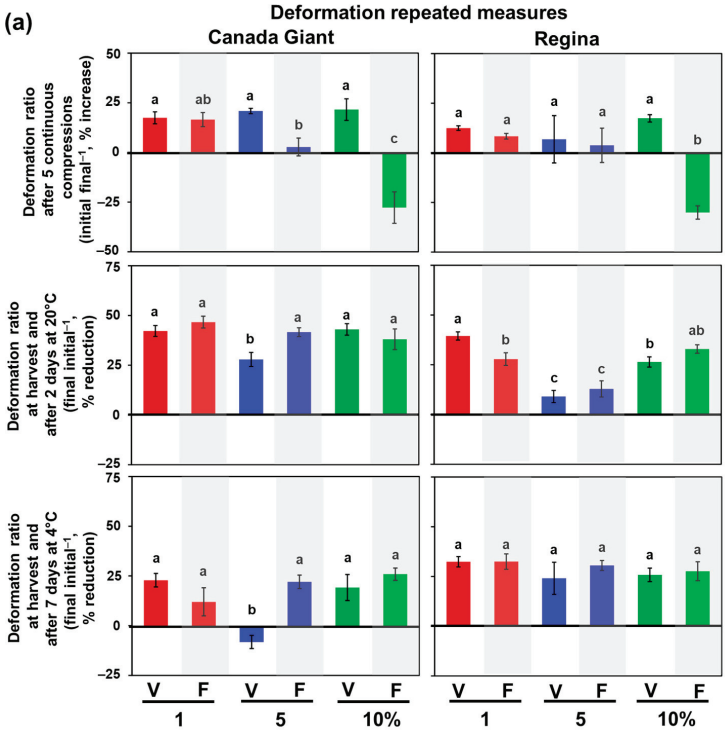

Figure 2. *Cont.*

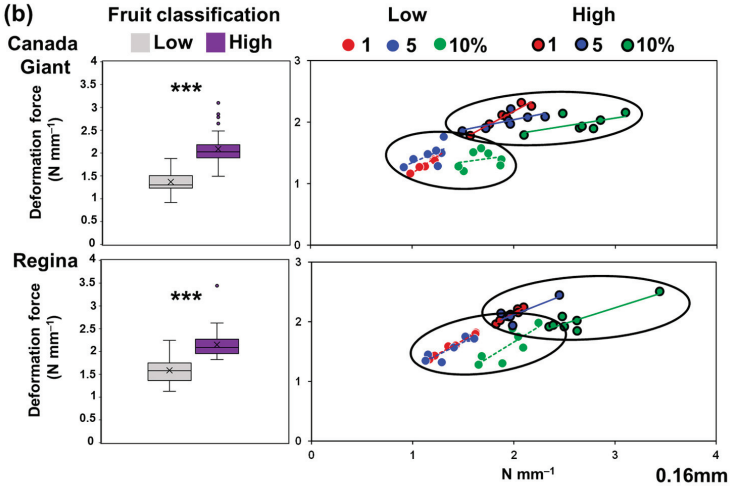

Figure 2. (**a**) Sweet cherry flesh firmness in response to fine continuous compressions following 2 days at 20 °C and 7 days at 4 °C in each treatment and cultivar. Values represent the ratio between each stage and the harvest. (**b**) Scatter plot among the fixed (x axis) distance and the corresponding varied (y axis) distance of flesh firmness classified into 'low' and 'high' by panelists. Each value represents the mean of 10 replications of individual fruit, and vertical bars represent the SD for the deformation ratio determinations. Different letters indicate significant differences among treatments based on Duncan's multiple range test ($p \leq 0.05$) or *t*-Student's test (*** $p \leq 0.001$). Data and linear regression with the R squared of each condition are provided in Table S1.

To further explore the association between sweet cherry flesh firmness measurement and hand sense, seven trained panelists classified 6 fruit into 'low' flesh firmness and 'high' flesh firmness (3 to each category) from a batch of 100 fruit from each cultivar. Panelists' assessments indicated that sweet cherries classified in the 'low' group had a normalized deformation force of 1.4 N mm^{-1}, while the 'high' group had 2.1 N mm^{-1} in Canada Giant, indicating a significant difference (Table S1 and Figure 2b). Moreover, Regina fruit with 1.6 N mm^{-1} and 2.1 N mm^{-1} were classified by panelists into the 'low' and 'high' groups, respectively, and they were also significantly differentiated between the two groups (Table S1 and Figure 2b). Subsequently, a scatter plot was constructed for both 'low' and 'high' flesh firmness classified cherries for each cultivar to examine the match to the linear regression model between the varied and the corresponding fixed distance. A high correlation in the linear models of the classified sweet cherries for both groups and cultivars was observed for the 1% deformation force with an R2 that ranged from 0.94 to 0.99 compared to the other conditions (5 and 10% deformation force) with an R2 that ranged from 0.07 to 0.8 (Table S1 and Figure 2b).

3.2. Monitoring Sweet Cherry Quality Traits

The weight loss and respiration rate of both cultivars were measured at 4, 24, and 48 h after treatment. Additionally, the membrane integrity of the fruit was assessed by measuring the relative electrical conductivity (REC) 48 h after treatment at 20 °C and 7 days after treatment at 4 °C. The weight loss of the fruit over the postharvest shelf life did not differ across treatments in both cultivars, except for a noticeable difference observed at the 48 h mark.

The treatment with a 10% deformation force showed an enhanced fruit weight loss compared to the control and the treatment with a 1% deformation force. Significantly, sweet cherries exhibited a weight loss of over 10% within a 48 h period at a temperature of 20 °C, as indicated in Table S2 and Figure 3a.

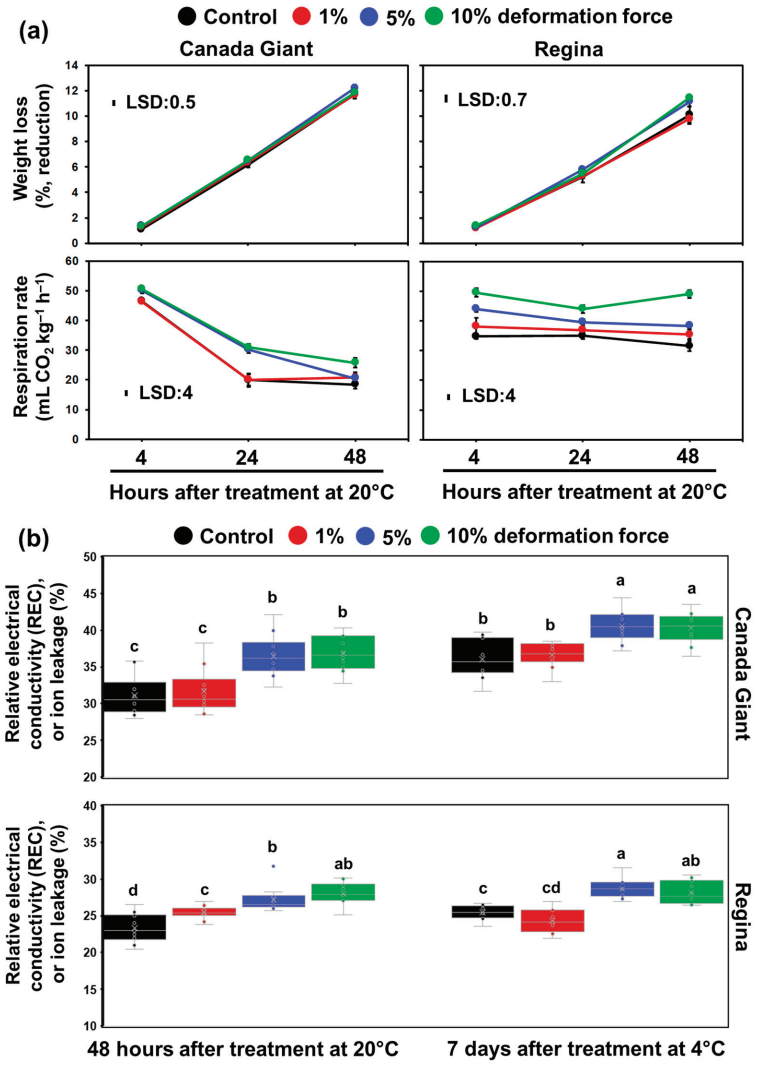

Figure 3. (**a**) Weight loss and respiration rate 4, 24, and 48 h after treatment at 20 °C, and membrane integrity (**b**) 48 h after treatment at 20 °C and 7 days after treatment at 5 °C of sweet cherries in each cultivar were determined. Each value represents the mean of 3 replications × 10 fruit in the case of physiological traits and 10 individually in membrane integrity determination. Differences among treatments were detected based on the least significant difference (LSD, $p \leq 0.05$) or different letters indicate significant differences among treatments based on Duncan's multiple range test ($p \leq 0.05$). Arithmetic data are provided in Table S2.

In addition, the respiratory activity of the fruit was often enhanced when subjected to deformation forces of 5% and 10%, regardless of the time point and cultivar. The only exception was seen after 48 h at 20 °C in the 5% deformation treatment of the Canada Giant cultivar. In contrast, the respiration rate of fruit subjected to a 1% deformation force did not show a significant impact (Table S2 and Figure 3a).

A reduction in respiration rate in Canada Giant during postharvest maintenance was observed, while the respiratory status of Regina fruit was not influenced during the postharvest period (Table S2 and Figure 3a). The relative electrical conductivity (REC) of

Canada Giant fruit ranged from 31 to 41% and increased in response to treatments 5 and 10% flesh deformation after 48 h at 20 °C and after 7 days at 4 °C, while there was no difference detected at these time points during REC determination at a 1% deformation force (Table S2 and Figure 3b). Moreover, the REC of Regina fruit ranged from 23 to 29% and increased in all treatments after 48 h at 20 °C and in 5% and 10% deformation treatments after 7 days at 4 °C. It is also worth noting that the REC for both cultivars increased in treatments of 5 and 10% deformation compared to a 1% deformation force (Table S2 and Figure 3b).

3.3. Primary Metabolic Profile of Sweet Cherry Fruit Exposed to Deformation Experiments

To better understand the variables contributing to fruit flesh deformation, we proceeded to examine the changes in the metabolic profile of the affected sampling areas 48 h after treatments at 20 °C using the GC-MS approach. In total, thirty-four polar metabolites in the exo-meso-carp tissue of the two sweet cherry cultivars were quantified and then clustered according to treatment and cultivar (Figure 4).

In response to treatments, 9 and 15 primary metabolites remained unaffected in Canada Giant and Regina, respectively, based on MANOVA. For instance, phosphoric acid, inositol, talose, and glucoheptonolactone showed no differences following treatments in both cultivars (Table S3). Generally, there was a clear separation in both cultivars due to hierarchical clustering analysis, leading to the first group where control and 1% deformation treatment grouped together and to the second group where 5% and 10% deformation treatments grouped together (Figure 4a). Furthermore, based on principal component analysis (PCA) for each cultivar, an obvious separation of the same groups (control and 1% deformation treatment compared to 5% and 10% deformation treatments) was detected, indicating that they are strongly related to PC1—principal component 1 (Figure 4b). In more detail, the variance of the metabolic data explained by the PCA model in Canada Giant was 91.1%, with 74.7% explained by PC1 and 16.4% by PC2, similarly in Regina, it was 90.1%, with 61.2% explained by PC1 and 28.9% by PC2 (Figure 4b).

To focus on the fruit metabolic alterations due to compression treatments, we studied metabolites that shifted their abundance in both cultivars. Thus, in both cultivars, an increase in mannitol and galacturonic acid in the 5 and 10% deformation treatments compared to control and the 1% deformation treatments was observed. Also, an increase in lactose was detected in the 10% deformation treatment compared to the control and the 1% deformation treatment (Figure 4a and Table S3). In Canada Giant, 11 primary metabolites (e.g., proline, fumaric acid, threonine, serine, arabinose, malic acid, ornithine, oxoproline, asparagine, quininic acid, and threonic acid) were increased in response to 5 and 10% deformation treatments, while sucrose was decreased. Furthermore, an increase in malonic acid and xylose were detected in the 10% deformation treatment, whereas a decrease in fructose, glucose, sorbitol, and maltose was recorded in this treatment (Figure 4a and Table S3). In Regina, an increase in mannobiose and cellobiose content in 5 and 10% deformation treatments were observed; however, a decrease in seven metabolites including proline, succinic acid, malic acid, asparagine, xylose, arabinose, and sorbitol was also observed. In addition, maltose and maltitol were increased in the 10% deformation treatment compared to the other applied treatments (Figure 4a and Table S3). The axis of principal component 2 (PC2) is mostly associated with the variation between the two greater compressions observed in the fruit subjected to a 5% deformation treatment compared to those that were subjected to a 10% deformation treatment in both cultivars (Figure 4b). Based on metabolic profile of the two cultivars from both hierarchical cluster analysis and PCA analysis (Figure 4), it is seen that the control and 1% deformation treatment in one group were grouped together, and 5% and 10% deformation treatments in the other group were also clustered. This indicates stronger changes in metabolite abundances after the application of 5% and 10% deformation treatments versus both control and 1% deformation treatment (Figure 4).

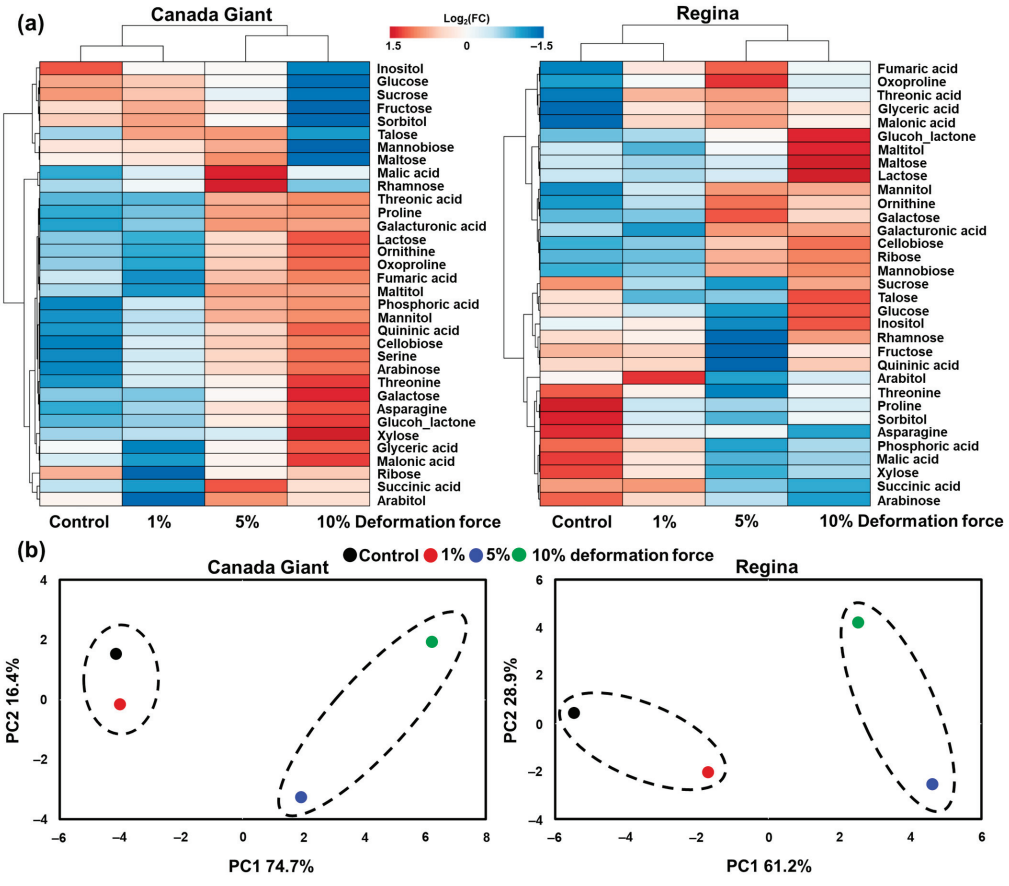

Figure 4. A heat map diagram and clustering analysis of primary metabolites were performed in deformation-related treatments of the two sweet cherry cultivars (**a**). Additionally, these metabolites were analyzed with principal component analysis (PCA) for each cultivar (**b**). Based on the grand mean of each cultivar and metabolite, an increase is depicted as red, and a decrease is depicted as blue (see color scale). Metabolite abundances were expressed as relative abundance compared to the internal standard of adonitol. Each metabolite is represented by 10 sweet cherries in three biological replications. Metabolites relative abundance data are provided in Table S3.

4. Discussion

Fundamental issues, such as the impact of compression due to firmness measurements on sweet cherry cell membrane integrity and cell metabolic status, have not been fully examined yet. In the current study, we investigated these aspects related to sweet cherry firmness, such as the optimum deformation force and the sensory discrimination of firmness, providing useful data in the food industry to develop a texture-based method of fruit quality monitoring. To achieve this, we conducted tests on the application utilizing three distinct fruit compression conditions (1%, 5%, and 10%) and a fixed compression distance condition of 0.16 mm. These tests were performed on two high commercial and traditional cultivars in Greece, as shown in Figure 1. An optimum number of one hundred tested cherries in each condition and cultivar were used to minimize variability [36] (Figure 1a). In addition, the selection of compression speed and loading direction was suggested to test the reliability of fast and accurate results, notably at the fixed compression distance of

0.16 mm (Figure 1a). For this reason, the compression speed was set to 20 mm s^{-1}, and the loading direction of the fruit was set to the small thickness dimension.

The demand for rapid and precise assessment of firmness arose primarily because of the short period of time available for handling sweet cherries and their transportation [21]. Additionally, the necessity for fast assessment of cherry attributes was reinforced by the implementation of hydro-cooling techniques to avoid the short period of life due to deterioration of the fruit [37,38]. A recent study indicates that the deformation leading to elastic, local plastic, or structural failure in strawberry fruit is highly influenced by the speed of compression and the direction of loading [39]. Consequently, the current firmness data may be affected by the selected speed of compression, but it also should be fast enough in sweet cherries to assess a specific fruit batch as soon as possible. Therefore, we have also chosen the loading direction with the small thickness dimension, since the probability of sweet cherries being automatically placed in this position is much higher than the large dimension. It is also noteworthy that the compression of fruit with a small thickness dimension equal to 1% was strongly correlated with the fixed compression distance of 0.16 mm on a linear regression with an R2 exceeding 0.96 in both cultivars (Figure 1b). Hence, a fixed distance in fruit deformation force below 0.2 mm enables the design of suitable postharvest mechanical handling facilities with elastic fruit deformation, assisting fruit classification based on the ripening stage [40]. In this regard, a non-destructive elastic deformation for the determination of tomato ripening stage was previously proposed [41]. In sweet cherries, a destructive elastic–plastic deformation when applied to the fruit's repeated measurement was observed; specifically, after five continuous compressions, an increase in recorded force was detected, leading to the hypothesis that this increase in fruit firmness should be taken into consideration in order to monitor the firmness declination during the postharvest life period (Figure 2a). A sweet cherry destructive method for firmness estimation should offer more precise measurement than non-destructive, yielding more accurate results. Furthermore, a destructive approach with a minimum impact on fruit physiology could provide accessibility to firmness while still preserving the fruit for further physiological analysis [42]. Although we can claim that deforming the fruit to 1% of the small thickness dimension had minimal effect on the fruit physiological responses (Figures 3 and 4), the non-destructive approach is well-defined, and concerns, among others, include the correlation of profiles in the infrared spectrum with physiological characteristics of the fruit such as firmness, SSC, DM and its biochemical traits [43].

Skin mechanical properties of sweet cherries are tightly linked with genotype, ripening stage, fruit water relations (including turgor, transpiration, and water uptake), and temperature [44]. Herein, we also performed sensory evaluation of sweet cherry firmness by hand, and cherries were split into 'low' and 'high' fruit firmness after squeezing them to the small thickness dimension. In these fruit, the firmness was also quantified using a texture analyzer. Data indicated that fruit characterized as low firmness had 1.30 and 1.58 N mm^{-1}, and those characterized as high firmness had 2.03 and 2.09 N mm^{-1}, respectively, in the cultivars Canada Giant and Regina (Figure 2b). According to previous studies [7,45] panelists' sensory assessment of firmness is highly sensitive. Moreover, firmness of sweet cherries has been linked with postharvest maintenance [6], as firmer cherries at harvest could remain firmer during the postharvest period than the softer ones [14]. Therefore, in this study, sweet cherry ripening characteristics, including fruit firmness, were periodically monitored during maintenance for 2 days at 20 °C following deformation force treatments (Figure 3a). Generally, there were no differences in sweet cherry weight loss after deformation treatments, with the only exception of an increase of 2 days in Regina after a 10% deformation force. Also, the fruit respiration rate displayed higher values in both cultivars after a 5 and 10% deformation force compared to either control or a 1% deformation force (Figure 3a). A rise in respiration activity had also been observed in plum fruit exposed to mechanical damage during the postharvest handling process [46]. A lower sweet cherry respiratory status is the optimal goal for depression in senescence and metabolic activity under postharvest conditions [10,47]. Hence, the observed increase in the respiration of

fruit exposed to 5 and 10% deformation treatments (Figure 3a) possibly gives rise to shifts in the fruit cell membrane status to reverse these adverse situations (Figure 3a). Indeed, the relative electrical conductance (REC), which reflects the cell membrane integrity, increased in response to 5 and 10% deformation treatments after 48 h at 20 °C, indicating a loosening of the cell membrane (Figure 3b). A strong positive correlation between REC and fruit damage due to vibration following wounding was recorded in strawberries [48]. In parallel, metabolic analysis and clustering revealed a clear separation between the first group, which includes control and a 1% deformation force, and the second group, which includes 5% and 10% deformation forces (Figure 4). Particularly, the increase in galacturonic acid in fruit treated with 5 and 10% deformation forces compared to the other group seemed to have a significant role in the observed disjunction (Figure 4a), since galacturonic acid accumulation, which is the main compound of pectin degradation, is associated with the activation of several pectin-related enzymes, such as β-galactosidase (β-Gal), polygalacturonase (PG), and pectinmethylesterase (PME) [14,49,50].

Polyol accumulation under stress conditions in higher plants has been well documented [51]. In this work, the detected increase in mannitol may indicate a fruit response to a stressful situation manifested as compression force (Figure 4a and Table S3). Although the metabolic changes in each cultivar showed distinct patterns, these changes led to the same result: an obvious separation of treatments of 5 and 10% deformation forces compared to control and a 1% deformation (Figure 4a and Table S3). For example, proline and oxoproline were accumulated in the aforementioned treatments (5% and 10% deformation forces) only in the cultivar Canada Giant (Figure 4a and Table S3), while these two compounds have been linked with stress conditions in sweet cherries [4,52]. On the contrary, the accumulation of mannobiose and cellobiose in the 5% and 10% deformation treatments (Figure 4a and Table S3) was a good example for sweet cherries of Regina cultivar, indicating that the cell wall collapsed, and these two constituents were released via hydrolysis from the cell wall and thereafter detected [53]. Undoubtedly, the metabolic response of fruit to high compression forces in both cultivars was differentiated between them, but the outcome was similar, namely the separation of high and low compression forces (Figure 4). The differences in sweet cherry firmness between 1% or specific deformation force at 0.16 mm and a 5% or 10% deformation force are illustrated in Figure 5.

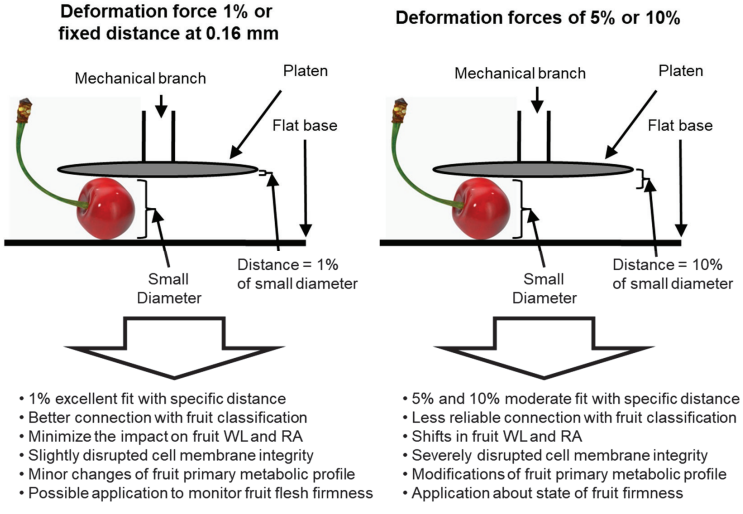

Figure 5. Schematic presentation of sweet cherry firmness measurements. Pros and cons during firmness determination when using a deformation force of 1% or a fixed distance of 0.16 mm on the left side and deformation forces of 5% or 10% on the right side. Abbreviations: WL, weight loss; RA, respiration activity.

5. Conclusions

Flesh firmness evaluation in sweet cherries is primarily determined by the deformation force applied at a distance equal to a different percentage compression distance of the fruit diameter. The current study supports the idea that a fixed distance of 0.16 mm for cherry fruit firmness evaluation, which is also the main goal for easy applications, fits better with a 1% deformation force determination than with a 5% or 10% deformation force. Moreover, the classification of fruit into low and high firmness by panelists demonstrated a stronger correlation with the application of a 1% deformation force and the fixed distance of 0.16 mm within each cultivar. The effect of a 1% deformation force on physiological traits such as weight loss, respiration activity, and membrane integrity was minimized compared to 5% or 10% deformation forces. Hence, in the fixed distance 0.16 mm or a 1% deformation force, the fruit's metabolic status was not dramatically influenced compared to 5% or 10% deformation forces, indicating a future perspective for monitoring sweet cherries during postharvest maintenance, either during cold storage or the shelf life with a minimum impact on fruit physiology. Additionally, control and 1% deformation treatment were clustered together concerning metabolic analysis, whereas 5% and 10% deformation treatments exhibited significant metabolic shifts compared to control and a 1% deformation treatment after 48 h. Finally, these findings should be tested on more sweet cherry cultivars in future studies, and extensive tests should also be accomplished to apply the proposed compression distance for sweet cherry firmness determination on a large scale.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10050435/s1, Table S1. Data on sweet cherry deformation force, repeated measurements, and classification.; Table S2. Data on sweet cherry physiological traits and membrane integrity.; Table S3. Quantification and descriptive data of primary metabolites in sweet cherries. Ref. [54] cited in Table S3.

Author Contributions: Conceptualization, M.M. and M.K.; methodology, M.K. and M.R.; software, M.K. and M.R.; validation, E.N., V.S.T. and M.M.; formal analysis, M.K., E.N. and V.S.T.; investigation, M.K. and M.R.; resources, M.M.; data curation, E.N. and V.S.T.; writing—original draft preparation, M.K.; writing—review and editing, M.M., E.N. and V.S.T.; visualization, M.K., M.R., E.N., V.S.T. and M.M.; supervision, M.M.; project administration, M.M.; funding acquisition, M.M. All authors have read and agreed to the published version of the manuscript.

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Chitosan Coating Incorporated with Carvacrol Improves Postharvest Guava (*Psidium guajava*) Quality

Chang Shu [1,2], Beatrice Kim-Lee [1] and Xiuxiu Sun [1,*]

[1] United States Department of Agriculture, Agricultural Research Service, Daniel K. Inouye U.S. Pacific Basin Agricultural Research Center, 64 Nowelo Street, Hilo, HI 96720, USA; chang.shu@usda.gov (C.S.); bkimlee311@gmail.com (B.K.-L.)

[2] Oak Ridge Institute for Science and Education, 1299 Bethel Valley Road, Oak Ridge, TN 37830, USA

* Correspondence: xiuxiu.sun@usda.gov; Tel.: +1-808-959-4307

Abstract: Guava (*Psidium guajava* L.) is an important economic crop grown widely in tropical and subtropical regions. Guava exhibits fast ripening and senescence as a climacteric fruit, causing a short shelf life and quality deterioration. Chitosan–essential oil nanoemulsions can be an edible coating used to improve postharvest quality attributes. In this study, chitosan was mixed with carvacrol to generate a nano-emulsoid solution containing 0.1 and 0.2% (*v*/*v*) carvacrol, using a sonic dismembrator. Guava fruit were coated with the above emulsion and postharvest quality parameters were measured during storage at 20 ± 1 °C and RH = 80 ± 5% for 8 days. The result illustrated that the particle size of the chitosan–carvacrol emulsions was nanoscale, and their high stability was demonstrated by the zeta potential and polydispersity index. Chitosan coating (2%, *w*/*v*, 310–375 kDa) containing 0.2% (*v*/*v*) carvacrol maintained postharvest quality compared to chitosan alone, with higher firmness, soluble solid content, total acid, and total phenol content, and lower weight loss and pericarp browning. The collective data were further verified by principal component analysis. A chitosan coating containing carvacrol can reduce postharvest losses. It can be applied as an effective strategy to improve postharvest fruit quality.

Keywords: edible coating; chitosan; carvacrol; guava; postharvest quality

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1. Introduction

Guava fruit (*Psidium guajava* L.) is a significant economic crop cultivated extensively in tropical and subtropical regions [1]. It is known for its delectable taste and nutritional richness, and recent research also reveals its medical values [2]. However, guava is a climacteric fruit that undergoes rapid ripening and senescence, resulting in a short storage period and shelf life along with sensitivity to pathogen infection, leading to considerable postharvest losses [3]. Also, guava is sensitive to cold temperatures, and storage at temperatures below 10 °C may result in severe chilling injury symptoms [4]. These all significantly limit the production and commerciality of guava with postharvest losses as high as 10–24% [5]. Therefore, it is urgent to explore feasible methods to reduce postharvest losses and improve the quality of guava.

Researchers and industry fields have explored various postharvest technologies and treatments, including 1-methylcyclopropene [6], novel cold storage technology [7], ionizing radiation [8], hot water treatment [9], modified atmosphere packaging [10], melatonin treatment [11], and edible coatings [3]. These approaches aim to extend storage time and shelf life, inhibit decay, and maintain the nutritional quality of guava fruit. Among them, edible coatings are recognized as promising strategies. Edible coatings are mainly made from food-grade biopolymers, including polysaccharides, lipids, proteins, or a combination thereof [12]. They form a physical barrier on the surface of the fruit, directly prevent the invasion of pathogens, and regulate the atmosphere exchange of the fruit, reducing the postharvest respiration rate and consequent weight loss [13]. Since edible coatings are sold

and consumed as part of the fruit, current research primarily focuses on the use of generally recognized as safe (GRAS) substances to ensure their non-toxicity and food safety [14]. The incorporation of functional ingredients (such as antimicrobial and antioxidant agents) into the coating matrix has been demonstrated to improve its physicochemical properties [15]. Given the short shelf life of guava, where the quality rapidly deteriorates upon ripening, the application of edible coatings holds significant potential.

Chitosan, a natural polysaccharide derived from chitin, has garnered significant interest due to its desirable properties, including biodegradability, biocompatibility, antimicrobial properties, and film-forming ability [16]. Notably, the U.S. Food and Drug Administration (FDA) has recognized chitosan as a GRAS polymer [17]. These unique characteristics position chitosan as an excellent candidate for developing edible coatings to preserve fresh produce. Numerous studies have demonstrated the effectiveness of chitosan coatings in reducing respiration rates and microbial decay, delaying senescence, and maintaining postharvest fruit quality attributes [18,19]. Carvacrol (5-isopropyl-2-methyl phenol), a monoterpene compound mainly extracted from oregano and thyme, has received considerable attention due to its broad-spectrum antimicrobial activity and GRAS certification and may act as a novel bio-preservative [20]. Incorporating natural essential oils into polysaccharide-based coatings provides an additional protective barrier to control the release rate and reduce the volatile/oxidation loss of essential oil [21] while simultaneously contributing to better barrier and mechanical properties of the coating [15]. Furthermore, by reducing the particle size of incorporated essential oils to the nanoscale through the emulsion, the specific surface area of oil droplets is significantly increased [22]. This results in improved stability as they become more uniformly distributed within the film-forming solution, thereby enhancing the utilization efficiency of essential oil [23]. Even though the use of chitosan coatings loaded with nanoscale essential oil has shown promising results in extending the shelf life of various fruits, their application on guava still needs to be further studied.

This study aimed to prepare a chitosan coating containing nanoscale carvacrol and apply it as an edible coating on guava fruit. The coating solution was characterized, and the fruit's shelf-life and quality attributes were studied to provide an effective strategy for improving postharvest guava quality.

2. Materials and Methods

2.1. Reagents and Fruit Materials

Chitosan and carvacrol were both purchased from Sigma-Aldrich (St. Louis, MO, USA). The molecular weight of chitosan is between 310,000 and 375,000 Daltons with a deacetylation degree over 75%. The purity of carvacrol is 99% and it is food grade. Tween 80 was purchased from Research Products International Corporation (Mt. Prospect, IL, USA). Analytical-grade acetic acid was purchased from Fisher Chemical (Fair Lawn, NJ, USA), with a purity $\geq$ 99.7%. Other chemicals not mentioned were of analytical grade.

Guava fruit (*Psidium guajava* L.) was harvested about 20 to 28 weeks after flowering and pollination in Hilo, Hawaii, in October 2022. The fruits were uniform in size (about 130 $\pm$ 15 g per fruit) and free of any mechanical damage or diseases. The fruit surface was washed with deionized water and dried at room temperature for later use.

2.2. Preparation of Coating Solutions

Preparation of coating-forming solutions referred to a previous study with some modifications [24]. Chitosan was dissolved in distilled water containing 1% (v/v) acetic acid and 0.15% (v/v) Tween® 80 to obtain a 2% (w/v) chitosan solution. Carvacrol was then mixed into the chitosan solution to prepare 0.1% and 0.2% (v/v) carvacrol coating solutions. The solution was stirred at 700 rpm for 40 min to let carvacrol distributed evenly, then the mixture was ultrasonically homogenized at 20 kHz for 10 min (Model 705, Fisherbrand, Waltham, MA, USA) to generate a nano-emulsion as the coating-forming solution. The coating solution was kept at room temperature until further usage.

2.3. Coating Treatment and Storage Condition

The coating-forming solution was manually spread on the fruit surface. Each fruit was coated with 1.0 mL of the above solutions; to ensure consistent coating on each fruit, excess solution flowed down from the fruit surface. The coated fruits were allowed to dry naturally at room temperature until the surface was completely dry and then stored at 20 ± 1 °C, RH = 80 ± 5% for 8 days to simulate shelf life. Fruit was sampled initially and at a 2-day interval during storage for quality evaluation. The whole experimental design is shown in Figure 1.

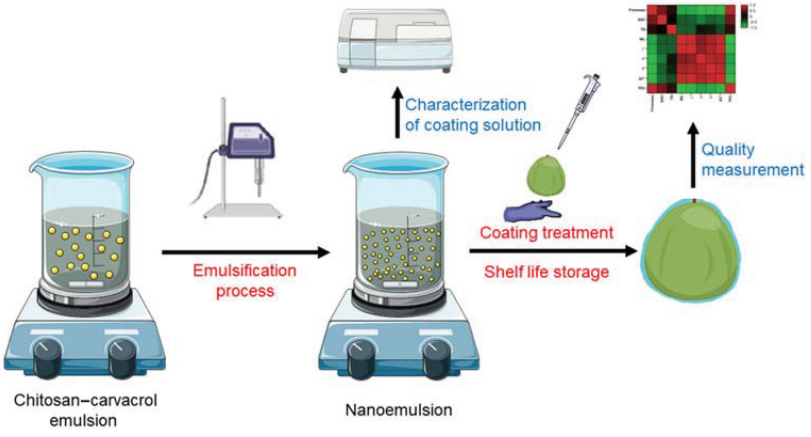

Figure 1. Schematic diagram of the experiment design.

2.4. Characterization of Coating Solution

The particle size, polydispersity index (PDI), and zeta potential of the samples were measured with the dynamic light scattering method using a Zetasizer analyzer (Zetasizer Ultrablue, Malvern Instruments Ltd., Worcestershire, UK). The data were analyzed by the XS Xplorer 3.2.0.84 software (Malvern Instruments Ltd., Worcestershire, UK).

2.5. Color Parameters

Peel surface color was measured on three fruits, and each fruit was measured at two opposite sites on the equator using a Minolta chromameter (model CR-300, Minolta Corp., Ramsey, NJ, USA) and recorded as CIE (International Commission on Illumination) L^*, a^*, and b^*. ΔE^* was calculated using the following Formula (1) to represent the total color difference when compared to the initial status.

$$\Delta E = \sqrt{(a_0 - a_1)^2 + (b_0 - b_1)^2 + (L_0 - L_1)^2} \quad (1)$$

2.6. Firmness

Fruit was peeled at two opposite sides of the fruit equatorial, and the tissue 30 mm under the epidermis part was measured. Pulp firmness was measured using a texture analyzer (Model Chatillon LTCM-100, AMETEK, Inc., Berwyn, PA, USA) equipped with a 60 mm diameter probe. The probe punctured the pulp 1.0 cm at a speed of 25.4 cm min^{-1}. The results were expressed in Newtons (N). In each replicate for each time point, a total of three fruits were randomly measured.

2.7. Total Soluble Solid Content and Titratable Acidity

Fresh pulp tissue was ground, and the juice was filtered through two layers of medical gauze. The total soluble solid content (SSC) was determined using a digital refractometer (PAL-3, ATAGO U.S.A., Inc., Bellevue, WA, USA), and the result was recorded as a percent-

age. The titratable acidity (TA) was measured with an acidity meter (GMK-835F, ATAGO U.S.A., Inc., Bellevue, WA, USA), which measures the total amount of hydrogen ions, and the result was expressed as a percentage.

2.8. Weight Loss

Weight loss was calculated as a percentage using the following Equation (2). The weight loss at each time point was fitted to linear models to estimate the daily weight loss.

$$\text{Weight loss (\%)} = (\text{Weight original} - \text{Weight measurement})/\text{Weight original} \times 100\% \quad (2)$$

2.9. Total Phenolic Content

The total phenolic content (TPC) was determined using a modified version of the Folin–Ciocalteu method described by previous research [25]. Frozen pulp (1.0 g) was mixed with 5.0 mL of 70% (v/v) ethanol in a pre-cooled mortar and homogenized at a low temperature. The mixture was then centrifuged at 16,000$\times$ g for 10 min at 4 °C, and the resulting supernatant was collected as the extract. To 0.15 mL of the above extract, 1.5 mL of Folin–Ciocalteu reagent (diluted 10 times) was added, mixed and stood by for 5 min. Subsequently, 1.5 mL of 6% (w/v) sodium carbonate solution was added to the mixture, followed by incubation in a water bath at 75 °C for 10 min. The solution was then rapidly cooled in an ice bath for 30 s. The absorbance was measured at 725 nm (SpectraMax M2, Molecular Devices, San Jose, USA), using the extraction solvent as the blank. Gallic acid was used the standard substance, and the results were expressed as mg kg^{-1} on a fresh weight basis.

2.10. Statistical Analysis

Principal component analysis (PCA) was applied to analyze the fruit quality parameters, which referred to a previous study [26]. The data were processed using Origin 2017 (OriginLab Corporation, Northampton, MA, USA) to calculate the eigenvalues, contribution, and factor scores (FAC) of the principal components. The Fernandez–Garcia definition was applied to explain the significance of each principal component in explaining the overall variance. This involved calculating the individual *F*-value (3) based on the FAC and eigenvalue, as well as the *F*-value for each observation (4). The average *F*-value was utilized as a ranking metric for comparing the different groups.

$$F_n = FAC_n \times \sqrt{\text{Eigenvalue}_n} \quad (3)$$

$$F\text{-value} = (F_1 \times Variance_1 + \cdots + F_n \times Variance_n)/\text{Cumulative} \quad (4)$$

All the data were organized and graphed using Excel (Microsoft Corp., Seattle, WA, USA), then analyzed using JMP statistical analysis software (version 16; SAS Institute, Cary, NC, USA). Analysis of variance (ANOVA) was used to evaluate the effect of the coating on guava quality, and Duncan's multiple range test with Holm correction was applied to determine significant differences ($p < 0.05$) among different groups at the same time point. At least three replications were conducted for all experiments to provide guava quality data.

3. Results and Discussion

3.1. Particle Size, Zeta Potential, and Polydispersity Index of Coating Solution

The application of edible coatings depends on different scenarios, such as immersion, spray, and artificial methods [27]. Therefore, the efficiency of the coating is related to the properties of its forming solution. Particle size is an important indicator of the uniformity and stability of the solution. The particle size of the chitosan solution was the smallest (Table 1), and the incorporation of carvacrol significantly increased the particle size of the so-

lution ($p < 0.05$). This may be due to the aggregation of essential oil droplets in the solution. Similarly, the addition of cinnamon essential oil increased the particle size of the chitosan film solution [16]. Emulsions can be classified into coarse emulsions (200 nm–200 μm) and nanoemulsions (0–200 nm) based on their particle size. Nanoemulsions have smaller droplet diameters, allowing for better distribution within the polysaccharide matrix and preserving the original membrane matrix structure, thereby minimizing adverse effects on membrane performance. Furthermore, nanoemulsions exhibit greater stability compared to macroemulsions and possess a higher surface area ratio, resulting in a slower release rate of essential oils and improved bioavailability [27]. The particle sizes of the prepared emulsions were all below 200 nm, which are consistent with previous reports [28,29] and can be applied as edible nano-coatings.

Table 1. Particle size, zeta potential, and polydispersity index of coating solutions *.

	Particle Size (nm)	Zeta Potential (mV)	Polydispersity Index
Chitosan	127.3 ± 2.62 c	55.46 ± 1.736 a	0.22 ± 0.04 a
Chitosan + 0.1% Carvacrol	144.3 ± 5.62 b	52.33 ± 2.039 ab	0.29 ± 0.06 a
Chitosan + 0.2% Carvacrol	186.4 ± 8.80 a	49.50 ± 3.224 b	0.31 ± 0.07 a

* The value is expressed as mean ± standard deviation ($n = 5$), the experiment was carried out three independent times. The different letters indicate significant differences (ANOVA, $p < 0.05$) among different groups in each parameter according to Duncan's multiple comparisons test.

The zeta potentials of all the samples were positive and over 40 mV (Table 1), indicating that the formed nanoemulsion has high stability. The polydispersity index was between 0.22 and 0.31, indicating that the essential oil was distributed uniformly in the emulsion, the applied emulsification in the experiment was effective, and the prepared nanoemulsion could be applied as a coating.

Zeta potential is an important indicator for evaluating the stability of the emulsion system. Positive values above +30 mV and negative values below −30 mV indicate that the particles in the system are stable. Chitosan amino groups are protonated at low pH values, resulting in their zeta potential being positive [29,30]. With the increasing carvacrol concentration, the zeta potential was observed to decrease significantly ($p < 0.05$), which may be due to the interaction of the carvacrol droplets with the free amino groups on the chitosan molecules, resulting in a slight decrease in stability [30]. PDI is an important parameter for characterizing the uniformity of the particle size distribution in emulsion systems. Dynamic light scattering (DLS) can be used to determine the size distribution profile of small particles in suspension or polymers in solution [31]. In this sense, the dispersity values are in the range from 0 to 1, with values between 0.1 and 0.25 indicating a narrow particle size distribution, while values above 0.5 indicate a wide particle size distribution. The PDIs of obtained nanoemulsions ranged from 0.22 to 0.31, which is within the range of the previous study [32], indicating a good distribution uniformity.

3.2. Fruit Appearance and Color Change

The fruit epidermis gradually turned yellow during storage, which is mainly due to the postharvest physiological process of the fruit, producing ethylene to promote the ripening of the fruit and cause the chlorophyll to decompose [33]. The control fruits were turning yellow on day 4 and exhibited wrinkling on day 8 (Figure 2A). The chitosan coating delayed yellowing, and it was slower than the control and no significant wrinkling occurred. The chitosan coating incorporating carvacrol significantly delayed fruit yellowing and reduced weight loss, maintaining the fruit appearance.

The biopolymer materials can form a selectively permeable film on the fruit surface, regulating the permeability of O_2 and enhancing the antioxidant system by respiratory metabolism regulation. The essential oil itself is hydrophobic, and the addition of the essential oil to the chitosan coating enhances the water barrier performance of the film

and reduces the water loss of the fruit due to respiration [18]. In addition, the strong antioxidant property of carvacrol contributes to improving fruit quality [34]. These results indicated that the carvacrol-incorporated coatings delayed senescence and maintained the fruit appearance. This result is consistent with the previous study, where the application of essential oils enhanced the barrier properties of chitosan coating, significantly decreasing the perishing process of postharvest tomatoes and strawberries [35].

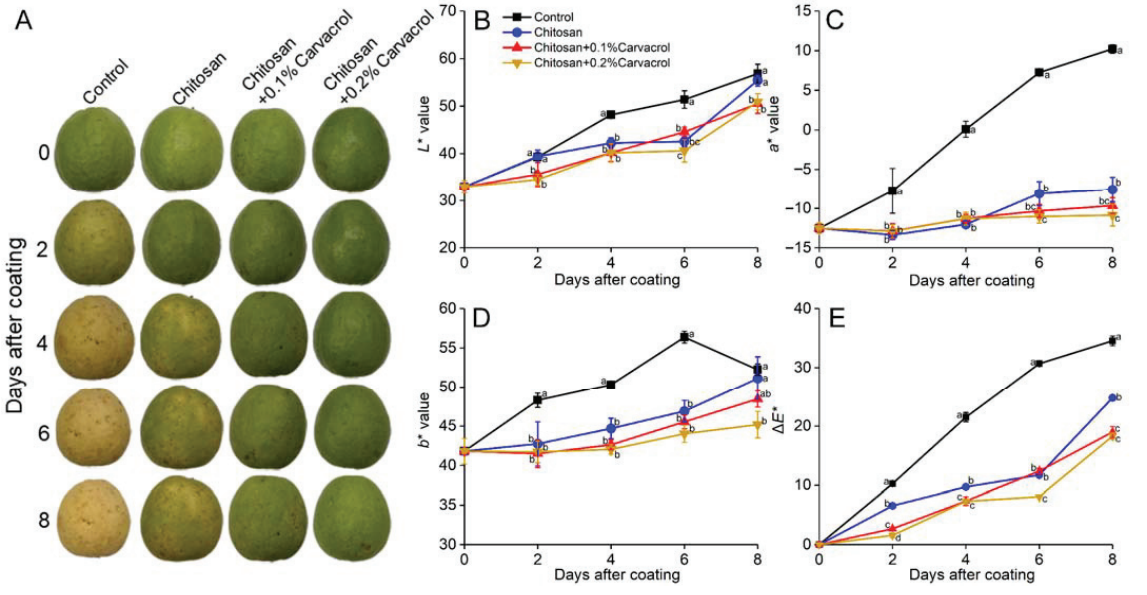

Figure 2. Effects of chitosan–carvacrol coating on appearance and peel color of postharvest guava fruit. The fruit appearance (**A**), peel L^* (**B**), a^* (**C**), b^* (**D**), and ΔE^* (**E**) in response to chitosan–carvacrol coating. Each value is the mean of three replicates. The vertical bars represent the standard deviation of the means. The different letters indicate significant differences (ANOVA, $p < 0.05$) according to Duncan's multiple comparisons test with Holm correction.

The a^* and b^* of all groups increased continuously, showing the loss of green and the increasing yellow and red of the peel (Figure 2C,D). The chitosan coating delayed yellowing, and chitosan incorporating carvacrol further delayed this trend. After storage for 8 days, the a^* of the control was the highest, which was 2.35-fold higher than the chitosan group; the a^* of the chitosan + 0.1 and 0.2% carvacrol groups were 27.64% and 43.78% ($p < 0.05$) lower than chitosan alone, respectively. The b^* of the control was also the highest during storage; at day 6, it was 20.24%, 23.81, and 28.08% ($p < 0.05$) higher than those of the chitosan and chitosan + 0.1 and 0.2% carvacrol groups, respectively. ΔE represents the total color difference compared with the initial state, and the ΔE of the control group was higher than the other three groups during storage, indicating that the color variation was greater, and the chitosan combined with carvacrol coating inhibited this significant change (Figure 2E). After 8 days of storage, the ΔE of the chitosan + 0.2% carvacrol group was the lowest, being 47.08% ($p < 0.05$), 26.22% ($p < 0.05$), and 3.63% lower than that of the control, chitosan, and chitosan + 0.1% carvacrol groups, respectively. These results indicated that the chitosan–carvacrol film could delay the color deterioration of the fruit epidermis, which was consistent with the conclusions of other polysaccharide–essential oil coating films [33,36].

In addition to controlling the fruit atmosphere, a small amount of chitosan entering the fruit will regulate the physiological metabolism and inhibit ripening and senescence [19].

Previous studies also reported that chitosan–essential oil coatings may regulate the activity of enzymes related to browning [37].

3.3. Fruit Firmness, Soluble Solid Content, Titratable Acidity, and Weight Loss

The firmness of guava decreased rapidly during storage, but the chitosan coating delayed this decrease. The control group showed the lowest firmness compared to that of the other three groups during the storage (Figure 3A). Both chitosan incorporated with 0.1% and 0.2% carvacrol coatings suppressed the decreasing firmness, which was 10.34% and 29.82% ($p < 0.05$) higher than that of chitosan alone after 8-day storage. The total soluble solid content (SSC) of the fruits increased slightly and then decreased during storage (Figure 3B). The SSC of the control fruit reached its peak on day 2 and then gradually decreased. The chitosan coating delayed the SSC peak until day 4, and it was 43.13% ($p < 0.05$) higher than the control at the end of storage. The consolidation of carvacrol further delayed the SSC decrease, its peak value was on day 6, and its content decreased slowly. Titratable acidity decreased constantly during storage, and the control group began to decrease rapidly from the second day, while all the coating groups remained at higher levels (Figure 3C). Chitosan + 0.2% carvacrol maintained the highest titratable acidity after 8 days, while the control was the lowest. The weight loss of all the groups increased constantly (Figure 3D). By establishing linear models of weight loss, the chitosan coating could slow down the weight loss of fruit from 2.46% per day in the control to 2.14%. The cooperation of carvacrol further inhibited weight loss, and the daily weight loss rates of chitosan + 0.1% and 0.2% carvacrol were 1.82% and 1.65%, respectively.

Firmness is one of the most important indicators of fruit quality, and its softening during postharvest storage is mainly due to the change in cell wall composition under the catalysis of various enzymes (such as polygalacturonase and pectin methylesterase), which decompose the middle lamella between cells. Oxygen is necessary for these enzymes, and the barrier characteristics of the coating may inhibit their activities [12]. The chitosan–carvacrol coatings delayed fruit ripening through respiratory regulation and suppressed the consumption of water, organic acids, and other substances. Previous studies have shown that chitosan coatings effectively reduced the respiration rate of fruit, maintaining SSC, titratable acidity, and weight loss [3,19]. Other coating materials also prolonged the shelf life of postharvest guavas. Gum arabic and *Aloe vera* gel extended guava shelf life, slowed weight loss, and resulted in higher titratable acidity [38], which is consistent with our results. The combination of a modified chitosan coating containing carvacrol nanoemulsions and pulsed light exhibited a high preservation efficiency on cucumber slices by producing positive effects on decontamination [39]. Further studies need to address the physiological changes in the fruit, including respiration rate, ethylene release rate, etc., to provide more comprehensive information on how the coating affects fruit physiology change. Since both chitosan and carvacrol exhibit antimicrobial properties, it is also important to study how the microbial parameters change in fruit surfaces/wounds, which provide decay and microbial-related information.

3.4. Total Phenolic Content

Polyphenols are important bioactive substances that contribute to the antioxidant activity of guava. During fruit ripening and senescence, polyphenols will gradually decrease, reducing the nutritional value of the fruit. Chitosan coatings incorporating carvacrol delayed the decline in total phenolic content (Figure 3E). On the last day of storage, the total phenolic content of the chitosan + 0.2% carvacrol was 17.14% ($p < 0.05$) higher than that of the control.

The decline in total phenolic content in guava is related to the higher respiration rate. Previous studies have shown that controlled atmosphere storage can slow down the ripening process and maintain the phenolic content [40]. The chitosan coatings prepared in this study may delay polyphenol decomposition by atmosphere regulation. Similarly, higher contents of polyphenols and flavonoids were observed in other edible coatings,

maintaining the antioxidant capacity and nutritional value of the coated guavas [41]. Polyphenols have been shown to be directly associated with antimicrobial activity. They also serve as signal molecules involved in defense responses against stress, affecting postharvest disease incidence [42]. Further study will determine whether the combination of chitosan and carvacrol in coatings affects polyphenol content by regulating antioxidant and antimicrobial activities, barrier properties, enzyme activities, pH alterations, and other potential pathways.

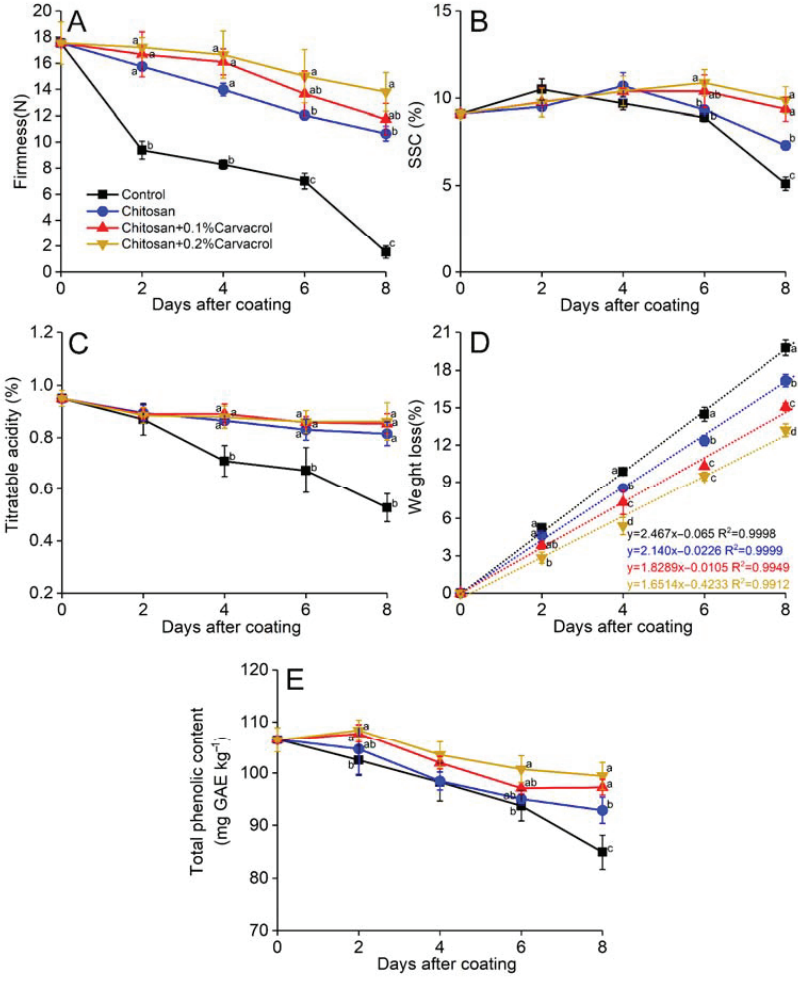

Figure 3. Effects of chitosan–carvacrol coating on quality parameters of postharvest guava fruit. Firmness (**A**), soluble solid content (SSC); (**B**), titratable acidity (**C**), weight loss (**D**), and total phenolic content (**E**). Each value is the mean of three replicates. The vertical bars represent the standard deviation of the means. The different letters indicate significant differences (ANOVA, $p < 0.05$) according to Duncan's multiple comparisons test with Holm correction.

3.5. Correlation and Principal Component Analysis of Fruit Quality Attribute Response to Chitosan–Carvacrol Coatings

Based on the data presented in Figure 4 and Table S1 in Supplementary Materials, it can be concluded that firmness, SSC, and TPC exhibited a negative correlation with weight loss and the color indexes. This suggests that with the fruit ripening, there was a consistent

decrease in firmness, SSC, and TPC accompanied by an increase in weight loss and color parameters. The correlation between titratable acid and the other indicators appeared to be relatively weaker; this may be due to cultivar differences. The initial titratable acid content was higher and remained higher during storage.

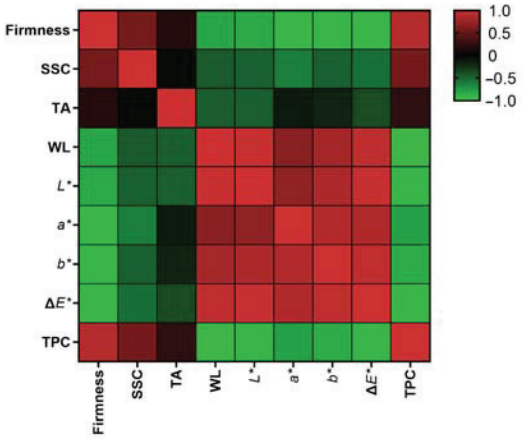

Figure 4. Pearson correlation analysis among the measured parameters. SSC: total soluble solid content; TA: titratable acidity; WL: weight loss; TPC: total phenolic content.

Principal component analysis (PCA) is a widely recognized method for reducing data dimensionality, which transforms a large amount of data into fewer dimensions while retaining the characteristics of the original data. All the measured indicators in this study were analyzed, and the cumulative contribution of the first five principal components obtained reached 99.0% (Table 2), which can explain the correlation characteristics of the data. Principal component 1 (PC1) explained 82.5% of the total variance and was primarily influenced by firmness, TA, and TPC. They clustered together in the score plot (Figure 5B), indicating close associations. PC2 explained 8.5% of the total variance of the original variables and was determined by the weight loss and color parameters, indicating a strong relationship between color variation and fruit weight loss. The relationship between these indicators was also observed in the PCA analysis of kiwi [43] and pear [44]. In the PC1 direction, there was a good distinction between the different storage times for each group (Figure 5A). At the beginning of storage, observation points were located in the negative half-axis of PC1, indicating good quality attributes. However, with prolonged storage, observations gradually shifted toward the positive half-axis of PC1. Notably, the last two data points of the control group were on the rightmost side of the PC1 axis, indicating the most significant quality deterioration. During the ripening, the SSC initially increased to a peak and then gradually decreased. The Cvc0.2_8 was located at the top of the positive axis of PC2, while the Con_8 treatment was at the negative half-axis of PC2, suggesting the SSC in the control group significantly decreased while the coating treatments maintained a higher level of SSC at the same time point, which was consistent with the actual measurement result.

Table 2. Eigenvalues and contribution rate of the principal components.

Principle Component	Eigenvalue	Percentage of Variance (%)	Cumulative (%)
1	7.423	82.479	82.479
2	0.762	8.467	90.945
3	0.471	5.238	96.183
4	0.160	1.780	97.963
5	0.097	1.083	99.046

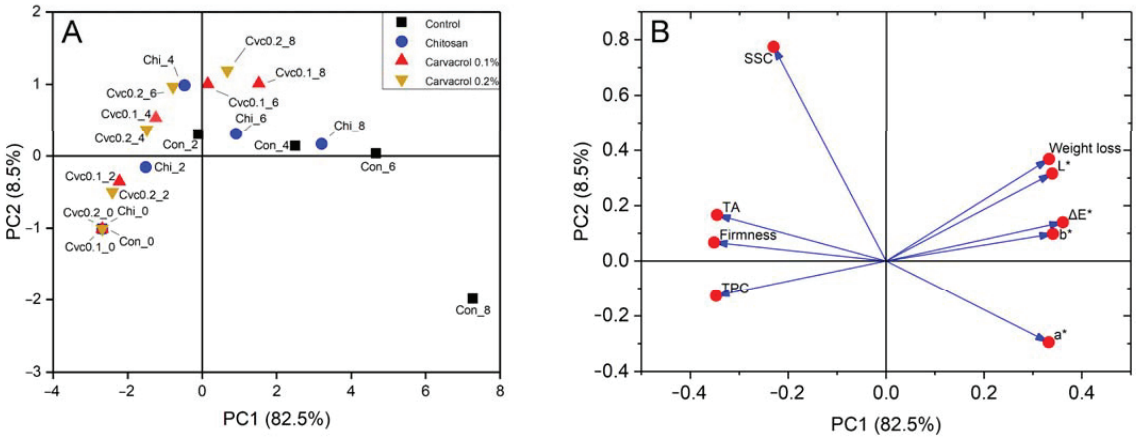

Figure 5. Principal component analysis (PCA) results of measured parameters. Score plot (**A**), loading plot (**B**). Con: control; Chi: chitosan; Cvc: carvacrol; SSC: total soluble solid content; TA: titratable acidity; TPC: total phenolic content. The number following the underscore indicates days after coating.

During the storage from day 2 to 8, the enclosed area of the control exhibited the greatest distance from the initial storage point, indicating a significant deterioration in fruit quality (Figure S1). The chitosan treatment shortened its enclosed area's distance on day 0, indicating an improvement in storage quality compared to the control. However, there was still some overlap between the chitosan treatment and the control group, suggesting that the improvement was not as pronounced. In contrast, the chitosan coating containing carvacrol was the closest to the initial point, and there was no overlap with the ensemble area of the control, indicating that the incorporation of carvacrol significantly improved fruit quality. This indicated that the chitosan coating containing carvacrol significantly enhanced the quality of the fruit. A similar result was also observed in the PCA conducted on the quality of litchi [45]. Considering the principal component score and loadings plots, chitosan coatings delayed fruit senescence, while the incorporation of chitosan and carvacrol coatings maintained better fruit quality compared to other treatment groups.

By evaluating the *F*-values of the component scores derived from PCA, *F*-values increased for each group with extended storage time, indicating quality deterioration compared to the initial state (Table 3). Based on the mean *F*-value average for each group, the four groups could be ranked in descending order: control > chitosan > chitosan + 0.1% carvacrol > chitosan + 0.2% carvacrol. This suggested that the control group experienced the most significant quality deterioration compared to the initial state of the fruit, while the chitosan + 0.2% carvacrol group exhibited the slightest change. This demonstrated that chitosan coatings incorporating carvacrol delayed the ripening and maintained the postharvest quality of guava fruit.

Further study is needed for the characterization and optimization of the coating, and more fruit physiological parameters need to be measured to provide more comprehensive insights into the coating's effectiveness. Also, the combination of chitosan and essential oil, multiple-layer coatings, and other coating materials should be further studied.

Table 3. Principal component score.

Group	Storage Time (d)	Name of Observations	FAC1	FAC2	FAC3	FAC4	FAC5	F1	F2	F3	F4	F5	*F*	*F* Average
Control	0	Con_0	−2.680	−1.010	−0.086	0.234	−0.104	−7.299	−0.880	−0.059	0.094	−0.031	−6.153	5.253
	2	Con_2	−0.094	0.304	1.245	0.485	−0.483	−0.255	0.265	0.854	0.194	−0.145	−0.185	
	4	Con_4	2.497	0.139	1.244	−0.065	0.338	6.802	0.121	0.853	−0.026	0.101	5.678	
	6	Con_6	4.668	0.036	1.396	0.604	0.234	12.716	0.032	0.957	0.242	0.070	10.596	
	8	Con_8	7.265	−1.990	−0.449	−0.763	−0.087	19.790	−1.735	−0.308	−0.305	−0.026	16.329	
Chitosan	0	Chi_0	−2.680	−1.010	−0.086	0.234	−0.104	−7.299	−0.880	−0.059	0.094	−0.031	−6.153	−0.243
	2	Chi_2	−1.513	−0.156	−0.274	0.007	0.203	−4.123	−0.136	−0.188	0.003	0.061	−3.445	
	4	Chi_4	−0.467	0.989	0.062	−0.325	−0.358	−1.272	0.862	0.042	−0.130	−0.107	−0.985	
	6	Chi_6	0.912	0.313	−0.258	−0.227	−0.699	2.484	0.272	−0.177	−0.091	−0.210	2.090	
	8	Chi_8	3.206	0.169	−1.556	0.817	0.014	8.734	0.147	−1.066	0.327	0.004	7.281	
Chitosan + 0.1% Carvacrol	0	Cvc0.1_0	−2.680	−1.010	−0.086	0.234	−0.104	−7.299	−0.880	−0.059	0.094	−0.031	−6.153	−2.002
	2	Cvc0.1_2	−2.222	−0.353	0.071	−0.214	0.355	−6.053	−0.308	0.049	−0.086	0.106	−5.066	
	4	Cvc0.1_4	−1.235	0.538	−0.063	−0.361	0.042	−3.364	0.469	−0.043	−0.144	0.013	−2.761	
	6	Cvc0.1_6	0.159	1.007	−0.107	−0.241	−0.294	0.433	0.877	−0.074	−0.097	−0.088	0.435	
	8	Cvc0.1_8	1.526	1.014	−0.679	0.403	0.052	4.156	0.884	−0.465	0.161	0.016	3.534	
Chitosan + 0.2% Carvacrol	0	Cvc0.2_0	−2.680	−1.010	−0.086	0.234	−0.104	−7.299	−0.880	−0.059	0.094	−0.031	−6.153	−3.008
	2	Cvc0.2_2	−2.410	−0.500	0.181	−0.173	0.377	−6.564	−0.436	0.124	−0.069	0.113	−5.502	
	4	Cvc0.2_4	−1.481	0.372	0.067	−0.408	0.310	−4.034	0.324	0.046	−0.163	0.093	−3.332	
	6	Cvc0.2_6	−0.775	0.967	0.206	−0.470	−0.111	−2.111	0.843	0.141	−0.188	−0.033	−1.686	
	8	Cvc0.2_8	0.681	1.191	−0.741	−0.004	0.523	1.856	1.039	−0.508	−0.002	0.157	1.632	

4. Conclusions

The result illustrated that a 2% (*w*/*v*) chitosan coating containing 0.1–0.2% carvacrol (*v*/*v*) maintained the postharvest quality of guava compared to the chitosan alone, with higher firmness, soluble solid content, titratable acid, and total phenolic content, with lower weight loss and peel yellowing. The results were verified by correlation and PCA analysis. Chitosan coatings containing carvacrol can be applied as an effective strategy to improve postharvest guava and other fruit quality.

Supplementary Materials: The following supporting information can be downloaded at https://www.mdpi.com/article/10.3390/horticulturae10010080/s1, Table S1: The R-value of Pearson correlation analysis among the measured parameters; Figure S1: Principal component score plots containing enclosed areas of each group.

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LED White Light Treatment Delays Postharvest Senescence of 'Zaosu' Pear Fruit with Inhibited Chlorophyll Degradation

Hongbo Mi [1], Xu Zhou [1], Jun Yang [1], Jingxin Chen [1,*] and Bin Liu [2,*]

[1] College of Food Science and Engineering, Bohai University, Jinzhou 121000, China; mihongbo1001@163.com (H.M.); zhouxu1009@163.com (X.Z.); yangjun980907@163.com (J.Y.)
[2] Hami Melon Research Center, Xinjiang Academy of Agricultural Sciences, Urumqi 830091, China
* Correspondence: chenjingxin180302@163.com (J.C.); liu.bin@xaas.ac.cn (B.L.)

Abstract: To investigate the effects of LED white light treatment (LWT) on the storage quality of postharvest 'Zaosu' pears, as well as its role in maintaining fruit greenness and delaying senescence, pear fruits were treated with intermittent irradiation using LED white light for 12 h per day, with a light source distance of 30 ± 1 cm and a photon flux density of 151 μmoL/(m^2·s) at 25 °C. The results showed that LWT preserved the postharvest quality of 'Zaosu' pear fruit by reducing weight loss and ascorbic acid degradation and promoting the ratio of sugar and organic acid. Meanwhile, LWT also substantially inhibited the respiration intensity and ethylene production during the storage process. Accordingly, the expressions of genes related to ethylene biosynthesis and signaling were reduced in LWT fruit. Notably, LWT retarded the decrease in chlorophyll content of fruit by increasing the activities of enzymes associated with chlorophyll synthase. Additionally, LWT also suppressed the chlorophyll degradation-related enzymes and their gene expressions in pear peel. These findings suggest that a moderate light irradiation can delay the de-green progress and benefit post-harvest storage of 'Zaosu' pear.

Keywords: illumination; pear fruit; chlorophyll degradation; fruit senescence

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1. Introduction

The 'Zaosu' pear (*Pyrus ussuriensis* Var. Zaosu) is a high-quality, high-yielding, strong resistance, and early maturing pear variety bred by the Research Institute of Pomology of the Chinese Academy of Agricultural Sciences (Xingcheng). It is also one of the most widely cultivated early maturing pear varieties in China [1]. The 'Zaosu' pear is favored by Chinese consumers due to its vibrant green color, minimal stone cells, abundant juice, and exquisite taste [2]. However, the high water content in the pear results in postharvest wrinkling and shrinkage, leading to physiological disorders and a decline in overall quality [3]. The pear fruit exhibits high respiration intensity and rapid nutrient consumption, which accelerate senescence and diminish its nutritional value [4]. Notably, the peel of the 'Zaosu' pear undergoes a rapid transition from green to yellow shortly after harvest, typically within ten days, which greatly compromises both the edible quality and commercial value of the fruit [5,6].

Currently, numerous approaches have been explored to preserve the green color of the fruit peel during storage and transportation. These include a controlled atmosphere, 1-methylcyclopropene (1-MCP), heat treatment, ozone, ethanol, plant growth regulators, plant extracts, and film preservatives [7–16]. Interestingly, researchers in the horticultural post-harvest field have shown great interest in light-emitting diodes (LEDs) due to their remarkable attributes. LEDs are recognized for being environmentally friendly, energy efficient, capable of close-range irradiation, and for generating minimal heat [17]. Research studies have demonstrated that LED light irradiation is highly effective in suppressing the growth of surface microorganisms on fruits and vegetables [18]. Furthermore, it has

been observed that it slows down ethylene release and respiration rate, thereby achieving successful post-harvest preservation [19]. In addition, fruits and vegetables treated with LED exhibit well-preserved nutritional value, flavor quality, and color, as evidenced by studies [19].

Based on the above, in this study, the 'Zaosu' pear was used as the experimental material to investigate the impact of LED white light treatment on the post-harvest quality and chlorophyll metabolism of the fruit during storage at 25 °C, providing an experimental basis and theoretical reference for the green preservation effect and storage preservation technology of 'Zaosu' pear fruits after harvest.

2. Materials and Methods

2.1. Plant Materials and Treatments

The 'Zaosu' pear fruit was harvested from a local orchard in Jinzhou City on September 10th, 2021 and transported to the Bohai University laboratory within 2 h after harvesting. A total of 112 fruits with a weight of 200–230 g, no diseases or pests, no mechanical damage, uniform size, similar color, and excellent texture were selected and stored in conditions of 25 ± 1 °C and RH 85–90%. The pear fruits were randomly divided into two groups: (i) control group: dark treatment group (control); (ii) LED white light treatment group (LWT): the fruit was placed in conditions of 25 ± 1 °C at a distance of 30 ± 1 cm from the light source, with a light density of 151 μmol/m^2·s, and subjected to intermittent light for 12 h/d (from 08:00 to 20:00 every day, remaining in darkness for the rest of the time). The specific experimental setup is shown in Figure 1. The pear fruit samples were turned over every 24 h and sampled on days 0, 5, 10, 15, 20, and 25. On the sampling day, color difference, weight loss rate, respiration intensity, and firmness were measured. The remaining parts (peel, flesh) were frozen with liquid nitrogen and stored in a −80 °C refrigerator for subsequent experiments.

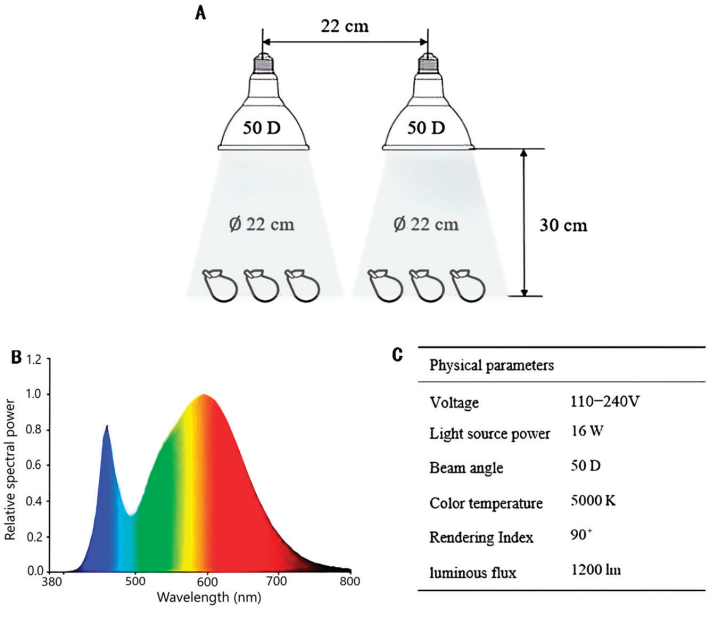

Figure 1. Schematic diagram of experimental apparatus (**A**), spectra (**B**), and physical parameters (**C**) of LED white light.

2.2. Color, Weight Loss, and Firmness

The fruit color was measured using a color spectrum CS-520 spectrophotometer (Suzhou Liuxinyan Precision Machinery Inc., Suzhou, China). Nine pear fruits were randomly selected from each group. Three equidistant positions were chosen on the equatorial part of the fruit, and the corresponding a* and b* values were measured and recorded. Subsequently, the h value was calculated using the formula [20]:

$$h^\circ = \arctan(b^*/a^*) \qquad (a^* > 0, b^* > 0)$$

$$h^\circ = 180 + \arctan(b^*/a^*) \qquad (a^* < 0, b^* > 0)$$

where the a* value represents the red–green chromaticity and the b* value represents the yellow–blue chromaticity. The h value has four color boundary points: h = 0° (red), h = 90° (yellow), h = 180° (green), and h = 270° (blue) [21].

For each group, five pear fruits were selected. The weight loss rate of the fruit was determined using a weighing method, and the following formula was employed to calculate the mass difference:

$$\text{Weight loss rate (\%)} = (\text{Weight before storage} - \text{Weight after storage})/\text{Quality before storage} \times 100$$

The fruit firmness was measured using the method described by Villarreal [6], using a digital fruit hardness meter (GY-4, Beijing Sunshine Yishida Technology Inc., Beijing, China) with a probe diameter of 11 mm and a testing distance of 10 mm. Three equidistant positions were selected on the equatorial part of the fruit, and the fruit peel, approximately 1.0 mm in thickness, was removed. For each group, nine fruits were randomly selected from each treatment group. Firmness was expressed in Newton (N).

2.3. Ascorbic Acid Content

The ascorbic acid (AsA) content in the fruit was determined using the 2,6-dichlorophenol indophenol titration method, as described by Skrovankova et al. [22] with some modifications. Five grams of frozen fruit powder was put into 100 mL of oxalic acid solution (20 g/L) and mixed thoroughly. The mixture was kept in dark conditions for 10 min and was filtered using a Whatman® qualitative filter paper. Ten milliliters of filtrate were transferred into a conical flask and titrated with a standard 2,6-dichlorophenol indophenol solution. Ten milliliters of oxalic acid solution (20 g/L) was used as a blank control. This was repeated three times for each sample. The content of ascorbic acid was expressed in mg/100 g.

$$\text{Ascorbic acid content}(\text{mg}/100\ \text{g}) = [V \times (V_1 - V_0) \times C/V_s \times W] \times 100$$

where V_1 represents the volume of dye consumed in the sample titration (mL); V_0 denotes the volume of dye consumed in blank titration (mL); C indicates the 1 mL of dye solution equivalent to the amount of ascorbic acid (mg); V_s expresses the volume of sample solution taken during titration (mL); V signifies the total volume of sample extraction solution (mL); and W refers to the sample weight (g).

2.4. Sugar-to-Acid Ratio

The content of total soluble solids (TSS) was measured using a PAL-1 refractometer (Tokyo, Japan). Nine 'Zaosu' pear fruits were randomly selected, and their flesh was cut and mixed thoroughly. The juice was extracted and centrifuged, and the supernatant was used to measure the value of TSS, which was expressed as a percentage.

The titratable acid (TA) content was determined using the acid-base titration method as described by Skrovankova et al. [22]. Approximately 1.5 g of pear fruit samples was weighed into 3.0 mL of CO_2-free distilled water and mixed thoroughly. The mixture was then centrifuged at 4 °C and 10,000 rpm for 20 min. Subsequently, 1.0 mL of the supernatant was taken, and 2 drops of 1% (*w*/*v*) phenolphthalein indicator were added. The volume of

0.1 M NaOH solution consumed during titration was recorded, and the titratable acid was expressed as a mass fraction (%). The conversion factor was calculated based on the malic acid (0.067).

The sugar-to-acid ratio was calculated as the ratio of soluble solid content to titratable acid content in the pear fruit.

2.5. Respiratory Intensity and Ethylene Production

The method for respiratory intensity and ethylene production measurement was in line with Sun's [23], with some modification. Nine pear fruits were randomly selected and weighed, then placed in a sealed desiccator at 20 °C. After sealing for one hour, one milliliter of the gas at the top of the container was collected for further analysis.

For gas chromatography analysis, the carrier gases used were 0.05 MPa of nitrogen, 0.1 MPa of hydrogen, and 0.1 MPa of air. The gas flow rate was set at 40.0 mL/min (7890A, Agilent Technologies Inc., Santa Clara, CA, USA). The respiration intensity and ethylene release were expressed as mg CO_2/(kg·h) and μL/(kg·h), respectively.

$$\text{Ethylene evolution}\left(\mu L \cdot kg^{-1} \cdot h^{-1}\right) = C \times V/W \times t \times 1000$$

where C represents the ethylene content in the sample gas determined by gas chromatography (μL/L); V denotes the volume of the glass container's confined space (mL); T indicates the measurement time (h); and W signifies the fruit weight (kg).

2.6. Chlorophyll Content and Related Enzyme Activities

As described by Esteban et al. [24], with some modifications, the chlorophyll content was measured via spectrophotometry. Approximately 1.0 g of pear peel sample was weighed and placed into 3.0 mL of pre-cooled extraction solution (80% (v/v) acetone: 95% (v/v) ethanol = 2:1). The mixture was stirred and kept at 4 °C in darkness for 30 min. The obtained suspension was centrifuged at 4 °C and 10,000 rpm for 15 min. The supernatant was collected and the absorbance values measured at 645 nm and 663 nm using a spectrophotometer (GS-520, Suzhou Liuxinyan Precision Machinery Inc., Suzhou, China). The chlorophyll content was expressed as mg/g FW (milligrams per gram of fresh weight). The contents of chlorophyll a and b, and total chlorophyll were calculated using the following formulas.

$$\text{Chlorophyll-a} = 12.72A_{663} - 2.59A_{645}$$

$$\text{Chlorophyll-b} = 22.88A_{645} - 4.67A_{663}$$

$$\text{Total chlorophyll content} = 20.29A_{645} + 8.05A_{663}$$

To determine the activities of chlorophyll metabolism-related enzymes (chlorophyll synthase, chlorophyll a oxygenase, chlorophyllase, Mg-dechelatase, pheophytinase, and pheophorbide a oxygenase) in the fruit peel sample, 1 g of fruit peel sample was taken and added into 9 mL of phosphate buffer (pH 7.2–7.4, 0.01 M elemental acid, hydrophosphate and sodium chloride). The mixture was homogenized in an ice bath, centrifuged at 4 °C and 4500× g for 20 min, and the supernatant was collected for further analysis. The plant enzyme-linked immunosorbent assay (ELISA) kits (Plant CAO ELISA Kit, Plant PPH ELISA Kit, Plant CS ELISA Kit, Plant Chkase ELISA Kit, Plant MDCase ELISA Kit, Plant PAO ELISA Kit, Shanghai Enzyme-linked Biotechnology Inc., Shanghai, China) were used. The ELISA assays were performed according to the kit instructions, with triplicate measurements for each group. The activities of enzymes were expressed as U/g FW (units per gram of fresh weight). A change of 0.01 per minute was considered as one unit of enzyme activity.

2.7. Fluorescence Quantitative PCR

The RNA extraction was performed using the CTAB method, referring to the method described by Chen et al. [25]. The reverse transcription was carried out using the FastKing RT Kit cDNA first strand synthesis kit (Cat # KR116-02, Tiangen Biochemical Technology Co., Ltd., Beijing, China). In line with previous research [26–28], PbNYC, PbNOL, PbCLH, PbSGR, PbPPH, PbPAO, PbRCCR, PbACS4, PbACO1, PbACO4, PbETR1, PbETR2, PbERS1, PbERS2, PbCTR1, PbEIN2, PbEIL1, and PbERF1 were selected, and their primers are listed in Table 1. The relative expression of the genes was calculated using the $2^{-\Delta\Delta CT}$ algorithm [29].

Table 1. Primers for genes selected for fluorescence quantitative PCR.

Target Gene	Gene ID	Primer Sequence
PbNYC	JN167997	F: GGCATAGCATGTGATGTTTG
		R: GCACCAGCATTGTTTATCC
PbNOL	JN167996	F: CCGTGAGGCAATAAAAATGATG
		R: CGCTTTGTTGCCCCGTAT
PbCLH1	JN168001	F: CAACGTTCATAGACTTCCCCTACG
		R: GGAGAGTACAAGGCAAGAGCTGC
PbSGR1	JN168000	F: ATTCAACAAGGTGGAGTGCTGG
		R: GCCGCTGTTGTTTTCCTGG
PbPPH	JN168999	F: CTGCTGCATCATTGGCCTC
		R: CTCGTAATATGGAGCTTCAGGC
PbPAO	JN168998	F: GCGACATCGACGAGCAAGTT
		R: TCGAAAGCGACCCACGATTC
PbRCCR	JN168002	F: TTCATAGACTTCCCCTACGTGTCG
		R: GGAGAGTACAAGGCAAGAGCTGC
EF1-α	AH009876	F: CAAGTATGCCTGGGTGCTTG
		R: TCAGCCTGTGAAGTTCCAGT

2.8. Statistical Analysis

All the data were expressed as mean ± standard deviation and analyzed using Excel 2016 software (Microsoft, Washington, DC, USA). SPSS 21.0 software (International Business Machines Corporation, Armonk, NY, USA) was used for one-way analysis of variance (ANOVA) with significant differences between groups at $p < 0.05$ (Duncan).

3. Results

3.1. Effect of LED White Light on the Postharvest Quality of Pear Fruit

During the entire storage period, the weight loss rate of pear fruit in the dark (control) and LED white light treatment (LWT) groups showed an increasing trend. The weight loss of the LWT group was significantly lower than the control group ($p < 0.05$), indicating that LED white light treatment, to some extent, inhibited the evaporation of water and respiration consumption of 'Zaosu' pear fruit during storage (Figure 2A).

Figure 2B shows that the levels of ascorbic acid (AsA) in both groups of pear fruit exhibited a decreasing trend during storage. However, by day 15, the AsA content in the LWT group was significantly higher than in the control group ($p < 0.05$). Therefore, it can be observed that LED white light treatment effectively inhibits the decline in AsA content in 'Zaosu' pear fruit during storage, particularly in the early stages of storage.

During the storage period, there was no significant difference in the firmness of 'Zaosu' pear fruit between the two treatment groups ($p > 0.05$, Figure 2C), indicating that LED white light treatment did not have an impact on the changes in fruit firmness during storage.

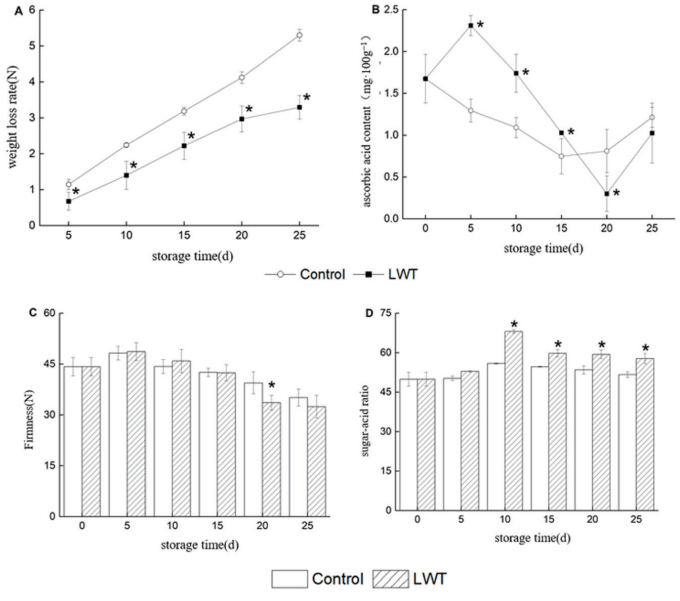

Figure 2. Effects of LED white light treatment (LWT) on weight loss rate (**A**), ascorbic acid content (**B**), firmness (**C**), and sugar–acid ratio (**D**) of pear fruit. The asterisk on the column represents the significant difference between groups ($p < 0.05$).

In both treatment groups, the sugar-to-acid ratio showed a similar trend of initially increasing and then decreasing, reaching its peak on day 10 (Figure 2D). The LWT group was significantly higher than the dark treatment group ($p < 0.05$), indicating that LED white light is beneficial in maintaining the favorable taste of 'Zaosu' pear fruit (Figure 2D).

3.2. Effect of LED White Light on Respiratory Intensity and Ethylene Production of Pear Fruit

As shown in Figure 3, 'Zaosu' pears exhibited typical peaks for respiration rate and ethylene production during storage. The respiration rate and ethylene production of the pear fruit in both treatment groups showed a trend of initially increasing and then decreasing, with peak values occurring on days 15 and 20, respectively. However, the respiration rate and ethylene production of pear fruit in the LWT group were significantly lower than in the control group ($p < 0.05$). This indicates that LWT can inhibit the respiration process and ethylene synthesis in pear fruit.

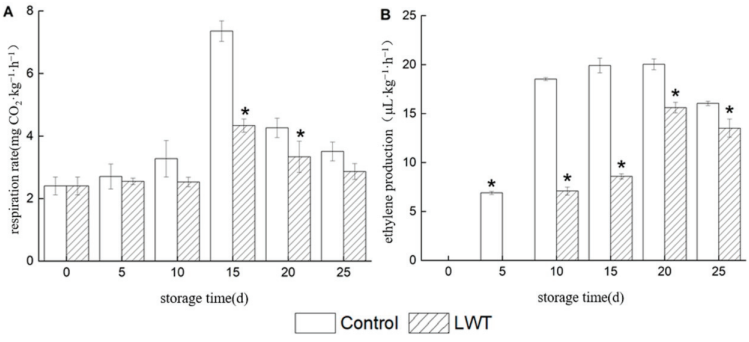

Figure 3. Effects of LED white light treatment (LWT) on the respiration rate (**A**) and ethylene production (**B**) of pear fruit. The asterisk on the column represents the significant difference between groups ($p < 0.05$).

3.3. Effect of LED White Light on the Expression of Genes Related to the Ethylene Biosynthesis Pathway in Pear Fruit

Throughout the entire storage period, the relative expression levels of PbACS4 and PbACO4 in 'Zaosu' pear fruit treated with LED white light showed a continuous increase, whilst their relative expression levels in the control group exhibited an initial increase followed by a decrease, with peak values occurring on day 20 (Figure 4A,C). Within the first 20 days of storage, the expression levels of PbACS4 and PbACO4 in the LWT group were significantly lower than in the control group ($p < 0.05$). The expression levels of PbACO1 in both treatment groups showed a trend of initially increasing and then decreasing, with peak values occurring on days 15 (control group) and 20 (LWT), and the LWT group was significantly lower than the control group ($p < 0.05$, Figure 4B).

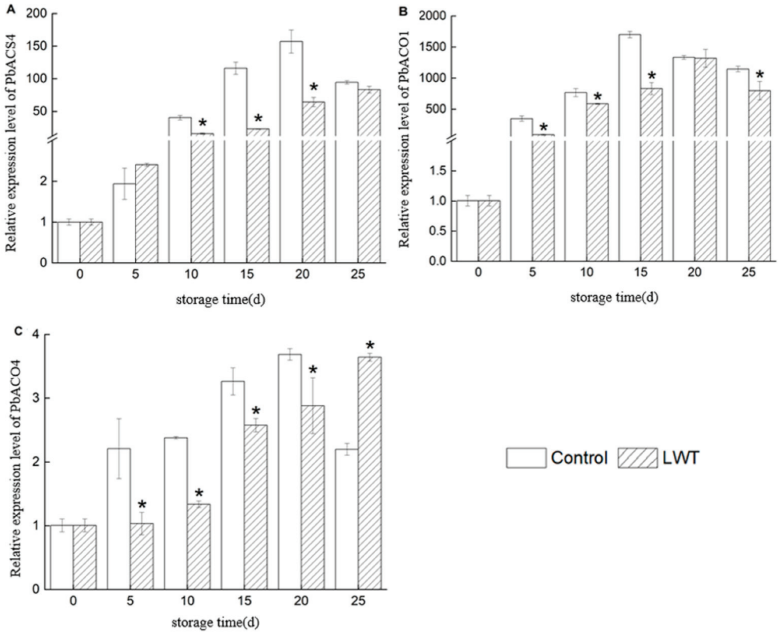

Figure 4. Effects of LED white light on the expression of *PbACS4* (**A**), *PbACO1* (**B**) and *PbACO4* (**C**) genes, which are related to the ethylene biosynthesis pathway of pear fruit. The asterisk on the column represents the significant difference between groups ($p < 0.05$).

3.4. Effect of LED White Light on the Expression of Ethylene Signal Transduction Pathway-Related Genes in Pear Fruit

Figures 5 and 6 show the expression levels of ethylene receptor genes (ETR1, ETR2, ERS1, and ERS2) and signal transduction factors (CTR1, EIN2, EIL1, and ERF1) during storage. Figure 5 shows that during storage, the expression levels of ethylene receptor genes in the control group pear fruit exhibited an initial increase followed by a decrease trend, reaching a peak on day 20, which was consistent with the change in ethylene release. Prior to the ethylene peak, the expression levels of ethylene receptor genes in LWT pear fruit were significantly lower compared to the control group ($p < 0.05$). Then, on day 25, the expression level of PbETR1 in the LWT group fruit was significantly lower than the control group ($p < 0.05$), while the expression levels of PbETR2, PbERS1, and PbERS2 in the LWT group fruit were significantly higher than the control group ($p < 0.05$).

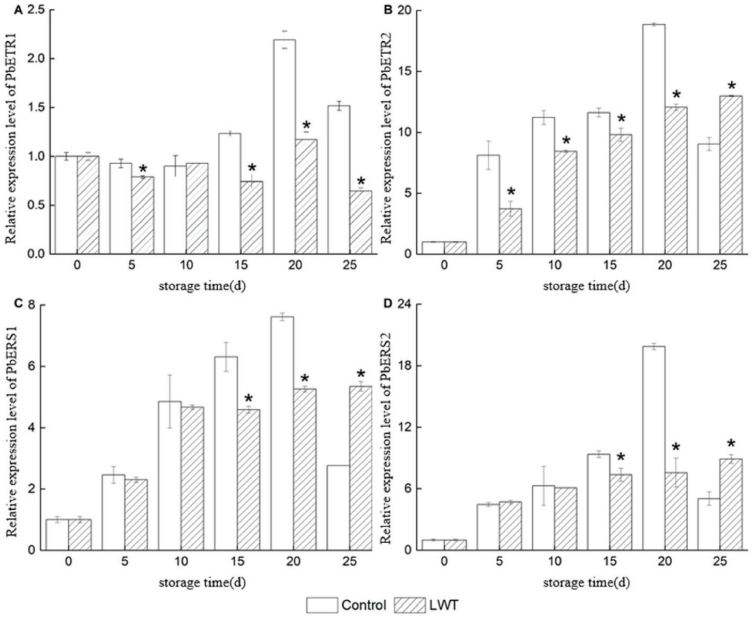

Figure 5. Effect of LED white light on the expression of *PbETR1* (**A**), *PbETR2* (**B**), *PbERS1* (**C**) and *PbERS2* (**D**) genes, which are ethylene receptors in pear fruit. The asterisk on the column represents significant differences between groups ($p < 0.05$).

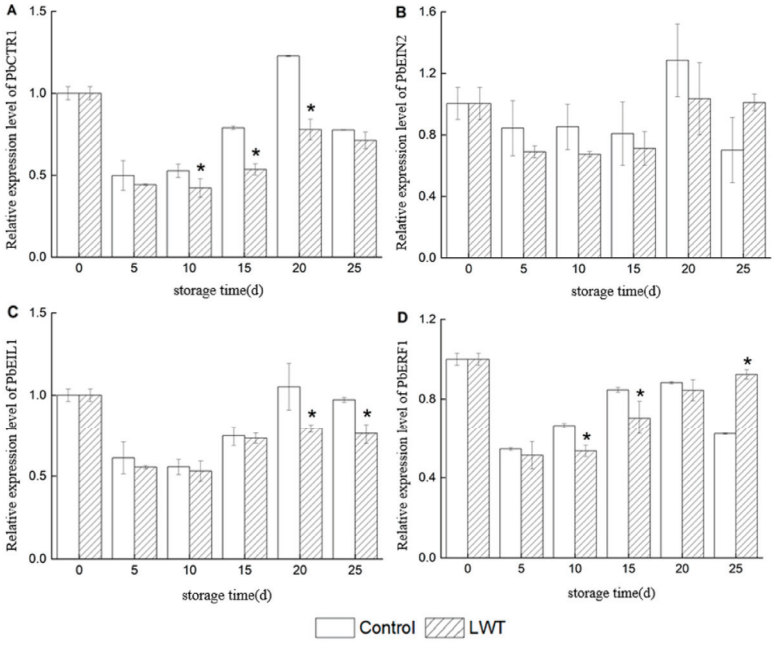

Figure 6. Effect of LED white light on the expression of *PbCTR1* (**A**), *PbEIN2* (**B**), *PbEIL1* (**C**) and *PbERF1* (**D**) genes, which are ethylene signal transduction factors in pear fruit. The asterisk in the figure represents significant differences between groups ($p < 0.05$).

Figure 6 shows that during storage, the ethylene signal transduction genes (PbCTR1, PbEIN2, PbEIL1, and PbERF1) in the pear fruit exhibited an initial increase followed by a decrease trend. Prior to the ethylene peak, the expression levels of PbCTR1 and PbERF1 in the LWT group fruit were significantly lower than the control group ($p < 0.05$), while there was no significant difference in the expression levels of PbEIN2 and PbEIL1 between the two groups ($p > 0.05$). After the ethylene peak, the expression level of PbERF1 in the LWT group fruit was significantly higher than the control group ($p < 0.05$), while the expression level of PbEIL1 in the LWT group fruit was significantly lower than the control group ($p < 0.05$).

3.5. Effect of LED White Light on the Color Change and Chlorophyll Content of Pear Fruit

Color change is an important indicator of quality decay of the fruit. As shown in Figure 7, the pear fruit in the control group exhibited significant yellowing of the skin after 15 days of storage, while the pear fruit in the LWT group displayed this phenomenon after 20 days of storage. This indicated that LED white light treatment delayed the occurrence of skin fading and yellowing in 'Zaosu' pear fruit.

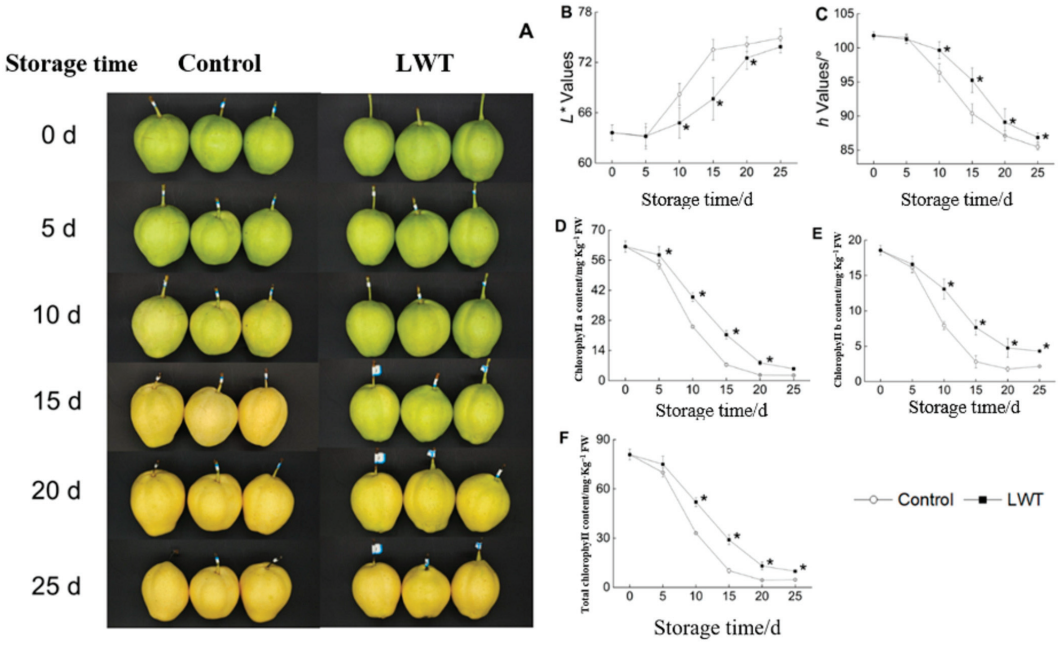

Figure 7. Effect of LED white light on color change ((**A**), pear fruit; (**B**), *L** valuses; (**C**), *h* values) and chlorophyll content ((**D**), chlorophyll a content; (**E**), chlorophyll b content; (**F**), total chlorophyll content) of pear fruit. The asterisk in the figure represents significant differences between groups ($p < 0.05$).

The skin greasiness can make fruit appear brighter and show higher L* values. During storage, the L* values of pear fruit in both groups showed an increasing trend, but the LWT group was significantly lower than the control group ($p < 0.05$, Figure 7B), indicating that LED white light treatment delayed the greasiness process of the pear skin.

The h values of pear fruit in both groups showed a decreasing trend (Figure 7C), indicating a gradual color transformation from green to yellow. Furthermore, the h value of the LWT group fruit was significantly higher than the control group ($p < 0.05$), which further confirmed that LED white light treatment effectively delayed the yellowing of 'Zaosu' pear fruit during storage.

During storage, the chlorophyll a, chlorophyll b, and total chlorophyll content of the pear fruit in both groups showed a decreasing trend (Figure 7D–F). However, the chlorophyll content of the pear fruit in the LWT group was significantly higher than the control group ($p < 0.05$), consistent with the trend of color change during storage. This indicated that LWT maintained the vibrant green color of the pear skin by inhibiting the degradation of chlorophyll in the fruit during storage.

3.6. Effect of LED White Light on the Activities of Enzymes Related to Chlorophyll Synthesis and Metabolism in Pear Fruit

During storage, the pear fruit in both groups showed a trend of initial increase followed by a decrease in CAO enzyme activity. The CAO enzyme activity in the LWT group reached its maximum value on day 20 of storage, while in the control group it reached its maximum value on day 15. The CAO enzyme activity in the LWT group was significantly higher than the control group (Figure 8A, $p < 0.05$). Similarly, the CS enzyme activity in the 'Zaosu' pear fruit subjected to dark treatment also showed a trend of initial increase followed by a decrease and reached its maximum value on day 20 of storage. The CS enzyme activity in the pear fruit subjected to LED white light treatment showed an increasing trend and was

significantly higher than the dark treatment group (Figure 8B, $p < 0.05$). This suggested that LED white light treatment enhanced the activity of chlorophyll synthesis-related enzymes.

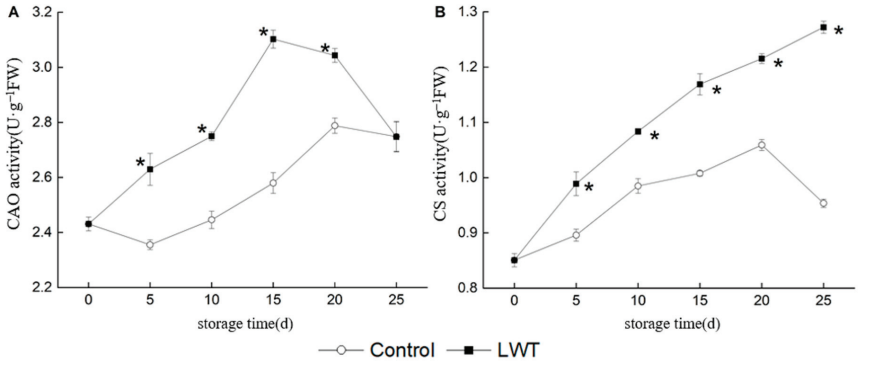

Figure 8. Effect of LED white light on the activities of CAO (**A**) and CS (**B**) enzymes related to chlorophyll synthesis in pear fruit. The asterisk in the figure represents significant differences between groups ($p < 0.05$).

3.7. Effect of LED White Light on the Activities and Gene Expression of Enzymes Related to Chlorophyll Metabolism in Pear Fruit

Chlorophyll degradation is the main reason for the yellowing of 'Zaosu' pears. As shown in Figure 9A, during the entire storage period, the Chlase activity in both groups showed a decreasing trend. The LWT group exhibited a particularly significant decrease on day 5, while a pronounced decrease in the control group occurred on day 20 of storage. The Chlase activity in the LWT group was significantly lower than the control group ($p < 0.05$). Additionally, the PAO activity in the LWT group of pear fruit was significantly lower than the control group (Figure 9B, $p < 0.05$). Furthermore, the expression levels of PbPAO, PbNYC, PbNOL, PbPPH, and PbRCCR genes in the LWT group of fruit were also significantly lower than the control group (Figure 10, $p < 0.05$). So, it can be observed that LED white light treatment to some extent inhibited the activity of chlorophyll degradation-related enzymes or the expression of enzyme genes.

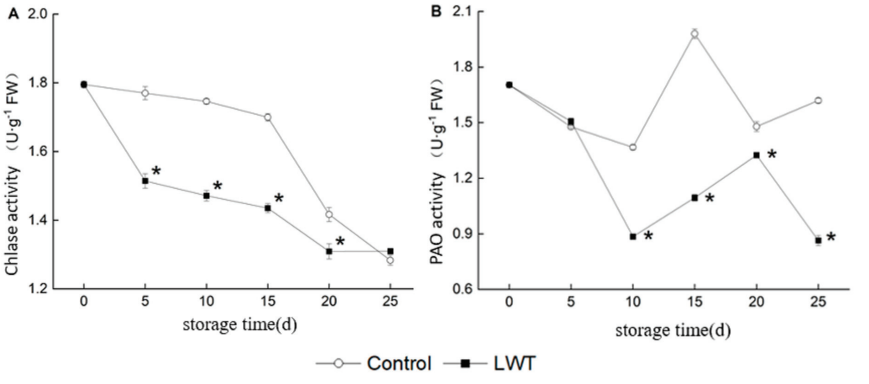

Figure 9. Effect of LED white light on the activities of Chase (**A**) and PAO (**B**) enzymes related to chlorophyll degradation in pear fruit. The asterisk in the figure represents significant differences between groups ($p < 0.05$).

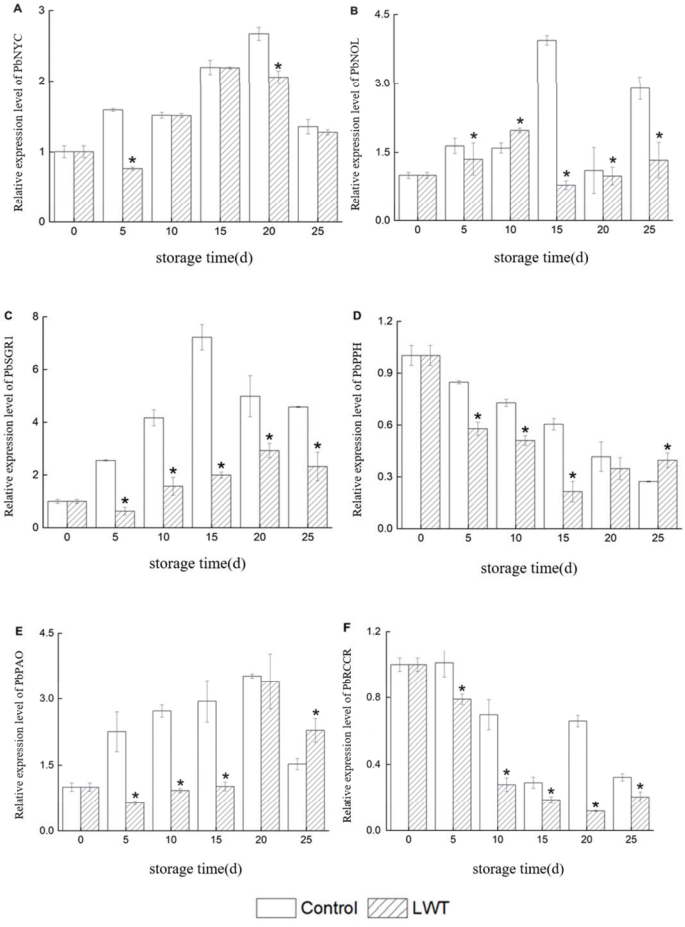

Figure 10. Effect of LED white light on the expression of *PbNYC*(**A**), *PbNOL* (**B**), *PbSGR1* (**C**), *PbPPH* (**D**), *PbPAO* (**E**) and *PbRCCR* (**F**) genes related to chlorophyll degradation in pear fruit. The asterisk in the figure represents significant differences between groups ($p < 0.05$).

4. Discussion

'Zaosu' pear fruit have a bright green skin and a crisp texture when harvested. However, this kind of pear fruit are prone to yellowing and have a short storage period [1]. In this study, LED white light treatment (LWT) inhibited weight loss in 'Zaosu' pear fruit during storage (Figure 2A). At the same time, LWT also substantially curbed the decline in ascorbic acid content in the pear fruit during the early storage period (Figure 2B,C), which is consistent with the findings of Joanna [30], who demonstrates that light exposure can prevent a decrease in ascorbic acid content in citrus fruit peel caused by bagging. Ascorbic acid (AsA) has been known to play a crucial role in scavenging free radicals, as well as in the protection and maintenance of the reduced state of compounds such as flavonoids and polyphenols [26].

The sugar-to-acid ratio is one of the important indicators that affect fruit texture and consumer preference [27]. LWT also effectively increased the sugar-to-acid ratio content of 'Zaosu' pear fruit (Figure 2D), thus maintaining their good taste. LED light treatment has been proved to be able to promote the accumulation of sucrose and fructose in fruit, affecting carbohydrate synthesis and transport, thereby influencing the soluble sugar content and the sugar-to-acid ratio [31].

'Zaosu' pear fruit treated with LED white light maintained a lower respiratory intensity and ethylene release (Figure 3A,B), contributing to the delayed ripening and aging processes of the fruit. This finding was consistent with the process of green-to-yellow color fading in the peel (Figure 3A,B). Similar results have been reported by Hasperué et al. [32], who show that the respiratory intensity and ethylene release of Brussels sprouts could also be reduced by LED light treatment, resulting in delayed aging and yellowing processes. In addition, the LWT suppressed the expression levels of ethylene synthesis and signal transduction-related genes and delayed the occurrence of the peak of ethylene production in pear fruit after harvest (Figures 4A–C and 5A–D). This result confirmed the findings reported by Lv et al. [33] in apple fruit.

The occurrence of peel yellowing is closely related to the degradation metabolism of chlorophyll in the fruit [34]. As the pear fruit ripened, their peel color changed from green to yellow (Figure 7A–C). During storage, LWT effectively inhibited chlorophyll degradation and peel yellowing of 'Zaosu' pear fruit (Figure 7D–F). Mehmet et al. [21] revealed that LED white light treatment could prolong the storage time of lettuce and showed it had strong effect on maintaining green color and reducing chlorophyll degradation. Additionally, Vergara et al. [35] found that in olives, LED white light treatment not only effectively maintained the content of chlorophyll but also reduced the activity of enzymes related to chlorophyll degradation metabolism, which may be the main reason for its inhibition of yellowing. In this study, LED white light treatment also effectively increased the activity of chlorophyll synthesis-related enzymes in 'Zaosu' pear fruit (Figure 8), such as chlorophyll synthase (CS) and chlorophyll a oxygenase (CAO), and reduced the activity of chlorophyll degradation-related enzymes (Figure 9) such as chlorophyllase (Chlase) and pheophorbide a oxygenase (PAO). The expression pattern of chlorophyll degradation metabolism-related genes in 'Zaosu' pear fruit further confirmed the above findings (Figure 10).

5. Conclusions

In summary, LED white light treatment (LWT) reduced the respiration rate and ethylene production of 'Zaosu' pear fruit and inhibited the expression of genes related to ethylene synthesis and signal transduction, thereby delaying fruit ripening and senescence. Meanwhile, LWT delayed the yellowing of the pear fruit by increasing the chlorophyll content and the activities of its synthesis-related enzymes and inhibiting the activities of chlorophyll degradation-related enzymes and their gene expression. Additionally, LWT effectively curbed the decline in ascorbic acid content in 'Zaosu' pear fruit and improved the sugar–acid ratio. Thus, LED white light treatment can effectively retain the green color and delay the yellowing of 'Zaosu' pear fruit, prolonging their post-harvest storage and shelf life.

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A Combinatorial TIR1-Aux/IAA Co-Receptor System for Peach Fruit Softening

Yutong Zhao [1,†], Qing Wang [1,†], Dan Guan [1], Haiqing Yang [2], Jianwei Wu [3,*] and Yueping Liu [1,4,*]

[1] College of Bioscience and Resources Environment, Beijing University of Agriculture, Beijing 102206, China; 202130111002@bua.edu.cn (Y.Z.); wangqing9803@163.com (Q.W.); guanadan@hotmail.com (D.G.)
[2] Pinggu District of Fruit Bureau, Beijing 101200, China; yanghaiqing115@163.com
[3] National Engineering Research Center for Information Technology in Agriculture, Beijing 102206, China
[4] Key Laboratory for Northern Urban Agriculture Ministry of Agriculture and Rural Affairs, Beijing University of Agriculture, Beijing 102206, China
* Correspondence: wujw@nercita.org.cn (J.W.); liuyueping@bua.edu.cn (Y.L.)
† These authors contributed equally to this work.

Abstract: Fruit softening is an important characteristic of peach fruit ripening. The auxin receptor TIR1 (Transport Inhibitor Response 1) plays an important role in plant growth and fruit maturation. Still, little research has been conducted on the relation of TIR1 to the softening of peach fruits. In this study, the hardness of isolated peach fruits was reduced under exogenous NAA treatment at low concentrations. At the same time, the low concentration of NAA treatment reduced the transcription level of *PpPG* and *Ppβ-GAL* genes related to cell wall softening and *PpACS1* genes related to ethylene synthesis. The transient overexpression of the *PpTIR1* gene in peach fruit blocks caused significant down-regulation of the expression of early auxin-responsive genes, ethylene synthesis, and cell wall metabolic genes related to fruit firmness. Through yeast two-hybrid technology, bimolecular fluorescence complementary technology, and a firefly luciferase complementation imaging assay, we were able to unveil an interaction between PpTIR1 and PpIAA1/3/5/9/27 proteins. Furthermore, it was determined that the interaction depended on auxin and its type and concentration. These results show that the PpTIR1-Aux/IAA module has a possible regulatory effect on fruit ripening and softening.

Keywords: peach; fruit ripening and softening; auxin; TIR1-Aux/IAA co-receptor

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1. Introduction

Auxin was the earliest plant hormone discovered, and it has recently played a notable role in the regulation of fruit ripening. Applying exogenous auxin during the early ripening stage of strawberry, grape, and tomato fruit can inhibit the ripening process [1–3]. However, applying auxin to treat apples, pears, and plums before fruit ripening can induce ethylene synthesis, thus promoting fruit softening and ripening [4–6]. Peach is a typical climacteric fruit. Studies have shown that the softening after peach fruit maturation is related to ethylene synthesis, which is regulated by endogenous auxin [7]. The abrupt change in ethylene at the mature stage of peach fruit was regulated by IAA (indole-3-acetic acid) [8]. Other studies have shown that inhibiting IAA synthesis during the ripening of hard peaches leads to a decrease in ethylene synthesis, which causes the fruit to soften [9,10]. At the same time, studies have shown an ethylene-independent auxin regulation pathway in peach fruit ripening [11].

Auxin regulates diverse physiological and developmental processes through the perception and transduction of auxin signals. The canonical auxin signaling pathway is comprised of the SCFTIR1/AFBs complex, which includes SKP1, Cullin, and the auxin signaling F-box protein. Additionally, it involves the participation of the transcriptional suppressor Aux/IAA (Auxin/Indole Acetic Acid) and the transcription factor ARF (Auxin

Response Factor) [12]. In tomatoes, the transgenic line with overexpression of the *SlTIR1* gene manifests changes in leaf morphology and fruit setting compared with the wild type [13]. Other studies on cucumbers have found that the auxin receptor gene may play an important role in plant height, leaf morphology, and parthenocarpy [14]. Overexpression of the plum *PslTIR1* gene in tomatoes decreased the height of transgenic plants and altered fruit development and fruit softening by controlling genes related to cell wall decomposition [15]. TIR1-like auxin receptors are involved in regulating plum fruit development [6].

Degradation of the Aux/IAA repressors is critical for auxin signaling. At present, 25 Aux/IAA genes have been identified in tomatoes that are involved in regulating auxin-mediated multiple signaling pathways [16]. Among them, SlIAA3 is an important factor in the cross-regulation of physiological responses by auxin and ethylene, which regulate tomato leaf morphogenesis, floral organ development, fruit set, and fruit development [17]. SlIAA9 resulted in abnormal leaf shape and parthenocarpy of tomato [18]. SlIAA17 plays a role in regulating fruit quality, and it is found that the SlIAA17 silencing line has larger fruit and thicker pericarp [19].

The regulatory mechanisms of Aux/IAAs on peach fruit development and maturation have also attracted much attention. The transient overexpression of the *PpIAA1* (*Ppa010303m*) gene in peach fruit can promote the expression of the *PpPG1* and *PpACS1* genes and result in earlier ripening and shorter postharvest storage, which indicates that PpIAA1 acts as a positive regulator to promote fruit ripening and softening. The overexpression of the peach *PpIAA19* (*Ppa011935m*) gene in tomatoes resulted in an increase in plant height, the number of lateral roots, and changes in parthenogenesis and fruit morphology [20]. The latest study showed that high levels of PpIAA13 (*Ppa010871m*) resulted in high expression of PpACS1, which increased ethylene production and peach fruit softening [21].

Although many studies have documented the influence of auxin on fruit ripening, the auxin signaling genes have not been investigated more. The whole genome analysis of the Aux/IAA and ARF gene families in peach fruit was conducted in our previous study [22–24]. A total of 4 TIR1/AFBs were identified in peach fruit. The transcript of PpTIR1 (*ppa003344m*) responds to exogenous auxin, and the expression level differs in peach fruit with different melting characteristics [22]. When PpIAAs were identified, the expression levels of 14 genes were higher in the melting "Okubo" than in the stony hard "Jing Yu" during almost all developmental stages. This strongly suggests that these genes may be related to auxin signaling during peach fruit ripening [23]. In this current study, we treated peach fruits with NAA (1-naphthylacetic acid). We found that low-concentration treatment delayed fruit softening and decreased the expression of cell wall-disassembling genes and ethylene biosynthetic genes. We also found that overexpression of the *PpTIR1* gene caused significant down-regulation of the expression of early auxin-responsive genes and cell wall metabolic genes related to fruit firmness. A combinatorial TIR1-Aux/IAA co-receptor system may be involved in this process. Therefore, the regulation of auxin on peach fruit softening is concentration-dependent. This study can enrich the theoretical research of drupe fruit ripening and lay a theoretical foundation for the hormone regulation measures of peach fruit softening.

2. Materials and Methods

2.1. Plant Materials and Treatments

Experimental samples of the melting peach "Okubo" were picked from the experimental orchard of the Beijing University of Agriculture (Changping District, Beijing, China). The fruits are at 37, 46, 55, 63, 70, 78, 84, 92, 98, and 110 days after full bloom (DAFB). We divided the development and maturation of peach fruit into four periods: the first rapid growth period (1 to 37 DAFB, S1), the hardcore stage (37 to 63 DAFB, S2), the second rapid growth period (63 to 84 DAFB, S3), and the mature period (after 84 DAFB, S4). The mature period was further divided into S4-1 (84 to 92 DAFB), S4-2 (92 to 98 DAFB), and S4-3 (after 98 DAFB).

Peach fruits "Okubo" at the developmental stage of S4-1 were selected and treated with deionized water (H_2O), 20 μM NAA, and 100 μM NAA, according to the preliminary results of our group. After washing with water and drying naturally, the peach fruit was soaked in the above three solutions, respectively. The solution was placed in a vacuum for 30 min, and the vacuum was slowly released to help the solution enter the peach fruit. After natural drying at room temperature, the treated peach fruits were stored under natural light for 14 days at 20 ± 2 °C. Samples were taken on the 1st, 7th, and 14th days after treatment, and the firmness of the fruits was measured.

2.2. Quantitative RT-PCR

Total RNA was extracted from peach fruit using the EASYspin reagent (Biomen, Beijing, China). Quantitative RT-PCR (qRT-PCR) was performed as described by Guan et al. [23]. The first-strand complementary DNA (cDNA) was synthesized using the TransScript First-Stand cDNA Synthesis SuperMix kit (TransGen Biotech, Beijing, China). The cDNA template for qRT-PCR analysis was diluted ten times with RNase-free water before use. TB Green Premix Ex Taq II (Tli RNaseH Plus) (Takara, Kusatsu Shiga, Japan) reagent was used to perform qRT-PCR analysis on the Applied Biosystems StepOnePlus system (Thermo Fisher Technologies, Waltham, MA, USA). *PpTEF-2* (Translation Elongation Factor 2) was used as an internal reference gene. Each line of treated peach fruit pieces was used to represent one biological replicate, and at least three technical replicates were analyzed for each biological replicate. The gene-specific primers used to detect the transcriptional level are listed in Table S1.

2.3. Agrobacterium-Mediated Infiltration

For overexpression of *PpTIR1*, the CDS fragment was ligated into the pCAMBIA3301-121 vector by the Seamless Cloning Kit (catalog no. D7010M; Beyotime, Shanghai, China) to generate overexpression constructs. The primers used are listed in Table S1. The resulting constructs were transferred into competent cells of *Agrobacterium tumefaciens* (strain GV3101). A transient expression followed previously published methods [23]. The fruit of "Okubo" in the developmental stage of S3 was used for infection. Six fruit pieces with a volume of about 1 cm^3 were taken on both sides of the ventral suture of peach fruit and cultured on MS medium for 24 h. Then, the pieces were soaked in the treatment solution and vacuum treated (−70 Kpa). The vacuum is slowly released to help the solution enter the pulp cells. After vacuum infiltration, the fruit pieces were washed three times with sterile water and cultured on MS medium in the growth chamber (20 °C, R.H. 85%) for 2 days, then quickly frozen in liquid nitrogen and stored at −80 °C for later use. Every single infection of peach pieces was used as one biological replicate, and three biological replicates were analyzed. The empty vector solution was used as a negative control.

2.4. GUS Histochemical Staining

The transiently overexpressed peach fruit pieces were cut into thin slices (1–3 mm). GUS staining solution was added to cover the material completely and placed at room temperature overnight. After that, the material was transferred to anhydrous ethanol for decolorization 2–3 times. The positive blue spots stained by the GUS solution were stable and did not fade with alcohol. The negative control was untreated peach fruit pieces, and the positive control was pieced transiently expressing pCAMBIA3301-121.

2.5. Subcellular Localization Analysis

The construction of the subcellular localization analysis was based on the cDNA of peach mesocarp. The CDS fragment of PpTIR1 was amplified by primers (Table S1). PpTIR1 without a stop codon and the full-length coding sequences of the genes were amplified by PCR and constructed into pBI121-GFP vectors by viscous terminal ligation. The successfully sequenced PpTIR1-GFP plasmids were transformed into the competent state of GV3101 *Agrobacterium tumefaciens*, and the bacteria identified were selected for an expanded culture

so that the final value of OD600 was 0.4. Tobacco leaves were injected with solution after being kept in darkness for 2–3 h at room temperature and cultured for 2 days. The marked areas of tobacco leaves were cut, and the GFP fluorescence signals were detected and photos taken by laser scanning confocal microscopy (Leica SP5, Leica, Wetzlar, Germany).

2.6. Yeast Two-Hybrid

The construction of a yeast two-hybrid vector was based on the cDNA of peach mesocarp. The CDS fragments of PpTIR1, PpIAA1, PpIAA3, PpIAA5, PpIAA9, and PpIAA27 were amplified by primers (Table S1). PpTIR1 without a stop codon and the full-length coding sequences of the genes were amplified by PCR and constructed into pGADT7 and pGBKT7 vectors by viscous terminal ligation. The PpTIR1-DBD and PpIAA1/3/5/9/27-AD plasmids were co-transformed into Saccharomyces cerevisiae strain AH109, and the yeast cells that contained these two vectors were screened on SD/-Trp-Leu media. When the transformed cells were inoculated on the strict four-deficiency plate SD/-Trp-Leu-His-Ade/X-α-Gal/auxin, different concentrations of NAA, 2,4-D, and IAA were added to check the effect according to previous research [6]. The colonies grew and turned blue, indicating that the plasmid was successfully constructed and that proteins interacted with each other. In addition, pGADT7 and pGBKT7 were used as negative controls.

2.7. Bimolecular Fluorescence Complementarities

The bimolecular fluorescence complementary vector was constructed using primers (Table S1) to amplify the CDS fragments, PCR to amplify the full-length coding sequence of the non-stop codon gene, and construction in pSPYNE173 and pSPYCE (M) vectors by viscous terminal ligation. The successfully sequenced PpTIR1-YNE and PpIAA1/3/5/9/27-YCE plasmids were transformed into competent *Agrobacterium tumefaciens* cells, respectively, to produce fusion proteins. Two types of bacteria containing different plasmids were mixed in an equal volume, and only 100 μM IAA was added simultaneously, according to the above results of the yeast two-hybrid. The bacterial solution was injected into tobacco leaves and cultured at room temperature for 2 days. A confocal microscope (Leica SP5, Leica, Wetzlar, Germany) was used to observe and take images.

2.8. Firefly Luciferase Fragment Complementary Image Technique (LCI)

The firefly luciferase fragment complementary image technology vector was constructed using primers (Table S1) to amplify the CDS fragments and PCR to amplify the full-length coding sequence of the non-stop codon gene. The vector was constructed in pCAMBIA1300-nLUC and pCAMBIA1300-cLUC vectors using a Seamless Cloning Kit (catalog no. D7010M; Beyotime, Shanghai, China). The PpTIR1-nLUC and PpIAA1/3/5/9/27-cLUC constructs successfully sequenced were transformed into *Agrobacterium tumefaciens* (strain GV3101), respectively. The suspension was prepared by mixing two types of construct solution with an equal volume, and only 100 μM IAA was added simultaneously, according to the above results of yeast two-hybrid. The suspension was injected into the back of tobacco leaves with a 1 mL syringe (without the needle). After culturing at room temperature for 2 days, the presence of fluorescence in the area where tobacco leaves were injected was determined by imaging in vivo (Tanon-5200muli, Tanon Science & Technology, Inc., Shanghai, China).

2.9. Statistical Analyses

All experiments were performed at least three times. All qRT-PCR reactions and other quantitative analyses were repeated at least three times. The Student's *t*-test was used to evaluate the significant differences.

3. Results

3.1. Low Concentration NAA Treatment Can Delay Fruit Firmness Decrease

Fruit firmness is an important quality index reflecting fruit texture and storage resistance, and it is also one of the important indexes reflecting fruit softening. As shown in Figure 1a, the fruit firmness of the "Okubo" peach decreased to a certain extent during fruit development and maturation. To clarify the effects of auxin on peach fruit firmness, "Okubo" peach fruits in the S4-1 period were treated with the exogenous hormone NAA. The results are shown in Figure 1b. The firmness of the peach flesh decreased with treatment time. However, on the 7th day of treatment, 20 μM NAA treatments delayed the decrease compared with the H_2O treatment ($p < 0.05$), and no obvious effect was found by 100 μM NAA treatments. There was no significant difference between any concentration of NAA treatments and control on the 14th day ($p < 0.05$). Taken together, these results suggest that low-concentration auxin delayed the firmness decrease in peach fruit.

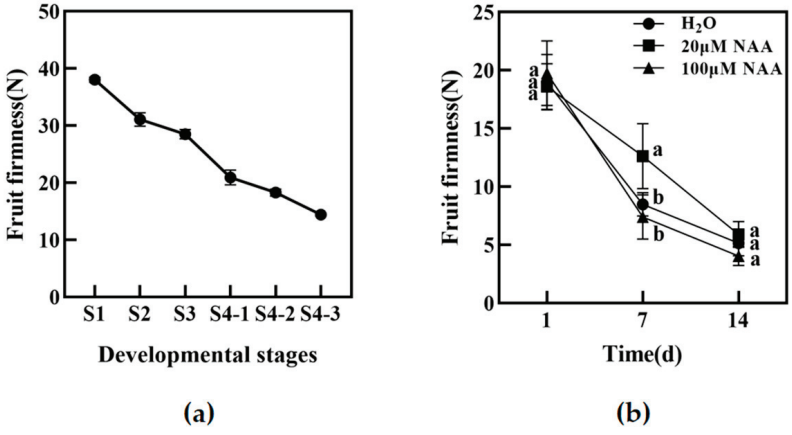

Figure 1. The firmness of peach fruit at different developmental stages (**a**) and the change in peach fruit firmness under NAA treatment (**b**). Vertical bars represent the standard deviation of the mean ($n = 3$). Different letters indicate significant differences between the groups ($p < 0.05$).

3.2. Low Concentration NAA Treatment Decreased the Activities of Softening-Related Enzymes in Peach Fruit

In order to explore the differences of peach fruit softening-related enzymes in different development stages, the relative expression levels of fruit cell wall degradation-related enzymes *PpPE* (pectinesterase), *PpPG* (polygalacturinase), *Ppβ-GAL* (β-galactosidase) and ethylene synthesis related enzymes *PpACO1* (ACC oxidase) and *PpACS1* (ACC synthase) were analyzed by qPCR. As shown in Figure 2a, the *PpPG* and *Ppβ-GAL* genes suddenly increased in the late stage of fruit development. In contrast, the change in the *PpPE* gene was not significant throughout the development period, and the expression levels of the *PpACO1* and *PpACS1* genes increased in the late stage of fruit development. When peach fruits in the stage of S4-1 were treated with different concentrations of NAA, it was found that 20 μM NAA treatment obviously reduced the transcription level of *PpPG* and *Ppβ-GAL* related to cell wall softening. Similarly, the expression of *PpACS1* genes related to ethylene synthesis was also affected ($p < 0.05$). However, there was no significant difference in the expression of these enzymes under 100 μM NAA treatment ($p < 0.05$).

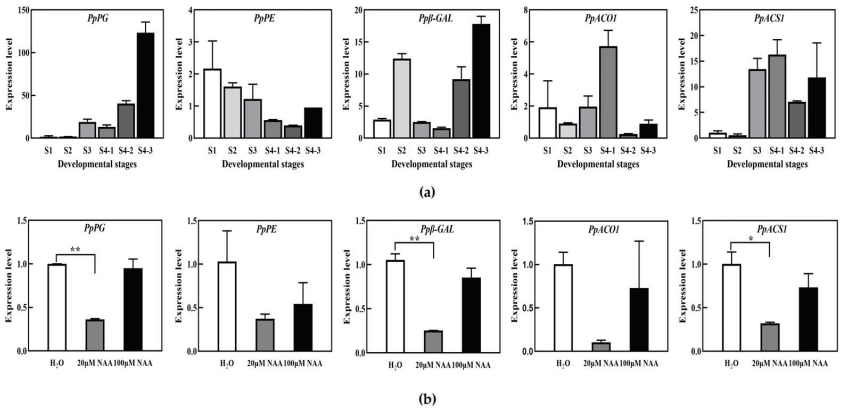

Figure 2. Expression levels of fruit softening-related genes and ethylene synthesis-related genes at different developmental stages (**a**) and under NAA treatment on peach fruits at the stage of S4-1 (**b**). Asterisks denote a statistically significant difference from the control (Student's *t*-test, ** $p < 0.01$; * $p < 0.05$).

3.3. Transient Overexpression of PpTIR1 Gene Affects Expression of Auxin Signal Transduction Factors and Fruit Softening Related Genes

To determine if PpTIR1 participates in the process of peach fruit ripening, the expression level of *PpIAAs* and *PpARFs* genes in the auxin signal transduction pathway and those related to cell wall degrading enzymes of fruit softening were detected using qRT-PCR (Figure 3). There were no blue spots on the peach fruit without transient expression, but blue spots appeared in the peach fruit when pCAMBIA3301-121 and pCAMBIA3301-121-PpTIR1 were transiently expressed, indicating that the transient expression of peach fruit was successful (Figure S1). After transient overexpression of the PpTIR1 gene in a piece of peach fruit, its expression increased significantly by approximately 36 times as much as that of the control ($p < 0.01$). The expression of the *PpIAA1/3/5/9/27* gene did not change significantly ($p < 0.05$), but the level of expression of some *PpARF* genes changed significantly. Among these, the expression of *PpARF2*′ and *PpARF4* genes was significantly lower by approximately 7 times and 3.7 times lower than that of the control ($p < 0.01$), respectively. The levels of expression of the *PpARF5* and *PpARF7* genes were lower than those of the control, with a decrease of approximately 3.7 times and 3 times ($p < 0.05$), respectively. In contrast, the levels of expression of the *PpARF10* and *PpARF12* genes were not different between the transgenic fruit and the control. The overexpression of *PpTIR1* affected the expression of some enzymes related to cell wall degradation, in which the expression of the *PpPG* gene decreased by approximately 1.7 times compared with the control ($p < 0.05$), while the expression of the *PpPE* and *Ppβ-GAL* genes did not change significantly ($p < 0.05$).

3.4. Yeast-2 Hybrid, BiFC, and Luciferase Reporter Assays Suggest That IAA and TIR1 Proteins May Directly Interact

A GFP fusion reporter was used to determine if the TIR1 protein localized to the nucleus in tobacco leaves. The coding region of the PpTIR1 gene was fused with that of the GFP protein to construct the fusion expression vector PpTIR1-GFP. The fusion expression vector was introduced into tobacco leaf back cells in vivo. Fluorescence microscopy revealed that the full-length PpTIR1:GFP fusions were localized exclusively in the nucleus (Figure 4a).

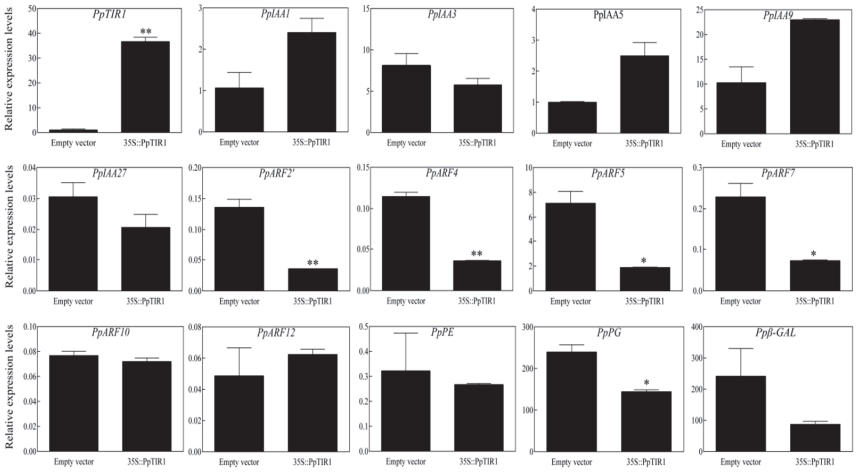

Figure 3. Effects of the over-expressed *PpTIR1* gene in an isolated peach fruit block on the relative expression levels of some PpIAAs, PpARFs, and enzymes related to cell wall degradation. Asterisks denote a statistically significant difference from the control (Student's *t*-test, ** $p < 0.01$; * $p < 0.05$).

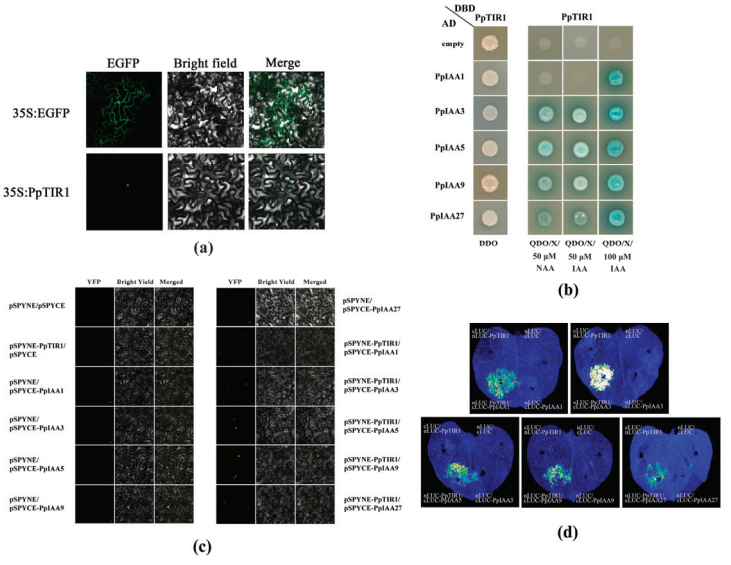

Figure 4. Subcellular localization of PpTIR1 proteins fused to the GFP tag and the interaction between PpTIR1 and some PpIAA proteins. PpTIR1-GFP fusion proteins were transiently expressed in leaves of *Nicotiana tabacum*, and their subcellular localization was determined by confocal microscopy (**a**). The yeast cells co-transformed with the recombinant plasmid could grow on DDO (SD/-Trp/-Leu) double-deficiency medium, indicating that the recombinant plasmid co-transformation was successful. Comparable results were observed on QDO/X (SD/-Trp/-Leu/-His/-Ade+X-α-gal) four-deficiency medium; greenish blue indicates positive interactions (**b**). The interaction between PpTIR1 and some PpIAA proteins was verified by bimolecular fluorescence complementation. Yellow fluorescence indicates positive interactions (**c**). A firefly luciferase complementation imaging assay was used to verify the interaction between PpTIR1 and some PpIAA proteins. There are four injection points on each tobacco leaf. The upper right is nLUC/cLUC; the upper left is cLUC/nLUC-PpTIR1; the lower right is nLUC/cLUC-PpIAA1, 3, 5, 9, 27, and the lower left is nLUC-PpTIR1/cLUC-PpIAA1, 3, 5, 9, 27 (**d**). Scale bar: 20 μm.

It has been reported that auxin is required to interact with the auxin receptor TIR1/AFBs and the Aux/IAA protein containing domain II. Consequently, this study involved screening Aux/IAA family members with domain II in peach. Five Aux/IAA proteins were selected, representing different sub-clades of Aux/IAAs, each playing distinct roles in mediating auxin responses. These five proteins were selected to determine their interactions with the PpTIR1 protein. The Aux/IAA proteins obtained were further studied to investigate their regulatory function in peach fruit firmness.

The constructed recombinant plasmid was co-transformed into AH109 yeast receptive cells, and the yeast two-hybrid results are shown in Figure 4b. The yeast cells co-transformed with the recombinant plasmid could grow on DDO (SD/-Trp/-Leu) double-deficiency medium, indicating that the recombinant plasmid co-transformation was successful. Comparable results were observed on the QDO/X (SD/-Trp/-Leu/-His/-Ade+X-α-gal) four-deficiency medium. The yeast co-transformed with the recombinant plasmid could not grow after adding 50 μM 2,4-dichlorophenoxyacetic acid (2,4-D). The mated yeast (DBD–PpTIR1 and AD–PpIAA3, PpIAA5, PpIAA9, or PpIAA27) could grow on the plates following the addition of 50 μM NAA or 50 μM IAA. Moreover, 100 μM IAA enhanced the interaction. The binding results confirmed the auxin-induced assembly of stable PpIAA:PpTIR1 co-receptors in yeast.

To further verify the interaction between PpTIR1 and PpIAA proteins, a BiFC assay was used. The yellow fluorescence signal of YFP was observed in the nuclei of tobacco leaf dorsal cells co-transformed with PpTIR1-NYFP and PpIAA1-CYFP, PpIAA3-CYFP, PpIAA5-CYFP, PpIAA9-CYFP, and PpIAA27-CYFP constructs in the presence of 100 μM IAA, and a YFP yellow fluorescence signal was not observed in the absence of auxin (Figure 4c). To provide additional evidence for the interaction between PpTIR1 and PpIAA proteins, the firefly luciferase fragment complementary image technique (LCI) test was used in this study. The fluorescence signals could be observed in tobacco leaf back cells co-infected with PpTIR1 and PpIAA1, PpIAA3, PpIAA5, PpIAA9, and PpIAA27 in the presence of 100 μM IAA. However, the signals did not appear in tobacco leaf back cells without auxin (Figure 4d). These results provided additional verification that the interaction between auxin receptor PpTIR1 and PpIAA proteins in peaches is dependent on auxin. These results confirmed that PpTIR1 interacts with PpIAA1/3/5/9/27.

4. Discussion

Peach is a kind of respiratory climacteric fruit, and its development and ripening processes experience a series of complex physiological and biochemical changes related to size, color, texture, flavor, and fragrance smell. Softening is the most significant textural change during the ripening and postharvest storage of peach fruit, which will affect the taste and shelf life of the fruit and the economic benefits of the peach industry. Therefore, a study on the mechanism of peach fruit softening has theoretical and practical significance.

It has been reported that there is a relationship between auxin and peach fruit development and softening during ripening. Previous research results from our group showed that the content of IAA in the hard fruit "Jingyu" was very low and did not increase during the late ripening stage. In contrast, the content of IAA in the rapidly dissolving fruit "Okubo" was significantly higher, which preliminarily revealed that the non-softening of hard fruit was related to low levels of IAA [23]. Therefore, our research focused on regulating peach fruit softening by auxin. To explore the relationship between auxin and softening, "Okubo" peach fruits were treated in vitro with exogenous NAA at different levels. The results showed that the firmness of the peach decreased after treatment with 20 μM NAA. However, 100 μM NAA had no obvious effect compared with H_2O treatment. These results indicated that low NAA concentrations could delay peach fruit's softening. Still, a report indicated a higher accumulation of auxin triggered the fast softening of peach fruit [25]. For other flesh fruits, previous studies have reported that exogenous auxin treatment can promote fruit ripening in pears [5]. NAA treatment accelerated the onset of ripening at a time when apple fruit could not ripen naturally [4,26]. However,

exogenous auxin treatment can inhibit fruit ripening and softening in strawberries [1] and grapes [27,28]. Therefore, the regulatory effect of exogenous auxin on fruit softening may be related to the type of fruit and the concentration of exogenous auxin.

In the auxin signal transduction pathway, after the TIR1/AFBs protein binds to auxin, the Aux/IAAs protein can be degraded through the ubiquitin degradation pathway. This degradation process alleviates the inhibition of transcription factor ARFs, allowing them to regulate the expression of a series of downstream auxin response genes. Therefore, it is of substantial significance to study the interaction between TIR1/AFBs and Aux/IAAs proteins to reveal the physiological function of auxin. Different TIR1/AFBs-Aux/IAAs co-receptors have different results in response to auxin. In *Arabidopsis thaliana*, rice, tomato, and plum, the interaction between TIR1/AFBs and Aux/IAAs proteins was found to depend on auxin. Still, there was also an auxin-independent interaction between *Arabidopsis thaliana* and rice [29,30]. In *Arabidopsis thaliana*, the interaction between AtTIR1, AtAFB1, AtAFB2, and AtAFB3 and the AtIAA3/5/7/8/12/28/29/31 protein depends on different concentrations of IAA [31]. In plums, PslTIR1, PslAFB2, and PslAFB5 can interact with the AtIAA7 protein in the presence of 100 μM IAA [6]. In this study, there was no interaction between PpTIR1 and the PpIAA1/3/5/9/27 protein in the absence of IAA and 50 μM 2,4-D, according to the yeast two-hybrid experiment. There was an interaction between PpTIR1 and the PpIAA3/5/9/27 protein, but the strength of the interaction differed depending on the concentration of NAA and IAA. In physiological experiments, 20 μM NAA had an obvious effect on the softening of peach fruits. In yeast two-hybridization, there was no effect of 20 μM NAA on the interaction between TIR1 and Aux/IAA. Compared with 50 μM IAA, 100 μM IAA can lead to an interaction between PpTIR1 and the Pp/IAA1 protein. Bimolecular fluorescence complementary and firefly luciferase fragment complementary image techniques were also used to prove the interaction between PpTIR1 and the PpIAA1/3/5/9/27 proteins under the condition of 100 μM IAA. These results showed that the response of different Aux/IAA factors to NAA or IAA varies in concentration and auxin type. Perhaps this difference enriches the multifunctional nature of Aux/IAA proteins. The interaction between PpTIR1 and PpIAA proteins may be an important regulatory process involved in activating downstream gene expression, thus realizing the biological function of auxin regulation.

The involvement of TIR1/AFBs in fruit ripening and softening has been reported in some studies. Currently, research on the TIR1/AFBs gene in fruit development, ripening, and softening is primarily focused on tomato fruit. In tomatoes, the overexpression of SlTIR1A affected flower morphology and fruit development, resulting in parthenocarpy formation. This led to the conclusion that SlTIR1A could interact with the SlIAA9 protein and regulate the expression of SlIAA9 and SlARF7 genes at the transcriptional level, thus affecting fruit setting. The overexpression of SlTIR1B would affect apical dominance, leaf morphology, and fruit formation. Other studies on SlTIR1 also showed that the overexpression of the SlTIR1 gene caused dwarfing, leaf morphological changes, and parthenocarpy in tomato plants. Simultaneously, the overexpression of the SlTIR1 gene led to a decrease in the expression of some early auxin response genes, such as SlIAA9, SlARF6, and SlARF7, while the level of expression of SlIAA3 increased [17]. A study on plums showed that the overexpression of the PslTIR1 gene led to early fruit setting before flowering, resulting in parthenocarpy and a decrease in the transcription of the IAA9 and ARF7 genes. It is hypothesized that PslTIR1 positively regulates auxin response and fruit set by mediating the degradation of Aux/IAA proteins, especially IAA9. In peaches, lower TIR1 protein levels trigger the stabilization of PpIAA13, leading to the accumulation of PpIAA13 protein, thus activating the expression of PpACS1 and promoting peach fruit softening [21]. In this study, we analyzed the expression of some auxin response genes and cell wall metabolic genes related to fruit firmness by transiently overexpressing the PpTIR1 gene in peach fruit. After overexpressing *PpTIR1* in peach fruit, the expression of the PpIAA1/5/9 gene increased (Figure 3). However, it did not reach a significant level, preliminarily indicating that these factors respond quickly to auxin and enhance

auxin signaling. The results indicated that the overexpression of the PpTIR1 gene in peach fruit resulted in a decrease in the expression of *PpARF2′*, *PpARF4*, *PpARF5*, and *PpARF7* genes, suggesting that the PpTIR1 gene may affect fruit development, ripening, and softening by regulating the downstream *PpARFs* genes. These *PpARFs* genes may play a negative feedback regulation of auxin signaling. Simultaneously, the overexpression of the PpTIR1 gene caused a significant decrease in the expression of PpPG, a gene related to fruit softening, indicating a close relationship between the PpTIR1 gene and peach fruit softening. In a study on plums, it was found that the firmness of plum fruit obtained by the overexpression of the *PslTIR1* gene was lower than that of wild-type fruit, and the expression level of soften-related genes in transgenic fruit was higher than that in wild-type fruit. These results show that PslTIR1 regulates fruit softening by controlling the level of enzymes related to cell wall decomposition [6]. However, whether PpTIR1 can promote or inhibit peach fruit softening needs further investigation.

5. Conclusions

In conclusion, PpTIR1 has a regulatory effect on fruit ripening and softening. The possible mode of regulation is through the interaction between TIR1 and the Aux/IAAs protein to activate the expression of downstream ARFs or other transcription factor genes. This activation, in turn, affects the level of enzymes related to cell wall degradation and ethylene synthesis, thereby facilitating the regulation of fruit ripening and softening.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9070734/s1, Figure S1: GUS staining to verify the success of PpTIR1 overexpression gene in an isolated peach fruit block; Table S1: Primers for vector construction.

Author Contributions: Writing—original draft, Y.Z.; methodology, Q.W.; formal analysis, D.G.; resources, H.Y.; software, J.W.; writing—review and editing, Y.L. All authors have read and agreed to the published version of the manuscript.

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Preharvest Applications of Oxalic Acid and Salicylic Acid Increase Fruit Firmness and Polyphenolic Content in Blueberry (*Vaccinium corymbosum* L.)

Jorge Retamal-Salgado [1,*], Geber Adaos [2], George Cedeño-García [3,*], Sebastian Camilo Ospino-Olivella [2], Rosa Vergara-Retamales [2], María Dolores Lopéz [4], Raúl Olivares [5], Juan Hirzel [1], Héctor Olivares-Soto [6] and Matías Betancur [4]

[1] Instituto de Investigaciones Agropecuarias, INIA Quilamapu, Av. Vicente Méndez 515, Chillán 3800062, Chile
[2] Faculty of Engineering and Business, Universidad Adventista de Chile, km 12 Camino a Tanilvoro, Chillán 3780000, Chile
[3] Facultad de Ingeniería Agronómica, Universidad Técnica de Manabí, Portoviejo 130105, Ecuador
[4] Department of Plant Production, Faculty of Agronomy, Universidad de Concepción, Campus Chillán, Chillán 3780000, Chile
[5] Driscoll's Sudamerica SPA, Camino a San Nicolas km 2, Chillán 3812120, Chile
[6] Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP), Campo Experimental Tecomán, Colima 28100, Mexico
* Correspondence: jorge.retamal@inia.cl (J.R.-S.); george.cedeno@utm.edu.ec (G.C.-G.)

Abstract: Blueberry exports that imply transport times of more than 25 d deteriorate their quality. The use of elicitors in preharvest has shown positive effects on the quality of berries such as grapes. The objective of this study was to evaluate preharvest applications (21, 14, and 7 d before harvest) of oxalic acid (OA) and salicylic acid (SA) on fruit firmness and phenolic compounds in blueberry. The treatments of 0, 2, and 4 mM OA in 'Kirra' and 0, 2, and 4 mM SA are in 'Stella blue'. With the earlier preharvest application, 'Kirra' presented better firmness than 'Stella blue'; however, 2 mM OA and SA in both cultivars increased fruit firmness, maintaining its weight and diameter with respect to the control. It should be noted that the treatment with 2 mM SA generated a 100% increase in polyphenolic content and antioxidant capacity ($p < 0.05$) in 'Stella Blue', with values close to 140 mg gallic acid 100 g^{-1} and 80 mg 100 g^{-1} fresh weight (FW), respectively. In Kirra, OA treatments did not have a significant impact on the polyphenol content, but 4 mM OA increased by 100% and 20%, total anthocyanin and antioxidant capacity of blueberry fruit, respectively. Based on our results, three pre-harvest applications of OA and SA during the fruit development until the beginning of ripening improve fruit firmness by up to 20% at different times of harvest.

Keywords: anthocyanins; antioxidant capacity; berries; oxalic acid; salicylic acid; *Vaccinium corymbosum*

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1. Introduction

Blueberry (*Vaccinium corymbosum* L.) is one of the richest sources of anthocyanins with high bioactive potential, compared to other berries such as strawberries, grapes, and raspberries [1], which has led to increasing demand worldwide. Harvest and postharvest are stages when fruit quality is altered, especially when the fruit is destined for long-term storage [2]. Blueberry-producing countries such as Chile export their fruit to countries with travel distances that exceed 25 d, which leads to fruit deterioration, reducing its postharvest shelf life in supermarkets [3]. In addition to the above, not all blueberry cultivars have the necessary quality at harvest to withstand long travel distances, with 'Duke' and 'Legacy' being among the cultivars that best withstand such trips [4], foregoing shows that the influence of genotype on plant resistance to abiotic conditions, such as temperature and radiation [5], as well as on fruit quality parameters, such as fruit firmness [6]. Given the agroclimatic conditions present in Chile, new cultivars introduced, such as Kirra and

Stella blue, have been well adapted, taking advantage of a ripening season mainly from December to February. However, the influence of their genotypes on the response of fruit physical–chemical characteristics to abiotic factors is still unknown, and only recently, their anthocyanin profile has been described [1].

Among the different techniques developed in blueberry postharvest to maintain fruit quality are those that control physical factors such as temperature [7], UV radiation, and inhibitors of fruit respiration, especially for modified atmosphere and controlled atmosphere systems [8]. However, other approaches have been implemented with the use of techniques based on chemical compounds, which have been called elicitors or inducers, that simulate physiological stress in the plant and induce a defense response by affecting its secondary metabolism and, consequently, increasing the biosynthesis of phytochemicals in the fruit [9]. These can be classified according to their origin as biotic that have a biological origin, such as coratine [10]; abiotic produced by environmental factors, either of physical origins, such as temperature or UV radiation; chemical, such as metal ions [9]; and phytohormones, among which are polyamines and spermine [11]. The use of elicitors helps in obtaining a plant and fruit more resistant to abiotic stress, also reducing the use of pesticides since the plant is able to synthesize phytoalexins [12].

Among the chemical elicitors, salicylic acid (SA) stands out; it is considered a natural phenolic compound and safe for commercial use [13]; when applied exogenously acts as a growth regulator and induces systemic acquired resistance in the plant, indirectly providing protection against abiotic stresses [14]. In addition, through systemic acquired resistance, SA induces some protein compounds in the fruit, such as glucanases, peroxidases, and chitinases, which are related to pathogenesis, hydrogen peroxide (H_2O_2) content, and other reactive oxygen species (ROS) [15]. Salicylic acid participates by regulating the enzyme phenylalanine-ammonia lyase [15], which induces the formation of phytoalexins and lignin through the phenylpropanoid pathway [12]; therefore, SA brings rigidity through lignin which is a polymer that is part of the cell wall [16], translating into better fruit firmness. On the other hand, oxalic acid (OA), another chemical elicitor that induces systemic resistance in the plant to face different diseases and/or increase firmness [17], has been tested in 'Sweet Heart' and 'Sweet Late' during preharvest, being able to significantly increase fruit firmness, as well as fruit size and weight postharvest [18]. However, the benefits of using elicitors on fruit quality may be subject to the concentration and timing of application for each plant species [14,19,20]. In addition, it has been shown that the increased phenolic content by OA applications may be the result of the activation enzyme phenylalanine ammonia lyase, which plays a key role in the phenylpropanoid pathway by catalyzing the conversion of phenylalanine to trans-cinnamic acid [21]. It has also been demonstrated that SA and its derivatives stimulate the accumulation of phenolic compounds in plant species before and after harvest due to the activation of phenylpropanoid metabolism [22], in addition to what has been observed in other studies, where SA improved the yield of carotenoids, through the regulation of biosynthetic genes of these compounds [23].

The fruit quality at harvest is essential for blueberries destined for export to arrive firm at their destination since quality decreases due to various postharvest factors such as constant handling, mainly during packing, where the berries suffer mechanical damage [24] that degrades the cellular network affecting their firmness, and the loss of moisture that decreases the fruit size [25]. On the other hand, chemical compounds of the fruit that provide its organoleptic characteristics and health benefits, for example, phenolic compounds that provide its aroma and color [12], can be affected since they act as a defense mechanism against abiotic factors that produce stress in the plant [6].

So far, there are few studies in fruit trees that evaluate the plant physiological response and fruit quality parameters by the use of elicitors applied in preharvest. For this reason, the objective of our study was to evaluate plant physiological and fruit physical-chemical response of new blueberry genotypes (Kirra and Stella blue) at harvest to oxalic and salicylic acids applied preharvest.

2. Materials and Methods

2.1. Characterization of the Study Site and Experimental Design

The study was carried out at the experimental station of Driscoll's Sudamerica SPA, located at kilometer 3 on the road to San Nicolás, Diguillìn province, Ñuble Region, Chile, from November to February 2019. The blueberry (*Vaccinium corymbosum* L.) cultivars used in the experiment were Kirra and Stella blue, which were established outdoors in individual pots of 15 L each, filled with a homogeneous substrate with pH 5.5 (Turba DSM2, KEKKILÄ S.A., Santiago, Chile), with an electrical conductivity 2.3 mS cm^{-1}, and NPK base fertilization of 15–12–29 kg m^{-3}. Each pot was irrigated using a drip irrigation system, with two irrigation laterals, with one dripper per lateral per plant, with a flow rate of 2 L h^{-1} per dripper (UniRam, Netafim, Hatzerim, Israel).

The maximum, minimum, and average air temperature (°C) of the study site was recorded every 30 min during the whole development of the crop, using Key Tag automatic sensors (Key Tag Recorders, USA). In parallel, the average soil temperature (°C) was measured with a digital thermometer (Multi Thermometer, Shanghai, China) at a depth of 0.2 m. Photosynthetic photon flux density (PPFD, μmol m^{-2} s^{-1}) was quantified according to the method proposed by Pinto-Poblete et al. [26] and Pinto-Morales et al. [27] using an AccuPAR LP-80 ceptometer (Decagon Devices, Washington, DC, USA), which delivers the average of 80 quantum sensors. Leaf temperature was measured during 1 d of fruit harvest with a portable OS-30p fluorometer (Opti-Sciences, Hudson, NH, USA) [28].

The experimental design corresponded to a randomized complete block design, where each elicitor was evaluated separately in different varieties. The treatments consisted of three doses of oxalic acid: 0 (control), 2, and 4 mM applied to 'Kirra' blueberries. And three doses of salicylic acid of 0 (control), 2, and 4 mM were evaluated on 'Stella blue'. The doses of the respective elicitors were selected as proposed by Zhu et al. [29]. The treatments were carried out by three applications of each elicitor on the respective cultivars, the first application being 21 d before harvest; the second application 14 d before harvest; and the third application 7 d before harvest, via foliar with a hand sprayer (Roots, Shanghai, China), with a total wettability volume per plant of 0.3 L $tree^{-1}$, maintaining a constant sprayer working pressure close to 1 ± 0.2 bar, the application characteristics of the elicitors were considered simulating a normal fumigation application in a potted blueberry orchard.

2.2. Plant Physiological Parameters

Maximum fluorescence intensity (F_m) and minimum fluorescence intensity (F_0) of chlorophyll were measured with a portable fluorimeter model OS-30p (Opti-Sciences) on fully sun-exposed leaves, mature and annual shoots located in the second third of the shoot, considering a total of 18 measurements per treatment [30,31], at five times of the day, 09:00, 11:00, 13:00, 15:00, 17:00 h, during a clear day. These results allowed quantification of the maximum photochemical efficiency of photosystem II (F_v/F_m) [32,33].

Stomatic conductance (g_s, mmol m^{-2} s^{-1}) was measured in parallel to chlorophyll fluorescence measurements, with a portable porometer model SC-1 (Decagon Devices) taking into consideration the same plant, shoot, location, and frequency criteria used in chlorophyll fluorescence measurements [34].

2.3. Yield and Physical-Chemical Parameters of Fruit

The first harvest was carried out when 5% of the total fruit on the plant was ripe, considering ripe fruit to be 100% blue. Subsequently, a total of seven harvests were completed at 4, 7, 11, 14, 18, and 21 d after the first harvest. At each harvest, all ripe fruits were harvested. Harvesting was carried out in the early hours of the day when ambient temperatures did not exceed 21 °C. At each harvest, the average weight of 100 fruits (g) per plant was measured using a precision balance (Precisa Instruments AG, Dietikon, Switzerland).

Immediately after harvest, fruit firmness and equatorial diameter were determined using FirmPro equipment (Happy Volt SPA, Santiago, Chile) [28,31,34]. Firmness was

expressed as the force in grams (g) necessary to deform the fruit in 1 mm (gf mm^{-1}). The equatorial diameter of the fruit was measured in mm, with an accuracy of ± 0.03 mm.

To carry out the chemical analysis of fruit, the fruit samples were stored at 7 °C in a container and, within the same day, were transferred to the Food Chemistry Laboratory of the Universidad de Concepción, where they were stored in a freezer (MDF-25U100, Antech Group, Hong Kong, China) at −20 °C for further analysis.

The total polyphenol content of the fruit was determined by the Folin–Ciocalteu method through a harvested sample of 0.25 g and 25 mL H_2O/MeOH/formic acid solution (24:25:1) for 1 h in a CPX 5800 Branson ultrasonic (Branson Ultrasonics Corp., Danbury, CT, USA). A calibration curve with gallic acid was used to calculate the polyphenol content, with concentrations between 0 and 1000 mg L^{-1} gallic acid according to the methodology proposed by Yıldırım et al. [35]. The standard curve equation used was 0.0013× + 0.0573 and R^2 = 0.99. The results are expressed as mg gallic acid 100 g^{-1} FW.

Total anthocyanins were determined by a differential pH technique according to the methodology described by Pinto-Morales et al. [27], where the molecular weight and molar absorbance of the anthocyanin pigment present in the highest proportion were used to calculate the total anthocyanin content. Data were expressed as mg 100 g^{-1} FW [36], a compound found in greater proportion in blueberry [31]. The DPPH antioxidant capacity was performed as described by Betancur et al. [37], the extract was diluted, and the DPPH solution was incorporated and kept in the dark for 1 h at room temperature. The readings in the spectrophotometer were determined at 515 nm, and the standard curve used was 0.0008× + 0.0272 and R^2 = 0.99. The results were expressed as μmol Trolox equivalent (TE) 100 g^{-1} FW [27].

2.4. Statistical Analysis

The data were analyzed by analysis of variance (ANOVA) with a significance level of $p < 0.05$. Comparison of means was performed by Fisher's test using the general linear and mixed models procedure of the INFOSTAT software (Infostat, Cordoba, Argentina) version 2018 software [38]. In addition, correlation and principal component analysis (PCA) were performed using mean-centered data based on eigenvalues using R software with the FactoMineR and ggplot2 packages [39].

3. Results

3.1. Environmental and Plant Physiological Parameters

Table 1 shows the environmental parameters of the study site, where the maximum, minimum, and average air temperatures were 32.0, 16.1, and 19.7 °C, respectively. Meanwhile, the maximum, minimum, and average soil temperatures were 30.6, 19.5, and 26.5 °C, respectively. The maximum, minimum, and average photosynthetic photon flux densities were 2407, 439, and 1495 μmol m^{-2} s^{-1}, respectively.

Table 1. Environmental parameters of the study site.

Value	Air Temperature (°C)	Soil Temperature (°C)	PPFD * (μmol m^{-2} s^{-1})
Maximum	32.0	30.6	2407
Minimum	16.1	19.5	439
Average	19.7	26.5	1495

* PPFD: Photosynthetic photon flux density.

Leaf temperature in both cultivars had the same trend during the different measurement hours of the day. The minimum and maximum leaf temperature for 'Kirra' was 26.4 and 35.5 °C, respectively (Figure 1a). On the other hand, in 'Stella blue', the minimum and maximum leaf temperatures were 25.9 and 33.8 °C, respectively (Figure 1b).

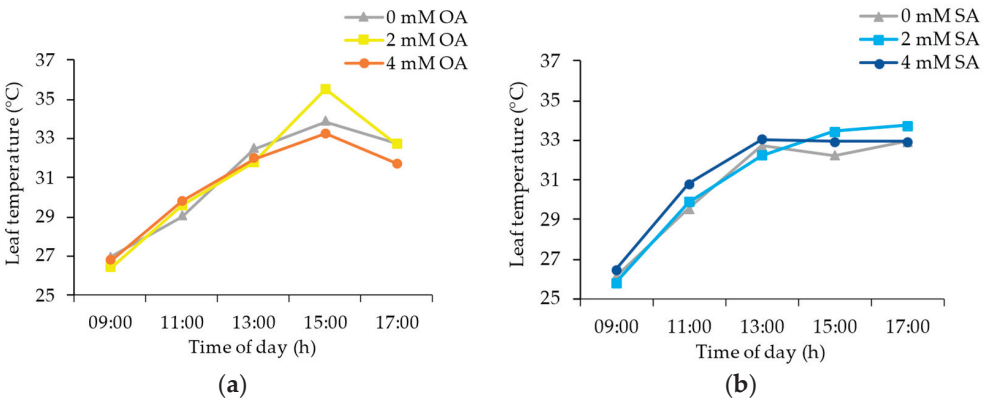

Figure 1. (**a**) Leaf temperature in plants of 'Kirra' blueberry under different doses of oxalic acid (OA): 0, 2, and 4 mM; (**b**) leaf temperature in plants of 'Stella blue' under different doses of salicylic acid (SA): 0, 2, and 4 mM, evaluated at different times of the day: 09:00, 12:00, 15:00, and 18:00 h.

Stomatal conductance (g_s) showed significant differences between treatments for 'Kirra' and 'Stella blue' under different concentrations of OA (Figure 2a) and SA (Figure 2b), respectively. In 'Kirra', 2 and 4 mM OA produced higher g_s during the first hour of measurement (09:00 h) compared to the control, reaching average values of 278 mmol m^{-2} s^{-1}. At 11:00 h, the treatments showed nonsignificant differences among them, with an average conductance of 245 mmol m^{-2} s^{-1}. The measurement at 13:00 h showed a significant increase in g_s, with the 2 mM treatment presenting a value of 284 mmol m^{-2} s^{-1}, higher than that presented during the first measurement. However, control and 4 mM OA treatments did not differ significantly, decreasing to a value of 199 mmol m^{-2} s^{-1}. The lowest value of g_s was recorded at 15:00 h, with 4 mM OA treatment being significantly superior to the other treatments with a value of 174 mmol m^{-2} s^{-1}. At 17:00 h, there was an increase in g_s in all treatments, with 4 mM OA treatment showing the highest conductance value at 260 mmol m^{-2} s^{-1}, significantly superior to the other treatments.

With respect to 'Stella blue', the measurement at 09:00 h indicated that 4 mM SA had a higher g_s than the other treatments, reaching 213 mmol m^{-2} s^{-1}. At 11:00 and 13:00 h, the treatments with doses of 2 and 4 mM SA were significantly higher than the control, without significant differences between them and with an average value of 236 and 237 mmol m^{-2} s^{-1}, respectively. At 13:00 h, there were nonsignificant differences between 2 and 4 mM SA treatments, but at 15:00 h, there was a significant decrease in conductance with the 2 mM SA treatment, reaching values similar to those obtained by the control treatment of 168 mmol m^{-2} s^{-1}. In the last hour of measurement (17:00 h), there was a generalized decrease in conductance values; however, the treatments 2 and 4 mM, without significant differences between them, were superior to the control treatment, which presented the lowest value recorded of all the measurements of the day, reaching 56 mmol m^{-2} s^{-1}.

The maximum photochemical efficiency of photosystem II (F_m/F_v) showed significant differences between treatments for 'Kirra' and 'Stella blue' under different OA (Figure 3a) and SA concentrations (Figure 3b), respectively. In 'Kirra', the F_m/F_v under the different concentrations evaluated had a general tendency to decrease until 13:00 h and then a sustained recovery in time until the end of the period evaluated (17:00 h). Significant differences were only observed at 11:00 and 13:00 h, when 2 and 4 mM OA showed a lower degree of stress during the first hours of the day compared to the control treatment, without significant differences between them and with average F_m/F_v values of 0.79 and 0.76, respectively (Figure 3a). Regarding 'Stella blue', F_m/F_v did not show a marked trend. At 09:00 h, treatments 2 and 4 mM SA did not show significant differences between them; they presented an average F_m/F_v value of 0.78, higher than the control treatment,

which presented an F_m/F_v value of 0.76. Moreover, significant differences were found between 11:00 and 13:00 h measurements, with the 2 mM treatment presenting the highest F_m/F_v values corresponding to 0.81 and 0.77 for both periods, respectively. In the last 2 h of measurement (15:00 and 17:00 h) nonsignificant differences were found between the different treatments.

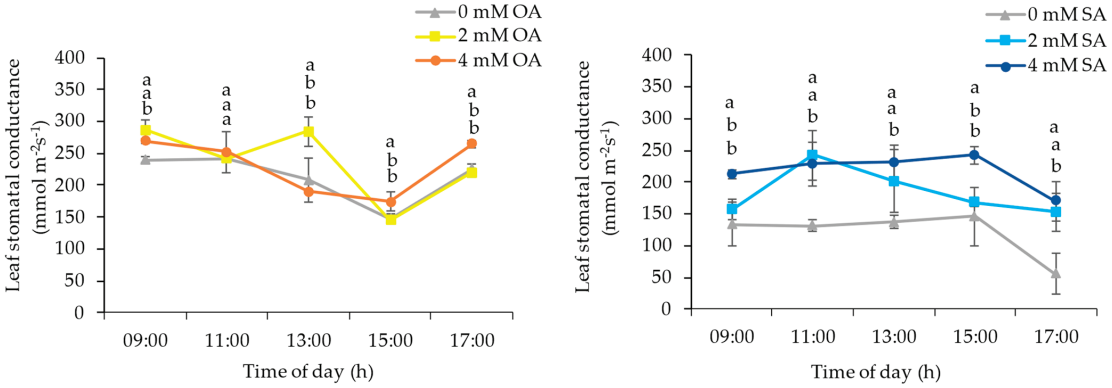

Figure 2. (**a**) Stomatal conductance in plants of 'Kirra' blueberry under different doses of oxalic acid (OA): 0, 2, and 4 mM; (**b**) stomatal conductance in plants of 'Stella Blue' under different doses of salicylic acid (SA): 0, 2, and 4 mM, evaluated at different times of day: 09:00, 12:00, 15:00, and 18:00 h. Different lowercase letters indicate significant differences between treatments according to Fischer's LSD test ($p < 0.05$). Mean ± standard error ($n = 3$). Bars correspond to experimental error for each treatment.

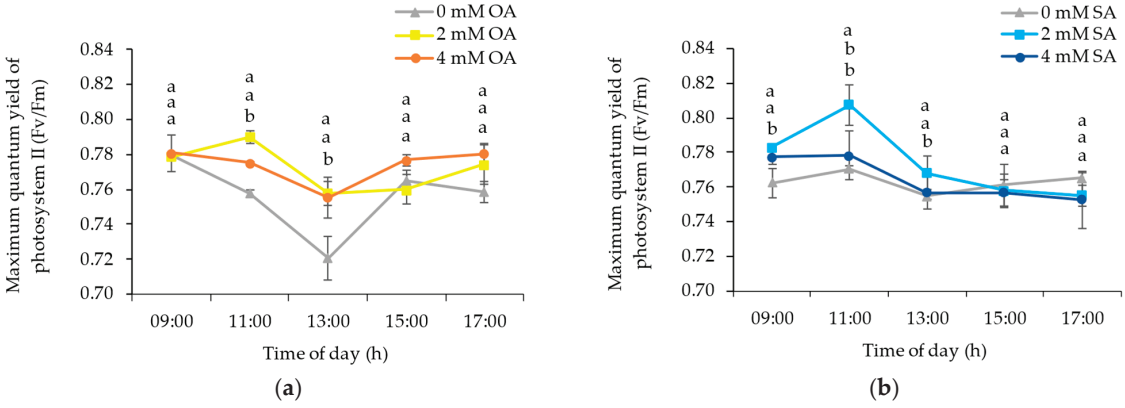

Figure 3. (**a**) Maximum photochemical efficiency of photosystem II (F_v/F_m) in plants of 'Kirra' blueberry under different doses of oxalic acid (OA): 0, 2, and 4 mM; (**b**) F_v/F_m in plants of 'Stella Blue' under different doses of salicylic acid (SA): 0, 2, and 4 mM, evaluated at different times of the day: 09:00, 12:00, 15:00, and 18:00 h. Different lowercase letters indicate significant differences between treatments according to Fischer's LSD test ($p < 0.05$). Mean ± standard error ($n = 3$). Bars correspond to experimental error for each treatment.

3.2. Physical–Chemical Parameters of Fruit

Fruit firmness showed significant differences between treatments for 'Kirra' and 'Stella blue' under different concentrations of OA (Figure 4a) and SA (Figure 4b), respectively. In

'Kirra', fruit firmness had a decreasing trend as days to harvest progressed, independent of treatment. During the first two harvests, nonsignificant differences were observed among the different treatments (Figure 4a) with values close to 200 gf mm^{-1}. In general, regardless of the treatment, a decrease in fruit quality was observed as time went by, until reaching firmness values close to 165 gf mm^{-1} in the control treatment. However, from the third harvest (7 d after the beginning of the harvest), an effect of the treatments was observed, with 2 mM OA treatment registering a lower rate of decrease in fruit quality, with a higher firmness than the control treatment ($p < 0.05$), and managing to maintain fruit firmness until the sixth harvest at values close to 200 gf mm^{-1}. In 'Stella blue', like 'Kirra', firmness decreased as time progressed (Figure 4b), but with a visible increase in quality in all harvests in the 4 mM SA treatment with respect to the control ($p < 0.05$), maintaining firmness above 200 gf mm^{-1} until the sixth harvest for 4 mM SA. It is important to note that the control treatment reached the lowest firmness values in all harvests, reaching values close to 150 gf mm^{-1}. In contrast, the 4 mM SA treatment obtained the highest firmness with values of 211 gf mm^{-1} (Figure 4b).

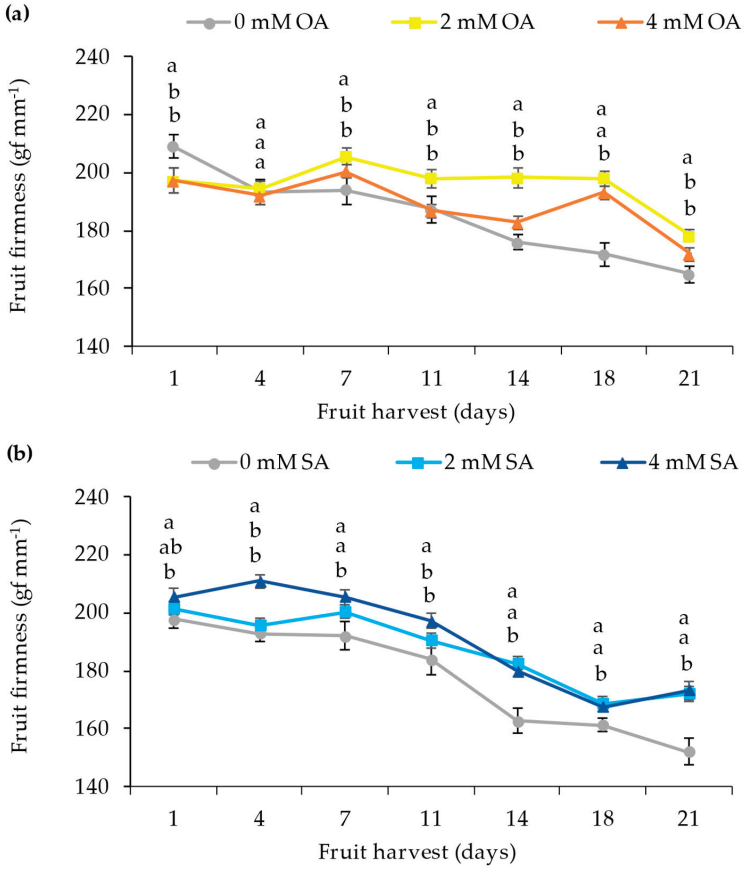

Figure 4. (**a**) Fruit firmness of 'Kirra' blueberry under different doses of oxalic acid (OA): 0, 2, and 4 mM; (**b**) Fruit firmness of 'Stella blue' under different doses of salicylic acid (SA): 0, 2, and 4 mM, measured at different harvest days 1, 4, 7, 11, 14, 18, and 21 d. Different lowercase letters indicate significant differences between treatments according to Fischer's LSD test ($p < 0.05$). Mean ± standard error ($n = 3$). The bars correspond to the experimental error for each treatment.

Regarding the physical parameters of the fruit, the average fruit weight (Figure 5a) only showed significant differences in 'Kirra', which was higher in the OA control treatment (1.8 g) compared to the 4 mM OA (1.5 g). On the other hand, the average fruit weight in 'Stella blue' (Figure 5a) did not show significant differences, with an average of 2.3 g.

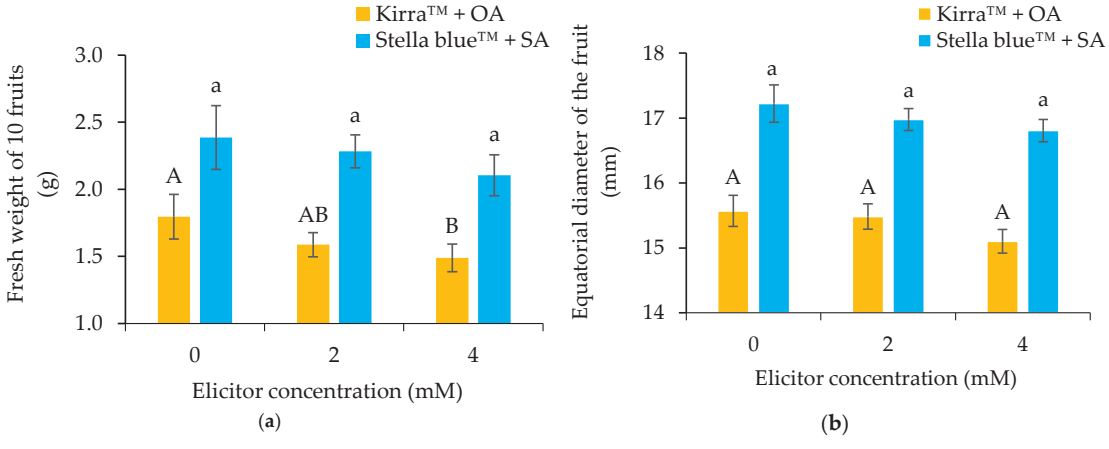

Figure 5. (**a**) Average fruit weight and (**b**) fruit equatorial diameter of 'Kirra' blueberry under different doses of oxalic acid (OA): 0, 2, and 4 mM; and of 'Stella blue' under different doses of salicylic acid (SA): 0, 2, and 4 mM. Different capital letters indicate significant differences between OA treatments according to Fischer's LSD test ($p < 0.05$). Different lowercase letters indicate significant differences between SA treatments according to Fischer's LSD test ($p < 0.05$). Mean ± standard error ($n = 3$). Bars correspond to experimental error for each treatment.

The equatorial diameter in both cultivars was not affected by treatments. In the 'Kirra', the average equatorial diameter was 15.4 mm, while in 'Stella blue', it was 17.0 mm.

The content of total polyphenolics, total anthocyanins, and antioxidant capacity of fruits in 'Kirra' and 'Stella blue' under different concentrations of OA and SA are shown in Figure 6. In 'Kirra', non-significant differences were observed for OA ($p > 0.05$), where the different treatments registered values close to 148 mg gallic acid 100 g^{-1} FW (Figure 6a). In contrast, the concentration of total polyphenolics in 'Stella blue' was significantly higher with 2 mM SA, registering a value of 139 mg gallic acid 100 g^{-1} FW. The 4 mM SA treatment with 77 mg gallic acid 100 g^{-1} FW was superior to the control ($p < 0.05$), which had 61 mg gallic acid 100 g^{-1} FW (Figure 6a).

The total anthocyanin content had a similar trend to the polyphenolic content of the fruit in 'Kirra' and 'Stella blue' treated with OA and SA, respectively (Figure 6b). In 'Kirra', the highest total anthocyanin contents were obtained by 4 mM OA ($p < 0.05$), with a value of 128 mg 100 g^{-1} FW, recording the 2 mM OA treatment 106 mg 100 g^{-1} FW, which in turn was superior to the control (62 mg 100 g^{-1} FW, $p < 0.05$) (Figure 6b). The anthocyanin content in 'Stella blue' was significantly higher with the 2 mM SA treatment, which presented a value close to 80 mg 100 g^{-1} FW, being 136% and 160% higher than 4 mM SA and control treatments ($p < 0.05$), respectively (Figure 6b).

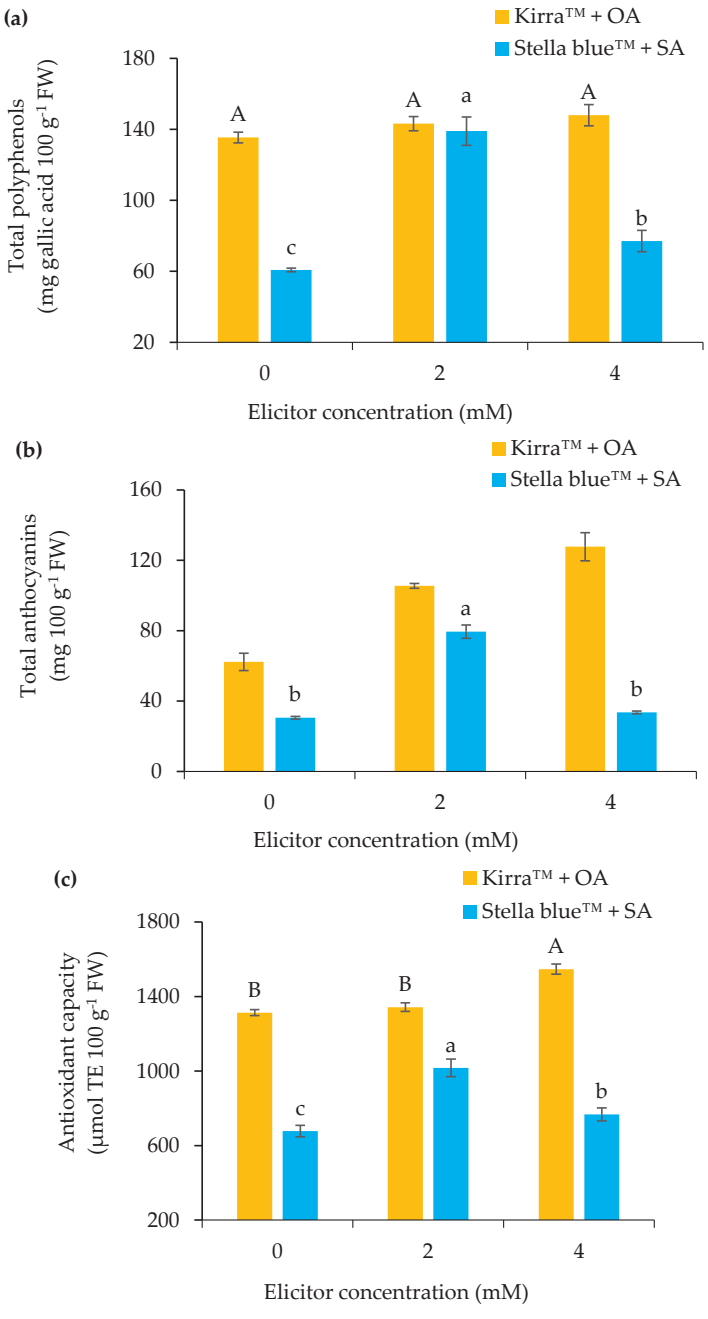

Figure 6. (**a**) Total polyphenolics content, (**b**) total anthocyanins, and (**c**) antioxidant capacity of 'Kirra' blueberry under different doses of oxalic acid (OA): 0, 2, and 4 mM; and of 'Stella blue' under different doses of salicylic acid (SA): 0, 2, and 4 mM. Different capital letters indicate significant differences between OA treatments according to Fischer's LSD test ($p < 0.05$). Different lowercase letters indicate significant differences between SA treatments according to Fischer's LSD test ($p < 0.05$). Mean ± standard error ($n = 3$). Bars correspond to experimental error for each treatment.

The antioxidant capacity of the fruit showed similar trends in anthocyanin and total polyphenolic content for both elicitors. The fruits of 'Kirra' had a higher antioxidant capacity with 4 mM OA, with a value of 1547 µmol TE 100 g^{-1} FW ($p < 0.05$) compared to 2 mM OA and control treatments, which did not show significant differences between them, and had an average value close to 1328 µmol TE 100 g^{-1} FW (Figure 6c). The fruits of 'Stella blue' had higher antioxidant capacity, with 2 mM SA reaching a value of 1018 µmol TE 100 g^{-1} FW being 33% and 50% higher than 4 mM SA and control treatments, respectively.

3.3. Correlations

The correlation matrix in 'Kirra' (Figure 7a) indicates that plant physiological parameters such as g_s and F_m/F_v were positively correlated with each other ($r = 0.63$) and with fruit firmness, having a correlation of $r = 0.66$ and $r = 0.69$, respectively. The higher the g_s and maximum efficiency of photosystem II, the higher the fruit firmness. Fruit physical parameters such as fruit weight and equatorial diameter correlated strongly with each other ($r = 0.94$) and negatively with F_m/F_v, with values of $r = -0.62$ and $r = -0.71$, respectively. Total polyphenolics content were positively related to total anthocyanin content ($r = 0.61$). Total anthocyanins were positively correlated with fruit firmness ($r = 0.56$) and with plant physiological parameters such as g_s ($r = 0.72$) and F_m/F_v ($r = 0.82$).

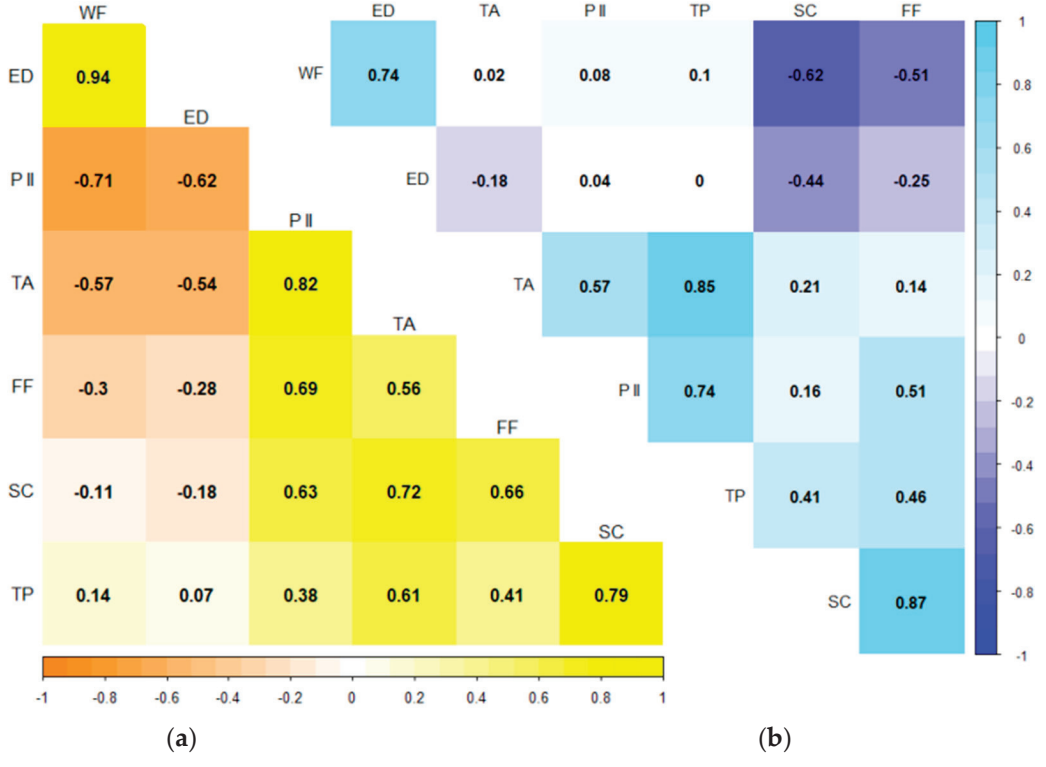

Figure 7. Correlation matrix between environmental and physiological variables of the plant and physicochemical parameters of the fruit of 'Kirra' (**a**) and 'Stella blue' (**b**) blueberries. SC: Stomatal conductance; PII: maximum efficiency of photosystem II; ED: equatorial diameter; WF: weight fruit; FF: fruit firmness; TP: total polyphenols; TA: total anthocyanins.

The correlation matrix in 'Stella blue' (Figure 7b) indicates that the physiological parameters of the plant, such as g_s and F_m/F_v, were positively correlated with fruit firmness,

with values of $r = 0.87$ and $r = 0.51$, respectively. Equatorial diameter and fruit weight had a strong positive correlation with fruit weight ($r = 0.74$) but a negative correlation with g_s ($r = -0.44$), the same as its fruit weight and stomatal conductance ($r = -0.62$). Total polyphenolics had a strong positive correlation with total anthocyanin content ($r = 0.85$). Total anthocyanins and total polyphenols had a positive correlation with F_m/F_v, with values of $r = 0.57$ and $r = 0.74$, respectively.

In 'Kirra' and 'Stella blue', principal component analysis (PCA) (Figure 8) was performed for six parameters: g_s, F_m/F_v, equatorial diameter, fruit weight, fruit firmness, total polyphenols, and total anthocyanins. In 'Kirra' (Figure 8a), the principal components PC1 and PC2 retained 23.3% and 56.9%, respectively. In contrast, in 'Stella blue', principal components PC1 and PC2 retained 31.1% and 45.8%, respectively. This represents all parameters as vectors in the biplot, while the vector length shows how well-represented the variables are in this plot. The approximate distance between the variables and their correlations confirms what was previously indicated in the correlation matrix for both cultivars (Figure 7). In 'Kirra', the treatments in the PCA are represented by numbers 1–3 for 0 mM, 4–6 for 2 mM, and 7–9 for 4 mM OA (Figure 8c). In 'Stella blue', the treatments in the PCA are represented by numbers 1–3 for 0 mM, 4–6 for 2 mM, and 7–9 for 4 mM SA (Figure 8d).

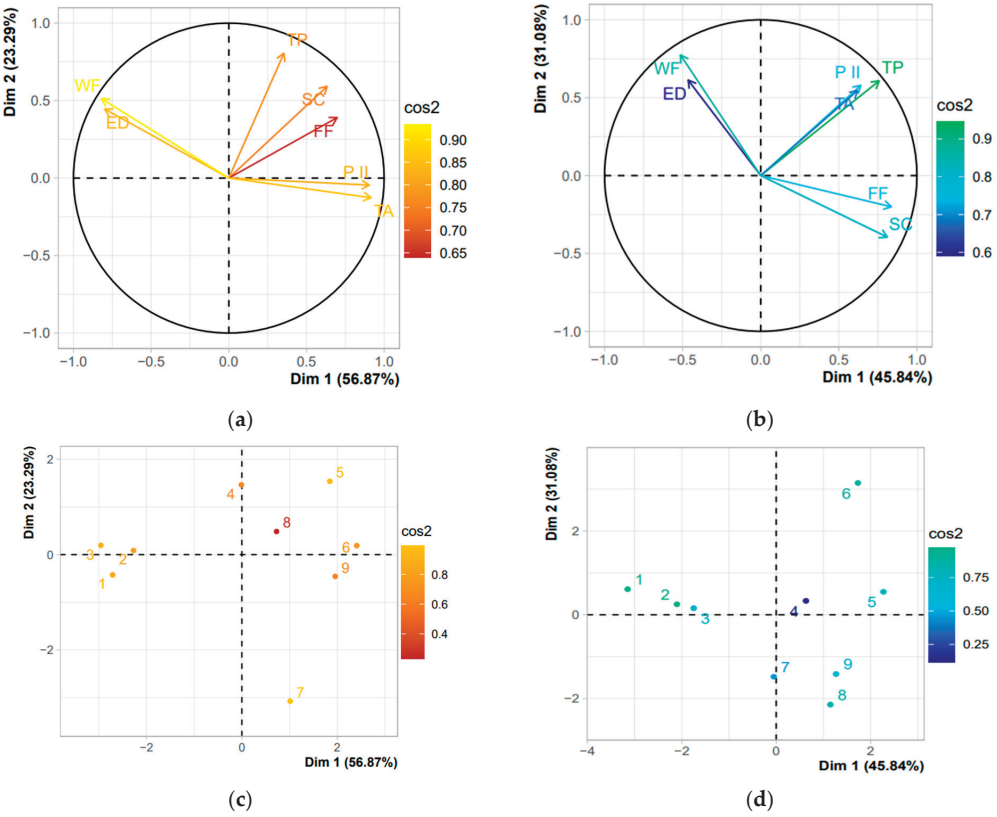

Figure 8. Principal component analysis (PCA) for variables for 'Kirra' (**a**) and 'Stella blue' blueberries (**b**) and PCA of individuals of 'Kirra' (**c**) and 'Stella blue' (**d**). SC: Stomatal conductance; PII: maximum efficiency of photosystem II; ED: equatorial diameter; WF: weight fruit; FF: fruit firmness; TP: total polyphenols; TA: total anthocyanins.

4. Discussion

4.1. Environmental and Plant Physiological Parameters

According to the present results, the environmental conditions such as air and soil temperature, as well as the photosynthetic photon flux density (PPFD) of the study site (Table 1), would not produce significant abiotic stress in blueberry plants because the average minimum and maximum air temperatures were 16.1 and 32.0 °C, respectively, and the soil temperature was 19.5 and 30.6 °C, for soil temperature. According to Chen et al. [40], blueberry plants suffer some degree of stress at temperatures above 40 °C. Moreover, it has been mentioned that leaf temperatures higher than 40 °C are capable of causing deterioration inside chloroplasts, decreasing the net photosynthetic rate [41]. However, in our study, leaf temperature did not exceed 35 and 36 °C for 'Kirra' and 'Stella blue', respectively (Figure 1a,b). The average daily photosynthetically active radiation, quantified as PPFD observed in this study, was 1495 μmol m^{-2} s^{-1}, agreeing with the average values of environmental measurements previously recorded for south-central Chile [42].

An important finding in the present investigation is that doses of OA and SA produced changes in the physiological state of the plant in 'Kirra' and 'Stella blue', respectively, represented as stomatal conductance and F_v/F_m. Stomatal conductance is an indicator of stomatal closure, which impedes gas exchange and water losses; therefore, less stressed plants will have greater gas exchange [36]. Likewise, higher values of F_v/F_m with respect to the values recorded earlier in the day indicate a higher rate of recovery of the photosynthetic apparatus [34]. At the beginning of the day, there was a greater response of g_s and F_v/F_m of the plant in both cultivars, which is consistent with what has been previously reported in 'Ochlockonee' blueberries (*V. virgatum* Aiton) [34]. In that period, 'Kirra' treated with 2 and 4 mM OA showed nonsignificant difference between them, being superior to the control ($p < 0.05$). However, in 'Stella blue' during the same period of the day, there was a greater response in g_s with 4 mM SA applications and greater F_v/F_m with 2 mM SA applications, both being superior to the control ($p < 0.05$). Salicylic acid is known to be one of the main regulators of growth and photosynthesis in plants under normal and stressful conditions [43]. According to studies, SA induces plant hormonal changes such as increases in abscisic acid (ABA) [44], decreased auxin signaling, or increased cytokinin synthesis [45], generating greater gas exchange, improved chloroplast differentiation, chlorophyll biosynthesis, and prevention of chlorophyll degradation [46]. Although, in our study, the values of F_m/F_v are slightly lower than those reported by Hao et al. [47] in *V. corymbosum* L., who indicate that at similar values of F_v/F_m, high net photosynthetic rates are recorded, which leads us to suppose that the elicitors evaluated in this study could be favoring a higher net photosynthetic performance of the plant. This is also consistent with the results found in other fruit species, such as grapevine [48] and pistachio [49].

Oxalic acid applications have been studied on fruit organoleptic characteristics generally in postharvest [50], so the mechanisms involved in plant physiological responses to preharvest OA applications are scarcely described. Nevertheless, it has been shown that chlorophyll fluorescence is involved in chloroplast activity [51] and can be increased by OA, preventing plant and/or fruit senescence or stress damage since it decreases cell membrane degradation. This is consistent with work carried out on fruit species such as kiwifruit [50] or plum [52], which showed less decrease in chlorophyll fluorescence with OA applications. The control treatment of 'Kirra' had maximum and minimum values of g_s up to 65% and 160% higher (Figure 2), respectively, than 'Stella blue', which would respond to the influence of the genotype on plant physiology [5]. This study is the first to evaluate the effects of OA and SA doses applied preharvest on the physiological parameters of 'Kirra' and 'Stella blue' blueberries, respectively.

4.2. Physical–Chemical Parameters of the Fruit

Fruit firmness decreased with advancing harvest dates in both cultivars (Figure 4). In blueberries and other fruit species, this is attributed to the phenological advancement of the

fruit ripening stage [7,53]. In 'Kirra', the 2 and 4 mM doses of OA were the treatments that presented higher fruit firmness with respect to the control towards the last harvest, i.e., the rate of decrease in firmness or loss of fruit quality was lower (Figure 4a). Postharvest fruit senescence is triggered by active oxygen species (AOS) largely involved in the ripening process and rupture of cell wall and membrane components [54]. In addition, they react with unsaturated fatty acids causing lipid peroxidation [53]. The present results are in agreement with other studies on fruit trees, where the decrease in quality loss was explained by the fact that OA was able to induce the activities of antioxidant enzymes in the fruit, such as superoxide dismutase, peroxidase, catalase, and ascorbate peroxidase, which counteract oxidative damage [7]. Likewise, it has been demonstrated that OA is able to increase lipoxygenase activity in peach fruit compared to fruit without OA, producing inhibition of lipid peroxidation and contributing to maintaining membrane integrity [7]. In addition, in climacteric crops such as mango, OA applications significantly reduced fruit ripening by inhibiting ethylene production [55]. On the other hand, applications of 2 and 4 mM SA on 'Stella blue' (Figure 4b) produced greater fruit firmness compared to the control. This is consistent with previous studies because SA applications slowed the loss of firmness in fruits such as plums [18] and sweet cherries [56]. It has been demonstrated that SA inhibits some cell wall degradation enzymes such as polygalacturonase, cellulase, and pectinmethylesteras [21]. Likewise, it has been indicated that SA could enhance the activity of antioxidant enzymes and inhibit oxidase activity by inhibiting the precursors of ethylene synthesis [57]. However, a previous study reported that SA treatment maintains fruit firmness by affecting the osmotic pressure of the cell wall and inhibiting the rate of fruit breakdown [58]. The results obtained in this research corroborate and extend the existing information on the benefits of the use of elicitors on fruit firmness.

Regarding fruit weight and diameter (Figure 5a,b), 'Kirra' was more sensitive to OA applications than 'Stella blue' with SA applications, the latter showing nonsignificant differences between treatments. Applications of 4 mM OA on 'Kirra' (Figure 5a) significantly decreased fruit weight with respect to the control, with a differential close to 21% lower. In addition, OA applications did not significantly increase fruit diameter (Figure 5b). These results contrast with previous studies, where these elicitor applications increase the physical parameters of the fruit as determined in this study [53–55]. Therefore, our results are related to the physiological state of the plant, which was more functional with elicitor applications, which may have led to higher fruit yield plant^{-1}, which often causes an inverse relationship with fruit physical parameters such as weight and size [59].

Regarding the fruit chemical compounds determined in this study (Figure 6), in 'Kirra', the total polyphenolic content was significantly higher with the 2 and 4 mM OA treatments with respect to the control, but without significant differences between them. These results suggest that the effects of OA on total phenol content do not depend on the concentration of the elicitor used in this study. The higher polyphenol content achieved with OA applications is in agreement with previous reports on peach [60], sweet cherry [61], banana [23], and mango [62] fruits. Phenolic compounds are an important group of secondary metabolites, which can influence berry quality, as well as the color, flavor, astringency, and functional properties of berries [63]. A probable explanation for the increase in polyphenols by OA application, this compound is considered a natural antioxidant by suppressing lipid peroxidation and reducing ascorbic acid oxidation [64]. In addition, it has been shown that the increased phenolic content by OA applications may be the result of the activation of the enzyme phenylalanine ammonia–lyase, which plays a key role in the phenylpropanoid pathway by catalyzing the conversion of phenylalanine to trans-cinnamic acid [60].

On the other hand, in 'Stella blue' the 2 mM SA treatment had a significantly higher polyphenolic content compared to the other treatments. It has been demonstrated that SA and its derivatives stimulate the accumulation of phenolic compounds in plant species before and after harvest due to the activation of phenylpropanoid metabolism [65], in addition to what has been observed in other studies, where SA improved the yield of carotenoids, through the regulation of biosynthetic genes of these compounds [66].

In both cultivars, total anthocyanin content and fruit antioxidant capacity had similar trends with respect to total polyphenolic content, being higher in 'Kirra' with an application of 4 mM OA and in 'Stella blue' with 2 mM SA. This is probably due to the fact that anthocyanins, characteristic pigments of blueberry fruits, account for up to 70% of the phenolic compound content of blueberries [67]. On the other hand, their unpaired electrons are hydrogen donors and can effectively scavenge reactive oxygen radicals that reduce the effects of oxidative stress [68], which is in agreement with the obtained results. In grapes, Champa et al. [69] observed that SA reduced anthocyanin degradation, while García-Pastor et al. [70] detected an increase in the anthocyanin content. Despite these findings, Perin et al. [63] indicate that intensive studies are still needed to explore the relationship between SA and the metabolic pathway of flavonoids in plants, as well as the application methods. Anthocyanins promote anti-inflammatory, immune, antioxidant, antitumor, and other physiological activities of the human organism [71]; for this reason, our research makes an important contribution to the knowledge of the influence on the quality of blueberry fruit by the effect of elicitors applied in preharvest.

4.3. Influence of Variables

The correlation matrix reaffirmed the importance of evaluating plant physiological response in conjunction with the physicochemical parameters of blueberry fruit treated with elicitors. Plant physiological response measured through g_s and F_v/F_m had a strong correlation with fruit firmness in 'Kirra' (Figure 7a) and 'Stella blue' (Figure 7b). This is consistent with previous reports; since the regulation of abiotic factors, the plant is able to improve its nutritional status and physiological efficiency, promoting photosynthesis and translocation of photoassimilates to different plant organs, including the fruit [34]. In our case, the use of elicitors in the different blueberry cultivars contributed significantly to improving the physiological processes of the plant and the firmness of the fruit compared to the postharvest control. It is known that once the fruit is harvested, softening begins immediately, which is the main limiting factor in the export of fresh fruit [3]. Without the use of elicitors, 'Kirra' presented better firmness than 'Stella blue'; therefore, the probable influence of genotype on this fruit parameter is highlighted [4]. However, the use of elicitors in preharvest significantly improved fruit firmness towards the last harvests, with 'Stella blue' having similar firmness values compared to 'Kirra'. On the other hand, it was possible to demonstrate that the concentration of bioactive compounds and antioxidant capacity of the fruit in both cultivars is closely related to the physiological conditions of the plant, presenting a positive correlation, and with respect to the physical parameters of the fruit, presenting a negative correlation (Figure 7a,b). Regarding the fruit chemical compounds determined in this study, elicitor applications led to their higher accumulation, which is probably due to a higher concentration caused by the loss of fruit weight and size, which is mainly water [56]. Principal component analysis of the variables (Figure 8a) corroborates the above, as there is a greater closeness in distance and colors between the aforementioned variables and treatments for 'Kirra' (Figure 7a,c) and 'Stella blue' (Figure 7b,d).

5. Conclusions

The applications of oxalic acid (OA) on 'Kirra' and salicylic acid (SA) on 'Stella blue' blueberry improved the physiological state of the plant, which led to higher fruit yield and an increase in the concentration of bioactive compounds in the fruit, such as polyphenolics and total anthocyanins, which, in turn, presented higher antioxidant activity. Although both elicitor concentrations (2 and 4 mM) showed better results in fruit firmness and chemical compounds with respect to the control treatment (0 mM). The treatment with 2 mM SA generated a 100% increase in polyphenolic content and antioxidant capacity ($p < 0.05$) in 'Stella Blue', with values close to 140 mg gallic acid 100 g^{-1} and 80 mg 100 g^{-1} fresh weight, respectively. In 'Kirra', OA treatments did not have a significant impact on polyphenolic content, but 4 mM OA increased by 100% and 20%, total anthocyanin and antioxidant capacity of blueberry fruit, respectively. Based on our results, three pre-harvest

applications of OA and SA, during the fruit development phase until the beginning of ripening, improve fruit firmness by up to 20% at different harvest times, reducing the loss of firmness during the advance of harvest. Despite these results, we believe that it is necessary to continue evaluating different doses of these elicitors and their combined effect on these and other blueberry cultivars, integrating different physical–chemical parameters of the fruit during postharvest storage.

Author Contributions: Conceptualization, J.R.-S. and G.C.-G.; methodology, J.R.-S., G.A. and R.O.; software, J.R.-S., M.B. and G.C.-G.; validation, G.A., S.C.O.-O., M.D.L. and R.V.-R.; formal analysis, J.R.-S., G.C.-G., M.D.L. and H.O.-S.; investigation, J.R.-S., R.O., G.A. and S.C.O.-O.; resources, J.R.-S., R.O. and G.C.-G.; data curation, J.H., G.A., S.C.O.-O., R.V.-R. and M.B.; writing—original draft preparation, J.R.-S. and G.C.-G.; writing—review and editing, J.H., H.O.-S. and M.D.L.; visualization, G.A., H.O.-S. and J.H.; supervision, J.R.-S., M.D.L. and M.B.; project administration, J.R.-S. and R.V.-R.; funding acquisition, J.R.-S. and G.C.-G. All authors have read and agreed to the published version of the manuscript.

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Effect of Nisin on the Quality and Antioxidant Activity of Fresh-Cut Pumpkins (*Cucurbita moschata* Duch.)

Ning Yuan [1], Yi Wang [1], Yuge Guan [2], Chen Chen [1,*] and Wenzhong Hu [1,*]

[1] College of Life Science, Dalian Minzu University, Dalian 116600, China; yn2822852617@163.com (N.Y.); w13936057663@163.com (Y.W.)
[2] School of Food and Health, Zhejiang Agricultural and Forestry University, Hangzhou 311300, China; gyg@zafu.edu.cn
* Correspondence: chenchen@dlnu.edu.cn (C.C.); hwz@dlnu.edu.cn (W.H.)

Abstract: Fresh-cut pumpkins refer to fresh pumpkin that has been graded, cleaned, peeled, sliced, preserved, and packaged. It has the qualities of freshness, nutrition, convenience, and being 100% edible. However, mechanical damages during the cutting processing can accelerate the quality deterioration, aging, and loss of nutritional values of fresh-cut pumpkins. Nisin, a natural preservative, has been widely used in fruits and vegetables with good preservation effects. To investigate the effect of different concentrations (0, 0.2, 0.4, and 0.6 g/L) of nisin on the quality of fresh-cut pumpkins, the critical indexes involved in weight loss, firmness, color, respiration intensity, reactive oxygen species (ROS) metabolism, ascorbate (AsA)—glutathione (GSH) cycle, and antioxidant capacity were monitored for fresh-cut pumpkins during storage at 4 °C for 10 days. The results showed that 0.4 g/L nisin was the best preservation concentration. Compared with 0 g/L nisin, 0.4 g/L nisin reduced the weight loss rate and whitening rate of fresh-cut pumpkins by 13.53% and 13.61%, inhibited respiration rate by 45.83%, and maintained hardness by 1.18 times. Meanwhile, 0.4 g/L nisin increased the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) and maintained higher contents of GSH and AsA. It prevented the rapid increase in ROS levels by improving antioxidant capacity, including DPPH, ABTS free radical scavenging rate, and T-AOC (total antioxidant capacity). The collected results showed that nisin has an obvious influence on the quality by regulating physiological and antioxidant activity metabolism. It is envisaged that the combination of nisin and physical and chemical preservation technology will further enhance the quality of fresh-cut pumpkins during storage in the future.

Keywords: fresh-cut pumpkins; nisin; reactive oxygen species; ascorbate-glutathione

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1. Introduction

Pumpkin is one of the most widely grown crops on the planet [1]. It is favored by consumers because of its sweet and mild flesh, as well as its high nutritional content [2]. Pumpkin polysaccharides [3], phenols (including flavonoids and phenolic acids), carotenoids [4], minerals (including potassium, phosphorus, magnesium, iron, and selenium), and vitamins (including B, C, E, and K) [5] are all found in pumpkin. These nutrients have antioxidant activity, which is useful for maintaining the body's immunological function and lowering the risk of some types of cancer, and they are especially vital for diabetics and those with vascular damage [6,7]. Thus, it is also regarded as a vegetable in many countries' healthy diets and traditional medicine. China is the world's largest producer of pumpkins. According to the Food and Agriculture Organization of the United Nations, China accounted for about one-third of the world's pumpkin production in 2021 [8]. Despite the fact that pumpkin is a store-tolerant crop, it is still wasted owing to quality degradation during the production, manufacture, transportation, retail, and home storage stages. Furthermore, the sheer size of pumpkins might make them difficult to transport and handle for the typical customer.

Fresh-cut pumpkins refer to fresh pumpkins that have been graded, cleaned, peeled, sliced, preserved, and packaged [9]. It has the qualities of freshness, nutrition, convenience, and being 100% edible. In line with consumer demands in the restaurants and retail markets for fresh-cut vegetables. However, mechanical cutting resulted in a number of quality changes that reduced the shelf life and marketability of fresh-cut pumpkins, including microbial infection, faster nutrient loss, yellowing and whitening of the cut surface, tissue softening, and flavor alterations [9]. For these reasons, food scientists have developed three different techniques. Firstly, the physical preservation method is the most widely employed for fresh-cut pumpkins because of its advantages of easy control of processing conditions and little influence on the structure and nutritional tastes, but it requires a relatively large investment in equipment [10]. Secondly, while the chemical preservation method has an excellent preservation effect, it uses chemicals that are easy to leave residue, which causes safety issues [11]. Finally, due to its safety, non-toxicity, and great efficiency, biological preservation technology has attracted special attention from scholars all over the world in recent decades, and it is utilized in a variety of foods [10].

Nisin, a bacteriocin produced by the Lactococcus lactis subspecies, is a safe and non-toxic natural biological preservative. Nisin has been widely used as a food preservative for more than 60 years. The U.S. Food and Drug Administration (FDA) classifies Nisin as Gras and other countries allow its use as a food preservative [12,13]. In addition, nisin can be rapidly digested and decomposed into various amino acids by protease, and it does not cause toxic side effects on the human body. Therefore, nisin has been widely used in the preservation of fruits and vegetables with good preservation effects [14,15]. Studies have shown that nisin combined with green tea (GTE), modified atmosphere packaging (MAP), and modified initial atmosphere (MIA) treatment on fresh-cut beet leaves greatly increased total polyphenol contents and antioxidant capacity [16]. Nisin and ε-polylysine with chitosan coating significantly ($p < 0.05$) inhibited respiration rate, decline of AsA, and white blush in fresh-cut carrots [17].

At present, there is no information available on the treatment of nisin on fresh-cut pumpkin to improve its quality and antioxidant activity during storage. Therefore, the purpose of this study is to investigate the effects of different nisin concentrations (0, 0.2, 0.4, and 0.6 g/L) on the weight loss, firmness, color, and respiratory intensity of fresh-cut pumpkins stored at 4 °C for 10 days. Additionally, a suitable concentration was chosen to measure the ROS level, antioxidant enzyme activity, antioxidant content, and antioxidant capacity of fresh-cut pumpkins in order to investigate how nisin affects their antioxidant activity. Simultaneously, the study provides a biological preservation strategy for maintaining the quality and extending the shelf life of fresh-cut pumpkins.

2. Materials and Methods

2.1. Processing and Treatments of Fresh-Cut Pumpkins

Pumpkins (*Cucurbita moschata*, Duch) of the cultivar "Luli" were obtained from a commercial farm near Wuchang City, Heilongjiang Province. The harvest area of "Luli" pumpkins was located in a high-cold and high-latitude area, with sufficient light in the growing season, long sunshine times, and a large temperature difference between day and night. The harvest area is fertile black soil, characterized by high organic content, fertile soil, and loose soil. The mature tropical pumpkins were harvested at the mature green 86 stage (65 days after flowering).

Pumpkins with uniform melon shapes and similar maturities, without diseases, pests, or mechanical damages, were selected and stored at 4 °C for later use. The pumpkins were soaked in 0.1% sodium hypochlorite water for 2 min, rinsed with distilled water, and then the skin and pulp were removed. The pumpkins were cut into 1/4 circular arc slices with a thickness of 0.5 cm and a width of 2 cm. Soak them in nisin (nisin A, purchased from Shanghai Yuanye Bio-Technology Co., Ltd., Shanghai, China) solution at a concentration of 0, 0.2, 0.4, and 0.6 g/L for 10 min, and then make fresh-cut pumpkins after being drained. Then, fresh-cut pumpkins were packed inside a polypropylene container with polyethylene

films and stored at 4 °C for 10 days. Samples were taken every 2 days to determine the optimal concentration of nisin for weight loss, firmness, color, and respiration. Then the samples of the control group and the optimal concentration treatment group were ground into a powder after being frozen with liquid nitrogen, then stored at −80 °C for antioxidant index measurements.

2.2. Weight Loss

The weight of samples was weighed using an electronic balance, and three sets were set up in parallel; thus, the weight loss was expressed as the ratio of the mass difference value to the initial fresh mass (%) [18].

2.3. Firmness

The firmness of fresh-cut pumpkin slices was measured by carrying out texture profile analysis (TPA) using a TA-XT texture analyzer. The cylindrical P5 probe with a diameter of 5 mm was pressed down the slice at a rate of 1 mm/s, the trigger force was 0.5 N, and the depth of penetration was 4 mm [19]. Different parts of fresh-cut pumpkins in each treatment group were measured, and the maximum force (N) was measured as the firmness.

2.4. Color

The color (L*, a*, and b*) of fresh-cut pumpkin slices was measured by a CR-400 colorimeter, where the L* value defines brightness, the a* value defines red-green, and the b* value defines yellow-blue. The whitening index (WI) indicates the degree of whitening on the cut surface of fresh-cut pumpkins [20]. The formula is as follows:

$$WI = 100 - \sqrt{(100 - L*)^2 + (a*)^2 + (b*)^2)} \quad (1)$$

2.5. Respiration Intensity

The respiration intensity of fresh-cut pumpkins was measured by a gas analyzer (F-940 STORE II, Felix, Camas, WA, USA) [21]. Place 50 g of fresh-cut pumpkins in a 550-mL sealed crisper to store the sample at 4 °C for 1 h. Then the CO_2 content in the crisper was measured for 25 s, and repeated three times. The respiratory intensity was calculated according to the following formula:

$$\text{Respiration Intensity (mg/(kg·h))} = ((C_1 - C_2) \div 100 \times V) \div (m \times t) \times 1.96 \quad (2)$$

where C_1 represents the CO_2 content (%) in the sealed crimp box, C_2 represents the atmospheric CO_2 content (%), V represents the container volume (mL), m represents the sample mass (kg), and t represents the placement time (h).

2.6. Superoxide Anion (O_2^-·) Production Rate

Approximately 0.2 g of the frozen sample was used for the O_2^-· rate of production assay according to the protocol of the OFR measurement kit (SA-1-G, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [22]. After adding reagents, the light absorption for each sample was determined by measuring the UV absorbances (A1) at 530 nm against a blank solution (A2) (ΔA = A1 − A2). A standard curve was generated for O_2^-· rate of production measurement (y = 0.0121x − 0.0027, R2 = 0.9980). Finally, the O_2^-· rate of production is measured by the following formula: (nmol/g·min) = (ΔA + 0.0027) ÷ 0.0121 × V1 ÷ (V2 ÷ V3 × W) × 2 ÷ T (V1: total response volume; V2: reaction sample volume; V3: extraction liquid volume; W: sample mass; 2: 2 molecules of O_2^- participating in the reaction to form 1 molecule of NO_2^-; T: reaction time.) Three independent replicates were used for each treatment.

2.7. H_2O_2 Content

Approximately 0.2 g of the frozen sample was used for H_2O_2 content assay according to the protocol of the H_2O_2 content measurement kit (H_2O_2-1-Y, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [22]. After adding reagents, the light absorption for each sample was determined by measuring the UV absorbances (A1) at 415 nm against a blank solution (A2) ($\Delta A = A1 - A2$). Regression curves were generated for the determination of H_2O_2 content under standard conditions ($y = 0.3744x + 0.0006$). Finally, the H_2O_2 content is measured by the following formula: $(\mu mol/g) = [(\Delta A - 0.0006) \div 0.3744 \times V1] \div (W \times V1 \div V2)$ (V1: sample volume in the reaction system; V2: extraction liquid volume; W: sample quality). Three independent replicates were used for each treatment.

2.8. Superoxide Dismutase Activity (SOD)

Approximately 0.2 g of the frozen sample was used for the SOD activity assay according to the protocol of the SOD measurement kit (SOD-1-Y, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [22]. After adding reagents, the percentage inhibition was determined by measuring the UV absorbance at 560 nm for each sample (A1) and the light absorption of the control solution (A2) (inhibition rate = $(A2 - A1) \div A2 \times 100\%$). One unit of SOD activity was defined as SOD enzyme activity in the reaction system when the percentage inhibition was 50%. Three independent replicates were used for each treatment.

2.9. Catalase Activity (CAT)

Approximately 0.2 g of the frozen sample was used for the CAT activity assay according to the protocol of the CAT measurement kit (CAT-1-Y, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [22]. After adding reagents, the light absorption for each sample was determined by measuring the initial UV absorbances (A1) at 240 nm against the absorbance value after 1 min (A2) ($\Delta A = A1 - A2$). One unit of CAT activity was defined as catalyzing 1 nmol of H_2O_2 degradation per minute per g of tissue at 240 nm. Three independent replicates were used for each treatment.

2.10. Ascorbate Peroxidase Activity (APX)

The APX activity determination method refers to Yan et al. [23,24]. The frozen, fresh-cut pumpkin tissues (5 g) were added to 20 mL of 0.1 mol/L potassium phosphate buffer (pH 7.5), and the supernatant was centrifuged at 12,000× *g* for 30 min at 4 °C as the enzyme extraction solution, which was stored at a low temperature for later use. APX was determined by mixing a 2.6 mL reaction buffer (containing 0.1 mmol/L EDTA and 0.5 mmol/L ascorbic acid) with 0.1 mL of enzyme extract, then adding 0.3 mL of a 2 mmol/L H_2O_2 solution to start the reaction. The absorbance value at 290 nm was determined within 15 s. One unit of enzyme activity was defined as U/g when the absorbance value at 290 nm of the APX enzymatic reaction system decreased by 0.01 per gram of the fresh-cut pumpkin sample.

2.11. Glutathione Reductase Activity (GR)

Approximately 0.2 g of the frozen sample was used for the GR activity assay according to the protocol of the GR measurement kit (GR-1-W, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [22]. After adding reagents, the light absorption for each sample was determined by measuring the initial UV absorbance (A1) at 240 nm against the absorbance value after 180 s (A2) ($\Delta A = A1 - A2$). One unit of GR enzyme activity was defined as catalyzing the oxidation of 1 nmol of NADPH per minute per g of tissue at 340 nm at pH 8.0 at a specified temperature. Three independent replicates were used for each treatment.

2.12. Reduced Glutathione Content (GSH)

Approximately 0.2 g of the frozen sample was used for the GSH content assay according to the protocol of the GSH measurement kit (GSH-1-W, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [25]. After adding reagents, the light absorption for each sample

was determined by measuring the UV absorbances (A1) at 412 nm against the absorbance of the blank solution (A2) (ΔA = A1 − A2). A standard curve was generated for GSH content measurement (y = 0.75x). Finally, the GSH content is measured by the following formula: (μmol/g) = ΔA ÷ 0.75 × V1 ÷ (V1 ÷ V2 × W) (V1: accumulation of supernatant liquid in the reaction system; V2: total volume of supernatant; W: sample quality). Three replicates were used for each treatment.

2.13. Ascorbic Acid Content (AsA)

Approximately 0.2 g of the frozen sample was used for the AsA content assay according to the protocol of the AsA measurement kit (AsA-1-W, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [22]. After adding reagents, the light absorption for each sample was determined by measuring the UV absorbances (A1) at 420 nm against the absorbance of the blank solution (A2) (ΔA = A1 − A2). A standard curve was generated for AsA content measurement (y = 0.0044x − 0.018, R2 = 0.9978). Finally, the AsA content is measured by the following formula: (μg/g) = (ΔA + 0.018) ÷ 0.0044 × V1 ÷ (W × V1 ÷ V2) (V1: reaction sample volume; V2: extraction liquid volume; W: sample quality). Three replicates were used for each treatment.

2.14. DPPH, ABTS Free Radical Scavenging Rate

Approximately 0.2 g of the frozen sample was used for the DPPH and ABTS clearance rate assays according to the protocol of the DPPH and ABTS measurement kits (DPPH-1-D and ABTS-1-D, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China), respectively [22]. After adding reagents, the light absorption of DPPH and ABTS for each sample was determined by measuring the UV absorbances (A1) at 515 nm against the absorbance of the blank solution (A2) (ΔA = A2 − A1), respectively. DPPH/ABTS free radical scavenging rate (%) = (A2 − A1) ÷ A2 × 100%.

2.15. Total Antioxidant Capacity (T-AOC)

Approximately 0.2 g of the frozen sample was used for the total antioxidant capacity assay according to the protocol of the FRAP measurement kit (FRAP-1-G, Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) [22]. After adding reagents, the light absorption for each sample was determined by measuring the UV absorbances (A1) at 593 nm against the absorbance of the blank solution (A2) (ΔA = A1 − A2). A standard curve was generated for FRAP content measurement (y = 1.2416x + 0.0134, R2 = 0.9996). Finally, the FRAP content is measured by the following formula: (μmol/g) = (ΔA − 0.0134) ÷ 1.2416 × V1 ÷ (V1 ÷ V2 × W) (V1: reaction sample volume; V2: extraction liquid volume; W: sample quality). Three replicates were used for each treatment.

2.16. Statistical Analysis

The data were presented as the mean ± SD (standard deviation). Originpro 2021 software (OriginLab., Northampton, MA, USA) was used for the drawing of figures. Using SPSS Version 26 (IBM Corp., Armonk, NY, USA), one-way analysis of variance (ANOVA) and minimal significance difference (LSD) were used to differentiate mean values at the $p < 0.05$ level and the correlation between parameters, using Heml Version 2.0 (The CUCKOO Workgroup, Wuhan, China) software to draw a heat map.

3. Results

3.1. Weight Loss and Firmness

Due to direct contact with air, the transpiration of fresh-cut pumpkins is rapidly accelerated, resulting in increased weight loss [26]. As shown in Figure 1a, the weight loss of fresh-cut pumpkins in all treatment groups showed an increasing trend. The values in the nisin groups were significantly lower than those in the control group at 0–6 days, and the weight loss in the nisin group with 0.2 g/L, which was almost the same as that of the control group, was higher than that in the other two nisin groups from 8 to 10 days.

However, the 0.4 g/L nisin had the lowest weight loss and the most obvious inhibition of water loss during the whole storage period. Compared with the control group, the weight loss rate at the end of the storage period in the 0.4 g/L treatment group was reduced by 13.53%.

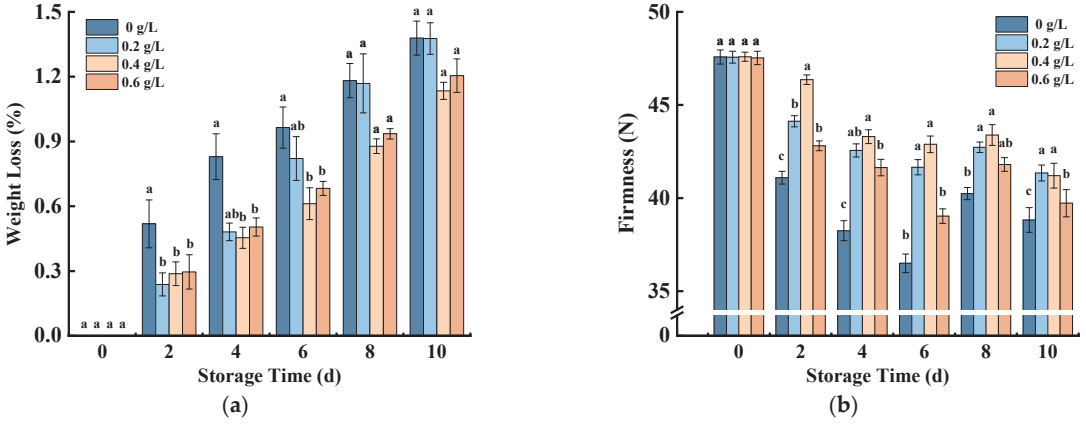

Figure 1. Effects of nisin treatment on weight loss (**a**) and firmness (**b**) in fresh-cut pumpkins. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

The firmness of fresh-cut pumpkins is easily affected by moisture content, lignin formation, and oxidation [27]. Figure 1b shows that the firmness of the four groups of fresh-cut pumpkins decreased rapidly and then increased slowly. During the whole storage period, the firmness of the 0.4 g/L nisin was higher than that of the other groups, and the value was the lowest in the control group. The 0.4 g/L Nisin most clearly preserved the hardness of fresh-cut pumpkin at 6 days, which was 1.18 times higher than the control group. The firmness increased slowly from 6 to 8 days, which may be due to the callus response of the pumpkin slices under the injury stress, which generated callus at the cut site, leading to the firmness increase of the fresh-cut pumpkins. Furthermore, applying a 1% nisin nano-coating to a white button similarly showed minimal weight loss and firmness [28].

3.2. Whitening Index (WI)

The surface of fresh-cut pumpkins easily produces a white substance when it loses water, and WI is used to indicate the degree of whitening [17]. Figure 2 shows an upward trend in the whitening index of fresh-cut pumpkins during storage, but nisin could effectively inhibit this change. It is possible that nisin reduced whitening on fresh-cut pumpkins by inhibiting surface weight loss. During the whole storage period, 0.4 g/L nisin-treated slowed the rise of the whitening index, and the values in all nisin-treated samples were lower than those in the control group. At the end of the storage period, the whitening index was reduced by 13.61% compared with the control group. Similar results were found in fresh-cut carrots [17] and white buttons [28], which indicated that nisin was extremely well maintained in the color of fruit and vegetables.

3.3. Respiration Intensity

Increased respiration rates can cause fruit and vegetable aging [29]. Figure 3 showed that the respiratory intensity of fresh-cut pumpkins in each treatment group decreased first and then increased during storage. The values reached their lowest intensity at 6 d and then rose. The respiratory intensity of all nisin-treated groups was lower than that of the control group, and the 0.4 g/L nisin group had a significant effect on the respiratory intensity

of fresh-cut pumpkins. Respiratory rates of fresh-cut pumpkins were greatly reduced by 0.4 g/L Nisin at 8 d and inhibited by 45.83% compared with the control group. Thus, it can effectively delay the aging process of fresh-cut pumpkins. In addition, similar results were found in fresh-cut carrots [17] and button mushrooms [30]. Those results indicated that nisin was extremely efficient in decreasing the respiration rates of fruits and vegetables.

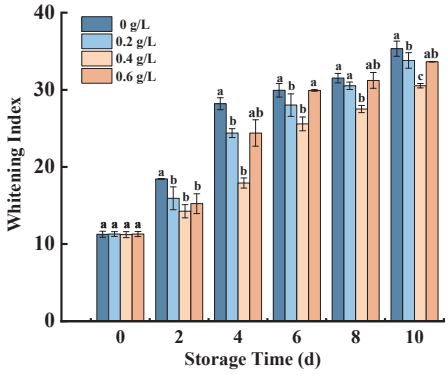

Figure 2. Effects of nisin treatment on WI in fresh-cut pumpkins. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

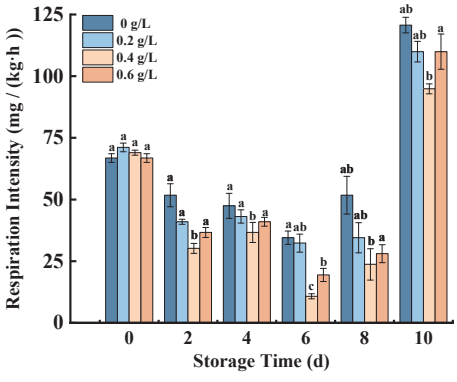

Figure 3. Effects of nisin treatment on respiration intensity in fresh-cut pumpkins. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

In conclusion, 0.4 g/L nisin treatment could effectively reduce the weight loss, hardness loss, whitening, and respiration intensity of fresh-cut pumpkins and better maintain the storage quality. Therefore, we selected the nisin concentration of 0.4 g/L for subsequent analysis of antioxidant activity indexes to explore the effect of nisin on the antioxidant capacity of fresh-cut pumpkins.

3.4. O_2^-· Production Rate and H_2O_2 Contents

Fresh-cut fruits and vegetables under cutting stress will trigger the production of a large number of ROS, such as O_2^-·and H_2O_2, in cells. However, excessive production and accumulation of ROS will seriously accelerate the aging of fruits and vegetables [31]. The O_2^-· production rate of fresh-cut pumpkins was at its highest rate at 0 d (Figure 4a). The control group showed a downward trend from 0 to 4 days and then slowly increased to 6 days and then decreased. After treatment with 0.4 g/L nisin, the O_2^-· production rate

of the nisin group was lower than that of the control group, except for the 4th day. Nisin reduced the $O_2^{-}\cdot$ production rate of fresh-cut pumpkins by 65.11% compared with the control group. Figure 4b showed that H_2O_2 content increased during the storage time, but the 0.4 g/L nisin effectively inhibited the accumulation of H_2O_2, compared with the control group. At day 8, 0.4 g/L nisin reduced the H_2O_2 content of fresh-cut pumpkins by 40% compared with the control. Similar results were found in mushrooms [30], indicating that nisin reduced $O_2^{-}\cdot$ production rate and H_2O_2 content.

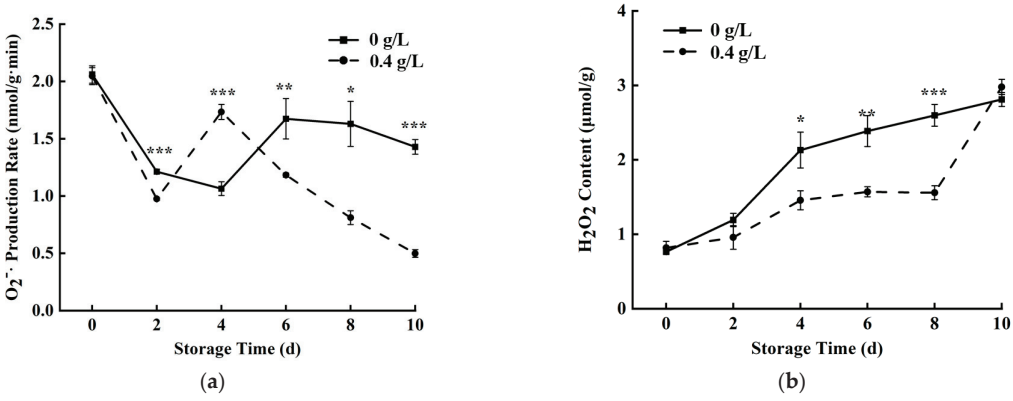

Figure 4. Effects of 0.4 g/L nisin treatment on $O_2^{-}\cdot$ (**a**) and H_2O_2 content production rate (**b**) in fresh-cut pumpkins (*, ** and *** indicate the significant difference between the 0.4 g/L nisin treatment and the control at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Vertical bars represent the standard deviation of the mean ($n = 3$)).

3.5. Antioxidant Metabolism-Related Enzyme Activities

SOD, CAT, APX, GR, and other enzymes work together to defend against the damage caused by reactive oxygen species or other peroxide free radicals to the cell membrane system, as well as maintain the balance of reactive oxygen metabolism and play an antioxidant role [32,33].

As can be seen from Figure 5a,c, the SOD and APX activities of fresh-cut pumpkins decreased first and then increased. Compared with the control group, 0.4 g/L nisin treatment could increase SOD activity at 2–4 days, while the trend in the later storage period was opposite to the early storage period. APX activity in the 0.4 g/L nisin group decreased from 0 to 4 days and then increased slowly from 4 to 10 days, whereas in the control group, it decreased to a nadir at 6 d and then increased, but it was always higher in the 0.4 g/L nisin group than that in the control group. Figure 5b showed that the CAT activity of fresh-cut pumpkins increased first and then decreased. CAT activity in the 0.4 g/L nisin group peaked at 6 d and in the control group at 4 d, but CAT activity was significantly higher in the 0.4 g/L nisin group at 0–6 days. Figure 5d showed that GR activity had been on the rise, and the activity of the 0.4 g/L nisin group was always higher than that of the control group during the storage. The activity of the 0.4 g/L nisin group was 759.51 U/g at 4 d, which was significantly higher than that of the control group.

3.6. Antioxidant Metabolism-Related Substances Contents

AsA and GSH are important antioxidants in the AsA-GSH cycle, and they complement each other in the process of scavenging reactive oxygen free radicals [34,35]. Figure 6a,b showed an increasing trend of both AsA and GSH in fresh-cut pumpkins. After 0.4 g/L nisin treatment, the AsA content of fresh-cut pumpkins accumulated rapidly from 4 to 10 days, which was significantly higher than that of the control group. In addition, the change trends of AsA content and APX enzyme activity were consistent, indicating that AsA can reduce the accumulation of H_2O_2 catalyzed by APX. Moreover, 0.4 g/L nisin treatment

could promote the accumulation of GSH content, which was higher than that of the control group during 4–10 days, which was conducive to the accumulation of antioxidants.

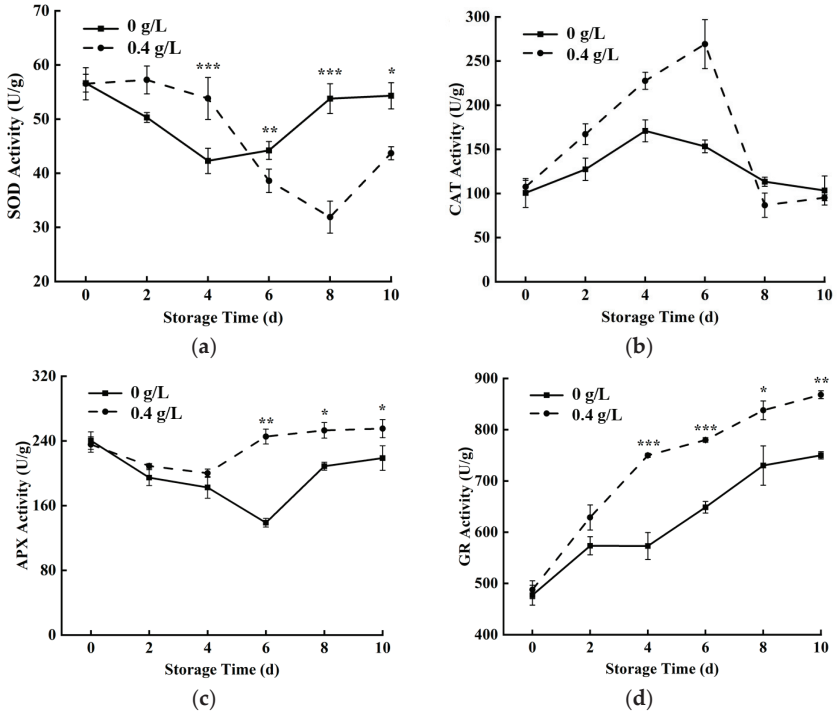

Figure 5. Effects of 0.4 g/L nisin treatment on SOD activity (**a**), CAT activity (**b**), APX activity (**c**), and GR activity (**d**) in fresh-cut pumpkins (*, **, and *** indicate the significant difference between the 0.4 g/L nisin treatment and the control at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Vertical bars represent the standard deviation of the mean ($n = 3$)).

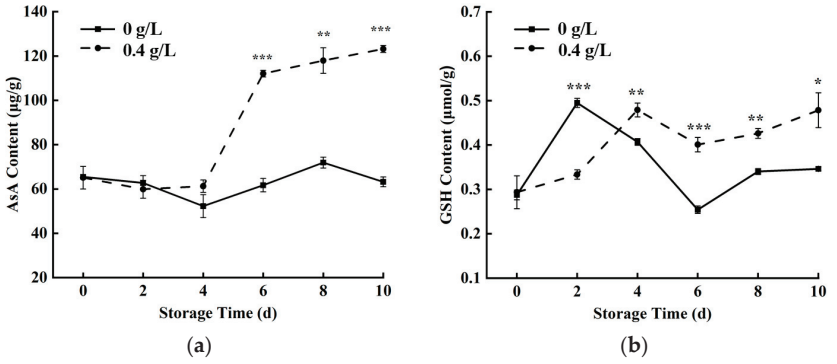

Figure 6. Effects of 0.4 g/L nisin treatment on AsA content (**a**) and GSH content (**b**) in fresh-cut pumpkins (*, **, and *** indicate the significant difference between the 0.4 g/L nisin treatment and the control at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Vertical bars represent the standard deviation of the mean ($n = 3$)).

3.7. Antioxidant Capacity

DPPH, ABTS, and T-AOC were used to evaluate the antioxidant capacity of fresh-cut pumpkins [32,36]. In the control group, DPPH, ABTS, and T-AOC of fresh-cut pumpkins showed an overall decreasing trend during storage (Figure 7a–c), and the scavenging ability of ROS decreased as a whole, but the antioxidant ability was improved after 0.4 g/L nisin treatment. The free radical scavenging rates of DPPH and ABTS reached their peaks on the second day, and the T-AOC was in an upward state, which was always higher than that of the control group, indicating that the 0.4 g/L nisin group had a higher antioxidant capacity.

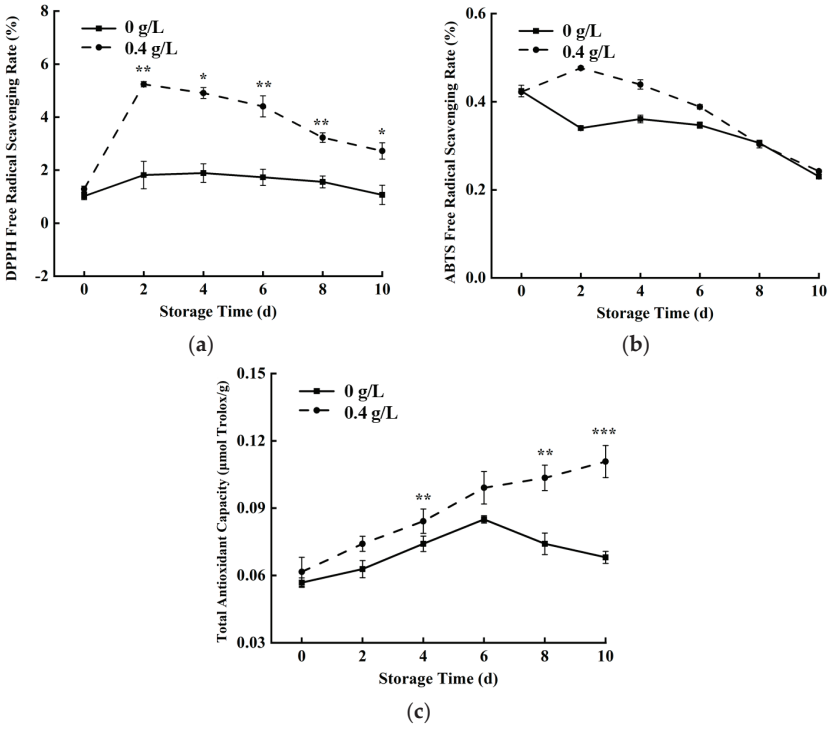

Figure 7. Effects of 0.4 g/L nisin treatment on DPPH (**a**), ABTS (**b**) free radical scavenging rate, and T−AOC (**c**) in fresh−cut pumpkins (*, **, and *** indicate the significant difference between the 0.4 g/L nisin treatment and the control at $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively. Vertical bars represent the standard deviation of the mean ($n = 3$)).

3.8. Correlation Analysis

The Pearson correlation coefficient between different parameters of fresh-cut pumpkins after 0.4 g/L nisin treatment is shown in Figure 8. Firmness was significantly positively correlated with APX and ABTS ($p < 0.05$) and significantly negatively correlated with H_2O_2 ($p < 0.05$). H_2O_2 was extremely significantly positively correlated with weight loss rate and WI ($p < 0.01$), while ABTS was extremely significantly negatively correlated ($p < 0.01$). ABTS was extremely significantly negatively correlated with weight loss and WI ($p < 0.01$). These results indicated that the antioxidant system of fresh-cut pumpkins was closely related to their storage quality. In addition, $O_2^{-}\cdot$ was extremely significantly negatively correlated with GR, GSH, and FRAP ($p < 0.01$), as well as significantly negatively correlated with AsA ($p < 0.05$), indicating that the AsA−GSH cycle was conducive to $O_2^{-}\cdot$ clearance.

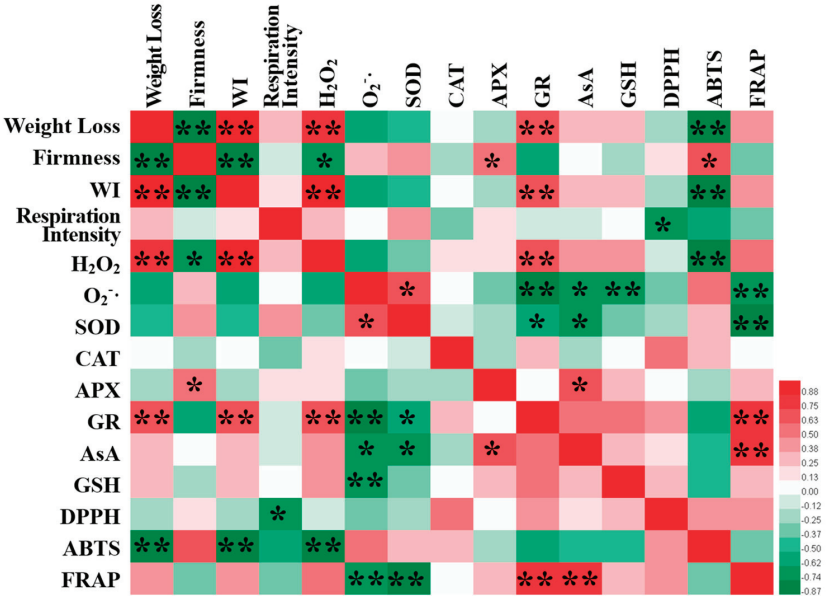

Figure 8. Pearson correlation matrix of each indicator. * and ** indicate that correlation between values reach the level of $p < 0.05$ and $p < 0.01$, respectively.

4. Discussion

Mechanically damaged pumpkin tissue is prone to a series of quality degradation problems such as cell rupture, water loss, and nutrient outflow, resulting in the weight loss rate decreasing, the tissue softening, the section turning white, and the respiration rate increasing [37,38]. Therefore, it is of great significance to explore safe and natural preservation technology to maintain the storage quality of fresh-cut pumpkins. Nisin, a safe food additive, is a safe and natural biological preservative agent, which has been widely concerned by researchers at home and abroad [39,40]. Many scholars have applied it to various foods and achieved good fresh-keeping effects, such as cucumber juice drinks [41], fresh-cut carrots [17], button mushrooms [30], fresh apple-kale blend juice [42], grape juice [43], fresh-cut beet leaves [16], fresh-cut onions [44], and fruit- and vegetable-based beverages [45]. In this study, it was found that nisin treatment could effectively inhibit the weight loss rate, the whiteness of the cut surface, the decrease in hardness, and the increase in respiration rate of fresh-cut pumpkins, as well as activate the antioxidant system and preserve the better quality of fresh-cut pumpkins.

Cutting stress leads to the accumulation of ROS and the aggravation of membrane lipid peroxidation, resulting in tissue oxidative damage and the acceleration of the aging process of fresh-cut pumpkins [46,47]. After broccoli was cut, high-intensity injury increased H_2O_2 content, low-intensity injury increased $O_2^{-}\cdot$ content, and SOD, POD, and CAT enzymes induced by the trauma accelerated the decomposition of ROS [48]. The antioxidant defense system is vital in the preservation of fruits and vegetables due to the fact that it can eliminate ROS and delay the aging process. SOD, CAT, APX, and GR are antioxidant enzymes that work together to maintain the metabolic equilibrium of ROS and function in an antioxidant role. SOD is a first-line defense system against ROS, capable of mutating $O_2^{-}\cdot$ into H_2O_2 and O_2. [49]. APX and GR serve as key enzymes in the AsA-GSH cycle [50]. APX and CAT enzymes catalyze the decomposition of H_2O_2 into O_2 and H_2O. GSH and AsA are important antioxidant substances in the ASA-GSH cycle, which are beneficial to maintain the activity of APX and provide ROS scavenging and reducing power [50,51].

In this study, ROS content was increased in the control group, while the production rate of $O_2^{-}\cdot$ and H_2O_2 content of fresh-cut pumpkins was decreased by nisin treatment almost during the storage period, which significantly inhibited ROS content. Additionally, nisin elevated SOD and CAT activities early in the storage period and APX activity later in the storage period. It is possible that SOD enzymes diminish $O_2^{-}\cdot$ production early in the storage period, whereas CAT and APX enzymes break down H_2O_2 later in the storage period, inhibiting ROS accumulation throughout the storage period. In the study of Agaricus bisporus using nisin in combination with chitosan and nano-silica, it was also found that the contents of $O_2^{-}\cdot$ and H_2O_2 could be maintained at the lowest levels, and the activities of SOD and CAT enzymes were significantly increased due to the addition of nisin [30]. Nisin treatment significantly raised the GR activity, GSH, and AsA contents of fresh-cut pumpkins, promoting the transport rate of the AsA-GSH cycle and increasing antioxidant capacity. Similar research has demonstrated that combining nisin and chitosan treatments could increase GR activity, GSH, and AsA contents in feijoa, improve fruit resistance, and so postpone plant senescence and fruit deterioration [52].

Furthermore, the correlation analysis results (Figure 8) show that $O_2^{-}\cdot$ was negatively correlated with GR, AsA, GSH, and FRAP, and H_2O_2 had a positive correlation with weight loss and the whitening index and was negatively correlated with hardness and ABTS. These findings revealed that ROS accumulation can lower the quality of fresh-cut pumpkins, but the antioxidant enzymes and substances are advantageous to ROS elimination.

In conclusion, fresh-cut pumpkins treated with nisin could increase the activity of the SOD enzyme in the early stage of storage to reduce the production rate of $O_2^{-}\cdot$ and activate the CAT and APX enzymes to remove H_2O_2, which results in reduced ROS levels. Meanwhile, GR activity and contents of GSH and AsA were also significantly increased, which promoted the transport rate of the AsA-GSH cycle and enhanced antioxidant capacity. Previous studies have also shown that nisin could enhance these antioxidant capacities in fresh-cut beet leaves [16], grape juice [43], fresh apple-kale blend juice [42], and Agaricus bisporus [30].

5. Conclusions

Nisin has a positive effect on the preservation of fresh-cut pumpkins. This study showed that 0.4 g/L nisin, compared with the control group, effectively reduced water loss, maintained firmness, inhibited whitening, and decreased the respiration rate for fresh-cut pumpkins to maintain a good appearance quality. Nisin could also reduce the accumulation of ROS ($O_2^{-}\cdot$ and H_2O_2) by enhancing the activities of SOD and CAT and regulating the conversion of antioxidant enzymes (GR and APX) and antioxidant substances (GSH and AsA) in the AsA-GSH cycle. In addition, combined with DPPH, the ABTS free radical scavenging rate, and T-AOC, nisin could effectively enhance the antioxidant activity of fresh-cut pumpkins. In conclusion, nisin treatment has the potential to maintain the preservation quality and delay the aging process during the storage period, which is a natural biological preservative that can effectively improve the quality of fresh-cut pumpkins.

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Different Cutting Methods Affect the Quality of Fresh-Cut Cucumbers by Regulating ROS Metabolism

Yuge Guan [1,*], Wenzhong Hu [2], Lei Wang [3] and Bailu Yang [4]

[1] School of Food and Health, Zhejiang Agricultural and Forestry University, Hangzhou 311300, China
[2] School of Pharmacy and Food Science, Zhuhai College of Science and Technology, Zhuhai 519041, China
[3] MOE Key Laboratory of Macromolecular Synthesis and Functionalization, Department of Polymer Science and Engineering, Zhejiang University, Hangzhou 310027, China
[4] Food Inspection Laboratory, Pingdu Municipal Inspection and Testing Center, Qingdao 266700, China
* Correspondence: gyg@zafu.edu.cn

Abstract: Fresh-cut cucumbers (*Cucumis sativus* L.) are appreciated by consumers for their convenience and freshness. In the process of home cooking and in the food industry, different cutting methods for cucumbers are needed. In order to explore the effect of cutting methods on the quality of fresh-cut cucumbers, cucumbers were cut into slices, pieces, and strips and whole cucumbers were used as the control. The results indicate that the vitamin C content of the sliced, pieced, and stripped cucumbers was gradually reduced, while the glutathione content increased significantly ($p < 0.05$) compared with the whole cucumbers. Furthermore, this study reveals that the fresh-cutting operation induced the production of ROS ($O_2^-\cdot$ and H_2O_2). Simultaneously, cutting activates phenylalanine ammonia-lyase and peroxidase activity, which enhanced the total phenol content by 1.35 times, 1.51 times, and 1.78 times in the pieced, stripped, and sliced cucumbers, respectively. This combines with the enhancement in the ascorbate peroxidase, glutathione reductase, superoxide dismutase, and catalase activity, contributing to the antioxidant capacity increasing by 1.14–1.95 times compared with the control. In conclusion, the degree of quality indexes was sliced > pieced > stripped. Therefore, this study provides useful information to illuminate the mechanism of the quality change in fresh-cut cucumbers subjected to different cutting methods and makes suggestions on the appropriate cutting style for the commercial or home use of cucumbers.

Keywords: fresh-cut cucumber; cutting methods; reactive oxygen species; quality

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1. Introduction

Fresh-cut fruits and vegetables are ready-to-eat fresh fruits and vegetables that have undergone various procedures such as classification, trimming, cleaning, cutting, coring, dressing, preservation, and packaging; these procedures make them convenient, safe, nutritious, and fresh to consume [1]. Additionally, the waste materials generated during the fresh-cutting process, such as skin residue and cores, can be recycled and reused, contributing to the reduction in urban domestic waste and environmental protection [2,3]. In recent years, fresh-cut fruits and vegetables have been increasingly favored by consumers due to their convenient characteristics of being ready-to-eat, ready-to-use, and ready-to-cook [4]. During fresh-cut produce processing, cutting operations may induce the explosion of reactive oxygen species (ROS) in the injured and surrounding tissues; this can lead to a faster deterioration process including oxidative browning, tissue softening, and development of off-flavors, thus limiting the shelf-life of fresh-cut produce [3,5].

Simultaneously, wounded products would activate various responses to regulate ROS levels, which allows for physiological modifications that aid in survival and protect the wounded tissue further. Studies have shown that the ascorbic acid–glutathione (AsA–GSH) cycle plays an important role in enhancing stress resistance and maintaining product quality [4,6,7]. Moreover, wounding stress would activate the phenylpropanoid metabolic

pathway to produce secondary metabolites, including phenolic compounds and lignin. This, in turn, enhances the adaptability of the organism to adapt to the stress caused by wounding. This phenomenon has been observed in various fresh-cut products such as apple [8], pitaya [9], potato [10], broccoli [11], carrot [12], lettuce [13], mushroom [14], onions [15], and celery [16].

Cucumber (*Cucumis sativus* L.) is an important vegetable crop with a vast cultivation area. It has become a very popular vegetable in people's daily diet, not only because of its sensorial properties and economic importance but also for its antioxidants, vitamins, pantothenic acid, and minerals that are important to maintaining human health [17]. In recent years, fresh-cut cucumbers have gained extensive worldwide attention due to their convenience and health benefits, and their application in the market has gradually increased [18,19]. Generally, during the processing of fresh-cut cucumbers, different cutting methods including cutting into pieces, strips, and slices were necessary for western vegetable salads and the prefabricated food industry or home use.

Previous studies have demonstrated that cutting styles have an obvious influence on the quality of fresh-cut carrots [20], onions [15], potatoes [10], and pitaya [9]. Interestingly, a previous study on fresh-cut broccoli revealed that the cutting type had no significant difference on the chlorophyll, total soluble solid, and reduced glutathione content [20]. However, previous research on fresh-cut cucumbers has primarily focused on preservation technology [18,19], and there is still a lack of scientific certification and systematic research on whether different cutting methods affect the quality of fresh-cut cucumbers.

Therefore, this study aimed to explore the mechanism of quality change based on the comparative analysis of the lightness, whiteness index, total soluble solid content, weight loss, respiration rate, firmness, and antioxidant substance content in fresh-cut cucumbers subjected to slicing, piecing, and stripping. Moreover, this study takes the ROS mechanism as the core, is focused on investigating the ROS content, ROS metabolism-related enzyme activities, and antioxidant substances content, in order to illuminate the mechanism of ROS in regulating the quality of fresh-cut cucumbers.

2. Materials and Methods

2.1. Sample Preparation and Treatment

Cucumber (*Cucumis sativus* L.) variety named "Zhongnong" was harvested in June 2022 and transported to the laboratory within 2 h. Fresh cucumbers, uniform in size, color, ripeness, and free of physical damage, were thoroughly washed with tap water and then sterilized in sodium hypochlorite (0.2 mL L^{-1}). After being rinsed twice with water, the selected cucumbers were cut into strips, pieces, and slices with a sharp knife. The wounded surface diameter of the strips, pieces, and slices was manually measured, and the wounding intensity (A/W) was defined as the ratio of the new surface area created by wounding in cm^2 divided by the tissue weight in g according to the method of Surjadinata and Cisneros-Zevallos [21]. The wounding intensities (A/W) of the whole, stripped, pieced, and sliced cucumbers were calculated as 0, 1.65, 1.76, and 3.20 m^2 kg^{-1}, respectively.

Fresh-cut cucumbers (100 g) were loaded into a polypropylene container and packaged with polyethylene films (Miuge Chemical Commodities Science and Technology Co., Ltd., Hangzhou, China). The whole cucumber was also packaged with polyethylene films at 4 °C, which was used as the control in this experiment. The polyethylene film sheets were 200 mm × 300 mm, and 0.01 μm thick, and the CO_2 transmission rate, O_2 transmission rate, water vapor transmission rate, and resistant temperature of the film were 363,000 cm^3 m^{-2} d^{-1} atm^{-1}, 10,030 cm^3 m^{-2} d^{-1} atm^{-1}, 25 g m^{-2} d^{-1}, and −60~110 °C. The samples including the whole cucumber and fresh-cut cucumbers were stored in the dark at 4 °C and 80–90% relative humidity for 5 d. The experiment was repeated using separate batches of "Zhongnong" cucumbers on two different occasions, with three replicates each time.

2.2. Colour Parameters, Weight Loss, Respiration Rate, and Firmness Assay

The color including lightness (L^*), redness (a^*), and yellowness (b^*) was evaluated according to the CIELAB colorimetric system using a CR400 colorimeter (Konica Minolta Inc., Tokyo, Japan). Three samples from each group were chosen to measure the surface color, taking an average of three surface measurements per sample. The whiteness index (WI) was used to evaluate the color of the sample; it was calculated as the following: $WI = 100 - [(100 - L^*)^2 + (a^*)^2 + (b^*)^2]^{1/2}$ [22]. Weight loss was measured according to the differential method [10]. The respiration rate was determined according to Zhou et al. [23], and the result was expressed as mg kg^{-1} h^{-1}. The firmness was determined according to the method reported by Hu et al. [10]. Briefly, the test speed was 1.0 mm s^{-1}, the penetration distance was 8 mm, and the measurement was averaged over six replicates corresponding to the maximum force (N).

2.3. Total Soluble Solid (TSS), Ascorbic Acid (AsA), and Glutathione (GSH) Content Assay

A total of 4 g of cucumber was ground with 20 mL distilled water and then filtered by filter paper (30–50 μm). The cucumber juice was used for the determination of the TSS content by using a hand-held refractometer (PAL-1, Aiago Co., Ltd., Tokyo, Japan) and the result was expressed as %.

The AsA content was analyzed according to the procedure used by Guan et al. [11]. Briefly, 5 g cucumber powder was mixed with 20 mL 2% oxalic acid solution. Then, the mixture was filtered and the supernatant was used to determine the AsA content (mg kg^{-1}) through 2,6-dichlorindophenol titration.

The method for the GSH content (μmol kg^{-1}) assay was performed according to the instructions of commercially available kits (Nanjing Jiancheng Technology Co., Ltd., Nanjing, China). Frozen cucumber powder (0.1 g) was mixed with 1 mL buffer and then centrifuged at 8000× *g* for 10 min at 4 °C, and the supernatant was used to measure the GSH content, and the GSH content was expressed as μmol kg^{-1}.

2.4. Total Phenols, Lignin Content, and PAL Activity Assay

The frozen cucumber sample (5 g) was mixed with 20 mL 80% ethanol, shaken well, and extracted in the dark at 40 °C for 40 min. After centrifuging at 15,000× *g* for 30 min, the collected supernatant was used to determine the total phenol content. The reaction system was used according to the method reported by Hu et al. [24], which consisted of 1 mL Folin-Ciocalteu reagent, 1 mL supernatant, 10 mL Na_2CO_3 solution, and 14 mL water, then, the absorbance value was determined at 750 nm. The result of the total phenol content was expressed as mg kg^{-1} from the gallic acid standard curve ($y = 0.0051x + 0.0178$, $R^2 = 0.999$).

The frozen powder of the fresh-cut cucumber was put into the air oven at 80 °C for the drying operation to constant weight, then passed through a 40-mesh sieve prepared for the lignin content assay. The lignin content was determined using assay kits (Suzhou Keming Biotechnology Co., Ltd., Suzhou, China) by measuring the absorbance at 280 nm, and the result was expressed as g kg^{-1}.

PAL activity was assayed as described by Hu et al. [24]. The crude enzyme was extracted by sodium borate buffer (50 mM, pH 8.5, containing 5 mM b-mercaptoethanol and 2 mM EDTA), and then L-phenylalanine (20 mM) was added before incubating for 60 min at 37 °C. The reaction was stopped by adding 0.1 mL of 6 M HCl, and the absorbance value was determined at 290 nm; 1 unit of PAL activity was equal to a change of 0.01 at 290 nm per min, and expressed as U kg^{-1} protein.

2.5. $O_2^{-}\cdot$ and H_2O_2 Content Assay

Frozen tissues (0.10 g) were homogenized with 1 mL of buffer coming from the $O_2^{-}\cdot$ and H_2O_2 kit (Suzhou Keming Biotechnology Co., Ltd., Suzhou, China). After centrifuging at 20,000× *g* at 4 °C for 15 min, the supernatants were collected and used for the $O_2^{-}\cdot$ and H_2O_2 content assay. The $O_2^{-}\cdot$ and H_2O_2 contents of the cucumbers were measured by the absorbance at 415 and 530 nm, respectively. The $O_2^{-}\cdot$ content was expressed

as mmol kg^{-1} based on the sodium nitrite standard curve, and the H_2O_2 content was expressed as mmol kg^{-1} based on the fresh weight.

2.6. Antioxidant Enzymes Activity Assay

The ascorbate peroxidase (APX), glutathione reductase (GR), peroxidase (POD), superoxide dismutase (SOD), and catalase (CAT) enzyme extractions were performed according to the instructions on the plant kits (Suzhou Keming Biotechnology Co., Ltd., Suzhou, China). The reaction system of the APX, GR, POD, SOD, and CAT activity determined the absorbance at 290, 340, 470, 560, and 240 nm, respectively. The antioxidant enzyme activities were expressed as U kg^{-1}, where $U_{APX} = 0.01 \times \Delta A_{290\ nm}$ per min, $U_{GR} = 0.01 \times \Delta A_{340\ nm}$ per min, $U_{POD} = 0.01 \times \Delta A_{470\ nm}$ per min, $U_{SOD} = 0.01 \times \Delta A_{560\ nm}$ per min, and $U_{CAT} = 0.01 \times \Delta A_{398\ nm}$ per min.

2.7. Antioxidant Activity Assay

The DPPH and ABTS free radical scavenging assays were performed according to the procedure of Chen et al. [8] with some modifications. The extraction method was the same as the extracts prepared for the total phenol assay, four times dilution of ethanol extraction solution for the detection of the DPPH and ABTS free radical scavenging capacity, and the results were expressed as nmol Trolox equivalents per kg (nmol Trolox kg^{-1}) of fresh tissue. The OH· scavenging activity (in nmol Trolox kg^{-1}) and ferric-reducing antioxidant power (FRAP) (in Trolox mmol kg^{-1}) were determined according to the instructions of the relevant kits (Suzhou Keming Biotechnology Co., Ltd., Suzhou, China).

2.8. Statistical Assay

IBM SPSS 20 (IBM Corp., Armonk, NY, USA) was applied to perform an analysis of variance (ANOVA). A significant difference was calculated by using Duncan's test with 95% level of confidence ($p < 0.05$) among the treatments.

3. Results

3.1. Colorimetric Values

Generally, the fresh-cutting process with wounding stress induces a variety of physiological responses and affects the quality of fresh-cut products [25]. Among these, the color and appearance quality are the important parameters that affect consumers' willingness to accept and buy fresh-cut products. Among these, the lightness (L^*) indicates the color depth, and the larger the lightness, the brighter the fresh-cut products. Furthermore, the WI could reflect the extent of the fade due to physiological disorders in the fresh-cut cucumber; the larger the WI, the more severe the fading phenomenon. In this study, the L^* and WI change in fresh-cut cucumbers subjected to three cutting methods, including stripping, piecing, and slicing, were explored. The results revealed that the L^* in the cutting treatment groups decreased, and the degree of the L^* level was stripped > pieced > sliced > whole (Figure 1a).

On the contrary, the WI of the fresh-cut cucumbers increased, especially after 2 days, when the WI increased sharply, and the degree of the WI value was sliced > pieced > stripped > whole (Figure 1b). At 5 day, the WI values of the sliced, pieced, and stripped cucumbers increased by 8.13%, 4.85%, and 3.95% compared with the whole cucumber, respectively, which demonstrated that the WI value increased as the wounding intensity increased. The above results proved that there was an obvious influence on the lightness and whiteness index in fresh-cut cucumbers with different cutting methods, which was similar to a previous study of fresh-cut potatoes [10].

3.2. Weight Loss and Total Soluble Solid

Cucumber is a vegetable with a high water content, thus, fresh-cutting and subsequent storage were easy to cause water loss and further resulted in a loss of quality in the fresh-cut cucumbers [18]. This study found that the weight loss in all the groups showed an upward trend during the storage period, among which, the maximum weight loss appeared in the

stripped group (Table 1). At 5 day, the weight loss in the stripped, pieced, sliced, and whole cucumbers was 1.26%, 1.13%, 0.72%, and 0.38%, respectively.

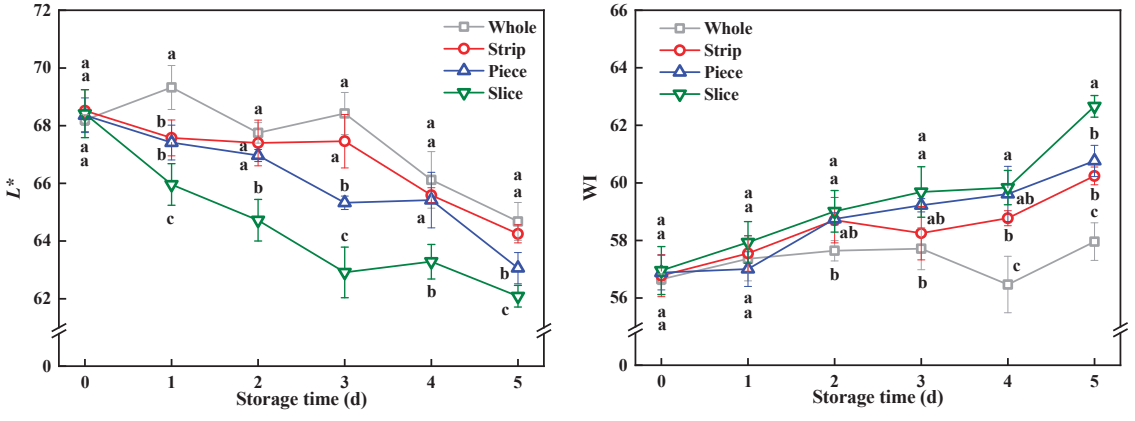

Figure 1. Effect of cutting methods on lightness (*L**) (**a**) and whiteness index (WI) (**b**) of fresh-cut cucumbers. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

Table 1. The effect of different cutting styles on the quality of fresh-cut cucumbers stored at 4 °C.

Storage Time (Day)	Treatment	Total Soluble Solid (%)	Weight Loss (%)	Respiration Rate (mg kg^{-1} h^{-1})	Firmness (N)	Ascorbic Acid (mg kg^{-1})	Glutathione (mg kg^{-1})
0	Whole	0.81 ± 0.03 a	0	51.64 ± 1.68 a	21.76 ± 0.90 a	19.97 ± 1.04 a	0.11 ± 0.004 a
	Stripped	0.82 ± 0.05 a	0	53.36 ± 0.98 a	21.83 ± 0.84 a	20.81 ± 0.94 a	0.12 ± 0.007 a
	Pieced	0.77 ± 0.09 a	0	52.09 ± 2.31 a	20.10 ± 1.21 a	21.93 ± 0.80 a	0.12 ± 0.008 a
	Sliced	0.82 ± 0.05 a	0	52.66 ± 0.75 a	20.64 ± 0.95 a	20.44 ± 1.31 a	0.11 ± 0.004 a
1	Whole	0.75 ± 0.05 b	0.09 ± 0.01 c	47.69 ± 1.39 b	19.84 ± 0.47 a	20.17 ± 0.75 a	0.10 ± 0.002 c
	Stripped	0.98 ± 0.04 a	0.29 ± 0.05 a	36.11 ± 1.20 c	17.05 ± 0.61 b	21.05 ± 1.41 a	0.13 ± 0.002 b
	Pieced	0.98 ± 0.04 a	0.24 ± 0.05 ab	44.80 ± 2.44 b	16.83 ± 1.02 b	20.64 ± 1.51 a	0.13 ± 0.005 b
	Sliced	0.98 ± 0.05 a	0.12 ± 0.08 b	56.18 ± 0.92 a	14.88 ± 1.49 c	15.62 ± 0.97 b	0.15 ± 0.007 a
2	Whole	0.78 ± 0.08 c	0.16 ± 0.02 d	55.30 ± 0.66 b	18.71 ± 0.76 a	18.69 ± 0.91 a	0.11 ± 0.003 c
	Stripped	1.03 ± 0.03 ab	0.60 ± 0.07 a	40.07 ± 0.70 c	15.82 ± 0.80 b	10.26 ± 0.81 c	0.13 ± 0.004 b
	Pieced	1.09 ± 0.04 a	0.46 ± 0.05 b	51.81 ± 2.25 b	15.09 ± 0.90 b	15.04 ± 1.32 b	0.14 ± 0.005 a
	Sliced	0.98 ± 0.06 b	0.31 ± 0.01 c	59.47 ± 1.86 a	11.93 ± 0.89 c	16.02 ± 1.41 b	0.15 ± 0.004 a
3	Whole	0.73 ±0.04 d	0.25 ± 0.04 d	36.32 ± 0.94 c	19.49 ± 0.94 d	19.34 ± 1.07 a	0.11 ± 0.004 b
	Stripped	0.88 ± 0.04 c	0.83 ± 0.09 a	59.49 ± 1.02 b	33.84 ± 0.83 a	14.91 ± 0.90 b	0.13 ± 0.005 a
	Pieced	1.08 ± 0.07 a	0.65 ± 0.03 b	62.31 ± 1.40 b	32.04 ± 0.65 b	15.00 ± 1.06 b	0.13 ± 0.006 a
	Sliced	0.92 ± 0.07 b	0.43 ± 0.09 c	72.30 ± 0.98 a	25.68 ± 0.84 c	16.20 ± 1.29 b	0.13 ± 0.007 a
4	Whole	0.82 ± 0.07 b	0.33 ± 0.04 c	30.15 ± 0.90 c	20.35 ± 1.28 b	18.61 ± 1.21 a	0.11 ± 0.004 c
	Stripped	1.01 ± 0.03 a	1.01 ± 0.10 a	59.04 ± 1.28 a	24.17 ± 0.67 a	15.43 ± 0.73 b	0.13 ± 0.003 b
	Pieced	1.01 ± 0.04 a	0.90 ± 0.07 a	54.20 ± 1.09 b	16.04 ± 0.84 c	16.58 ± 1.41 ab	0.12 ± 0.004 b
	Sliced	0.93 ± 0.06 a	0.57 ± 0.09 b	59.62 ± 2.06 a	15.36 ± 1.19 c	18.17 ± 0.98 a	0.14 ± 0.006 a
5	Whole	0.78 ± 0.04 c	0.38 ± 0.03 d	32.17 ± 0.93 c	19.34 ± 1.23 a	17.85 ± 1.47 a	0.10 ± 0.002 d
	Stripped	1.10 ± 0.03 a	1.26 ± 0.03 a	35.87 ± 1.72 b	15.76 ± 1.12 b	16.99 ± 1.03 a	0.11 ± 0.003 c
	Pieced	1.01 ± 0.04 b	1.13 ± 0.03 b	37.65 ± 0.92 b	12.69 ± 0.68 c	15.86 ± 0.85 a	0.12 ± 0.005 b
	Sliced	0.93 ± 0.08 b	0.72 ± 0.01 c	66.46 ± 2.16 a	11.54 ± 1.00 c	17.76 ± 1.22 a	0.13 ± 0.004 a

Note: Data are expressed as the mean ± SD (n = 3). Values with different letters are significantly different at $p < 0.05$ among the treatments.

Soluble solid is one of the important indexes to judge the nutritional quality of fruits and vegetables [26]. As shown in Table 1, compared to the whole sample, the fresh-cutting treatment enhanced the total soluble solid (TSS) content in the cucumbers. After storage for 5 day, the TSS values of the stripped, pieced, and sliced cucumbers were 1.10%, 1.01%, and 0.93%, which was 41.02%, 29.49%, and 19.23% higher than that in the control group in the same period, respectively. Interestingly, the trend in the TSS content was consistent with the trend in the weight loss, and the significant positive correlation between the weight

loss and TSS content confirmed that the fresh-cutting operation increased the weight loss and then resulted in a higher concentration of soluble solids in the samples. Furthermore, previous studies in fresh-cut broccoli [7], onion [27], and ginger [28] reported that the cutting treatment accelerates the decomposition of macromolecular substances into small carbohydrate substances to provide adequate energy required in the metabolic process. Therefore, we extrapolated the degradation of substances such as protein and starch which may be another reason for the TSS content enhancement in fresh-cut cucumbers.

3.3. Respiration Rate and Firmness

Respiratory intensity is an important condition to maintain the freshness of agricultural products, and also a necessary index which affects the shelf life of fresh-cut fruits and vegetables [29]. The present study of fresh-cut cucumbers revealed that the respiration rate was decreased during 1–2 day of storage in the stripped and pieced samples, then increased and decreased again in the later storage time (Table 1). Whereas the respiration rate of the sliced cucumbers was active through cutting in the entire storage period, and the highest respiration rate appeared in the sliced samples subjected to the highest wounding intensity. With the extension in the storage time, the respiration rate of the sliced, pieced, and stripped cucumbers reached the peak value on the third day, and they increased by 99.04%, 71.56%, and 63.79% compared with the whole sample. The above results revealed that the cutting resulted in physiological stress on the plant tissues, triggering the respiratory metabolism in the fresh-cut cucumbers [1,23]. The respiration rate levels in the fresh-cut cucumbers were sliced > pieced > stripped > whole during the later storage time, which indicated that different cutting methods have a significant influence on the respiratory metabolism in fresh-cut cucumbers, and this may be an important reason that directly affected the cucumber tissue senescence [30].

The firmness showed the same downward trend from the beginning to the first 2 day of storage; this may be because the cell tissue in the fresh products was destroyed by cutting and then led to the increase in pectinase activity, which accelerated the decomposition of the cell wall and tissue softening in the fresh-cut cucumbers [31,32]. In addition, the process of hydrolysis of starch and degradation of pectin may be another reason for the declining firmness of the sample [33]. However, at a storage time of 3 day, the firmness of the sliced, pieced, and stripped samples increased rapidly to 1.32, 1.64, and 1.74 times that before storage. The reason for this result may be that the wound-induced callus and closing layer at the wound site then contributed to the substantial increase in firmness at a storage time of 3 day [34].

3.4. Reactive Oxygen Species (ROS) Content

In this study, to evaluate the different cutting methods for the production of ROS, we measured the $O_2^{-}\cdot$ and H_2O_2 content in fresh-cut cucumbers. The results showed that fresh-cutting induced the production of $O_2^{-}\cdot$, and the highest values were found in the sliced sample at 2 day; it was increased by 3.07-folds compared with the whole group (Figure 2a). However, the highest levels in the pieced and stripped samples appeared at 1 day and 3 day, respectively, which indicated that the different cutting styles have a significant influence on the ROS production in fresh-cut cucumbers.

Similarly, the H_2O_2 content was enhanced obviously in the cucumbers after the cutting operation, and the maximum values of the sliced, pieced, and stripped samples were increased by 11.20 times, 7.35-times, and 4.89 times, respectively, compared with the control group. During the whole storage time, the H_2O_2 values were sliced > pieced > stripped > whole (Figure 2b), which revealed that the H_2O_2 content increased as the wounding intensity increased. This may be caused by the cutting operations attacking the unsaturated fatty acids in the cell plasma membrane and organelle membrane, leading to the decrease in the activity of cell membrane-binding enzymes, which destroys the normal redox balance of cells, thus causing the explosion of ROS [3,35]. Furthermore, the correlation analysis showed that there was a significant positive correlation between the H_2O_2 content and

WI, whereas there was a negative positive correlation between the H_2O_2 content and *L** (Figure 3, demonstrating that the H_2O_2 content plays an important role in the whiteness phenomenon of fresh-cut cucumbers. The results in fresh-cut pitaya [2], apple [36], carrot [5], potato [23], and onion [15] have also proved the accumulation of ROS could further influence the quality such as browning, softening, and secondary metabolites synthesis.

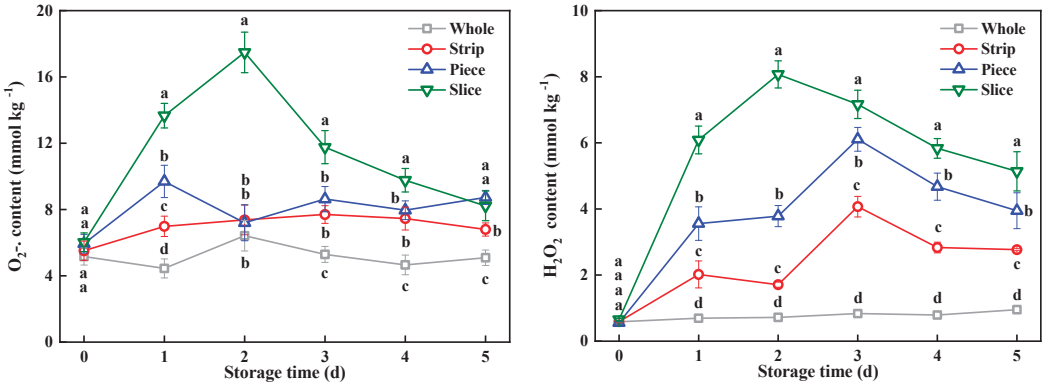

Figure 2. Effect of cutting methods on $O_2^{\cdot-}$ content (**a**) and H_2O_2 content (**b**) in fresh-cut cucumbers. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

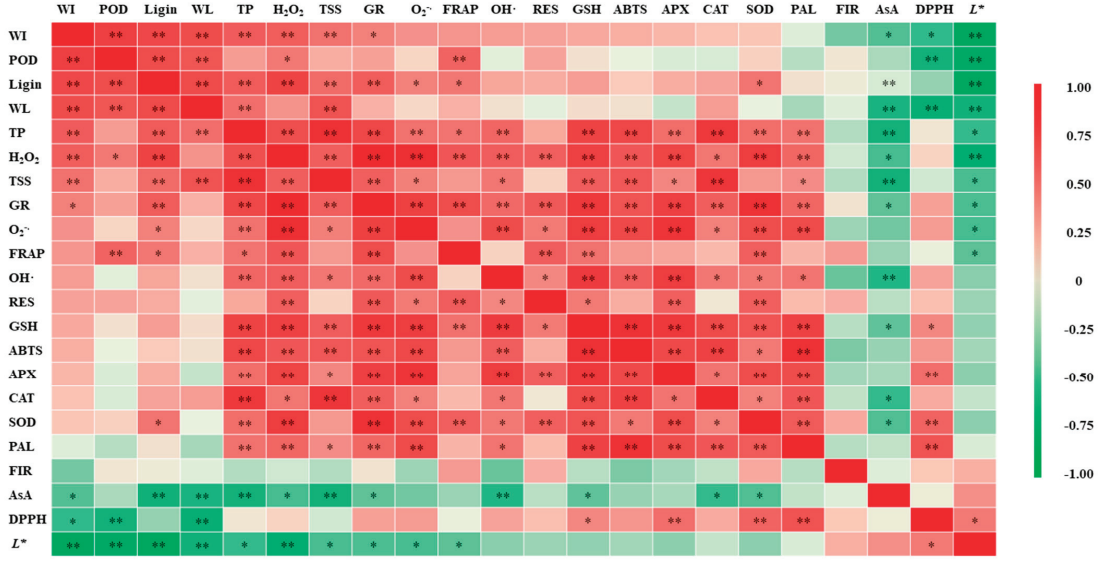

Figure 3. Pearson correlation matrix for each index. * $p < 0.05$, ** $p < 0.01$. WI, whiteness index; POD, peroxidase; WL, weight loss; TP, total phenols content; TSS, total soluble solid; GR, glutathione reductase; FRAP, ferric−reducing antioxidant power; RES, respiration rate; GSH, glutathione; APX, ascorbate peroxidase; CAT, catalase; SOD, superoxide dismutase; PAL, phenylalanine ammonia−lyase; FIR, firmness; AsA, ascorbic acid; *L**, lightness.

3.5. Ascorbic Acid–Glutathione Cycle-Related Indexes

According to Table 1, the ascorbic acid (AsA) content in the cutting-treatment cucumbers exhibited a downward trend until 2 day of storage, and the AsA content declined by

45.07%, compared with the original values. The reason for the decrease in the AsA content may be that the injured tissue of the fresh-cut cucumbers is exposed to the air, which will increase the contact area between the wounded tissue and air, and accelerate the loss rate of the AsA content [4]. On the other hand, the fresh-cutting instantly triggers the burst of ROS and then activates the decomposition of AsA to enhance the resistance to wounding stress and further protect the wounded tissue, which resulted in the decrease in the AsA content [12]. It is worth mentioning that the AsA content of the fresh-cut cucumbers was increased slightly after 2 day of storage, and the sliced samples had the highest AsA content (17.76 mg kg^{-1}) at 5 day, and there was no significant difference with the control group.

As another important component of AsA–GSH in plants, glutathione (GSH) is a tripeptide-containing sulfhydryl group with a strong antioxidant capacity [7]. Contrary to the changing trend in the AsA content, the GSH content increased during 1–2 day of storage, then decreased (Table 1). The enhancement in the GSH content in the early stage may be used to clear the ROS in the fresh-cut cucumbers and then reduce the oxidative stress [37]. Hu et al. reported that the cutting operation enhanced the GSH content generation triggered by activating the antioxidant defense mechanism in fresh-cut potatoes, thereby increasing the ability to resist wounding stress [37]. Meanwhile, Xia et al. [38] revealed that exogenous GSH treatment alleviates the aging damage of oat seeds by regulating the GSH content to maintain the integrity of the mitochondrial structural and functional systems. In our study, we found that the GSH content is significantly negatively correlated with the AsA content, which indicates the antioxidant enzymes catalyze the rapid decomposition of AsA in the process of scavenging ROS and then provide the necessary prerequisite for GSH synthesis, and further increase the GSH content [39].

In the AsA–GSH cycle, ascorbate peroxidase (APX) and glutathione reductase (GR) are the major enzymes [40]. In this study, we found that the APX activity was enhanced in the first 2 day of storage, and it increased by 118.97%, 68.97%, and 12.07% in the sliced, pieced, and stripped cucumbers, respectively, compared with the whole cucumber in the same period (Figure 4a). The APX activity decreased after 3–5 day of storage, and this changing trend was opposite to that of the AsA content, which indicated that APX catalyzes the conversion of AsA to dehydroascorbic acid with the reduction reaction of H_2O_2, thereby further scavenging free radicals in fresh-cut cucumber cells [41].

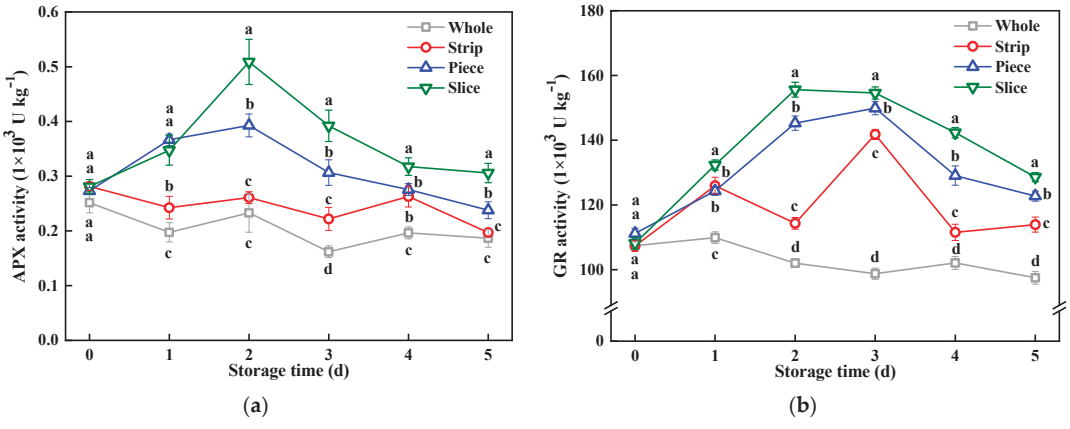

Figure 4. Effect of cutting methods on ascorbate peroxidase (APX) (**a**) and glutathione reductase (GR) (**b**) activity of fresh−cut cucumbers. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

Generally, GR catalyzes the conversion of oxidized GSH into reduced GSH with the participation of NADPH, thus limiting the operation efficiency of the AsA–GSH cycle. As shown in Figure 4b, the GR activity in the fresh-cut cucumbers increased and then

decreased with the extension in the storage time, and the levels of the GR activity were the same as the APX activity, that is, sliced > pieced > stripped > whole during the storage time. At 3 day, it reached 154.59, 149.88, and 141.79 U kg^{-1} in the sliced, pieced, and stripped samples, respectively. In the early storage period, the increase in the GR activity may promote the conversion of oxidized GSH into reduced GSH, thereby increasing the accumulation of reduced GSH [40,42].

3.6. Phenylpropanoid Metabolism-Related Indexes

As the most important antioxidants in fruits and vegetables, phenolic compounds are synthesized through the phenylpropanoid metabolic pathway [43,44]. A previous study has reported that wounding stress induces the accumulation of phenols intended to achieve healing and defense [5]. In this study, we found that the total phenol (TP) content increased following all treatments and the highest value was found in the pieced samples at the earlier storage time (Figure 5a). However, the TP contents were arranged as whole < pieced < stripped < sliced later in the storage duration. At 5 d of storage, the TP content increased by 1.35 times, 1.51 times, and 1.78 times in the pieced, stripped, and sliced samples, respectively.

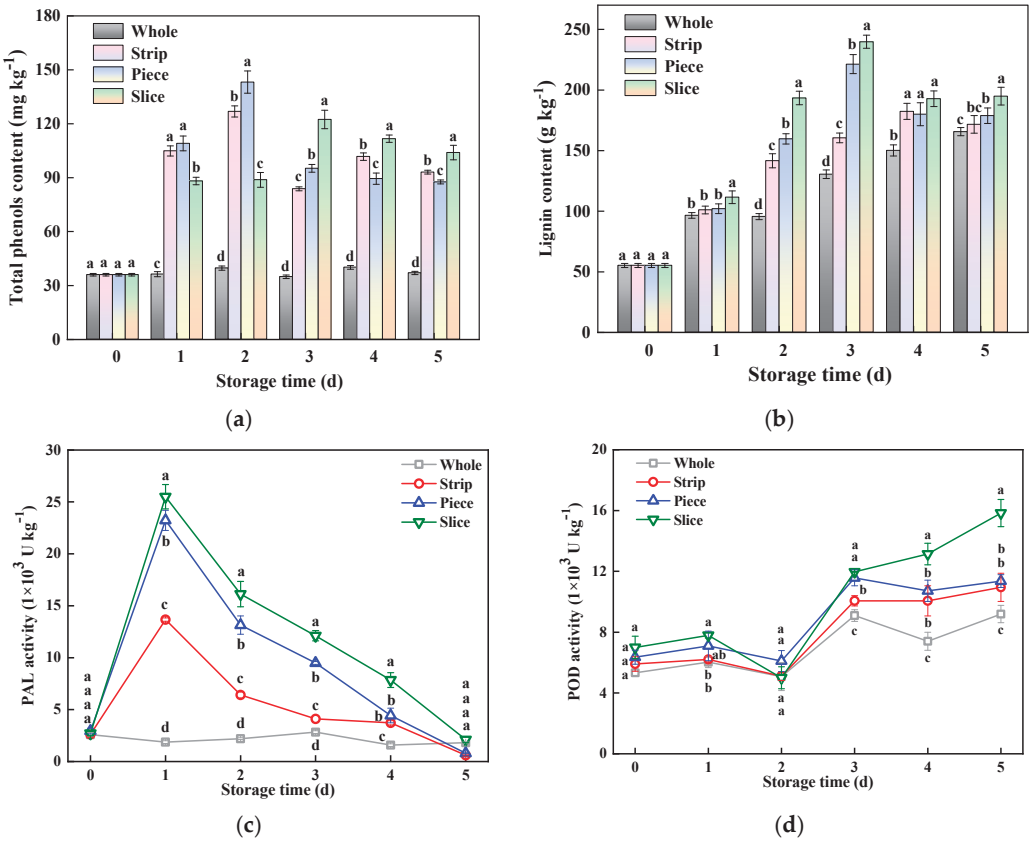

Figure 5. Effect of cutting methods on ascorbate peroxidase (APX) (**a**) glutathione reductase (GR) (**b**) phenylalanine ammonia−lyase (PAL) (**c**) and peroxidase (POD) (**d**) activity in fresh−cut cucumbers. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

According to Figure 5b, the lignin content increased in all the cutting treatment groups, and it was higher than that in the control samples. The highest lignin content in the control samples was 165.69 g kg^{-1}, while the contents in the stripped, pieced, and sliced samples were 182.38 g kg^{-1}, 221.43 g kg^{-1}, and 239.90 g kg^{-1} ($p < 0.05$), respectively. Meanwhile, we found that there was a significant positive correlation between the lignin content and TP content (Figure 3). In addition, the lignin content was positively correlated with the WI, whereas there was a significant negative correlation with the L^* (Figure 3), which demonstrated that the synthesis of lignin accelerated the occurrence of the whiteness phenomenon in the fresh-cut cucumbers. A previous study of fresh-cut potatoes also proved that lignin could enhance the resistance of injured parts to mechanical damage [23,45].

Phenylpropanoid metabolism plays an active role in improving the resistance of fresh-cut fruits and vegetables [46]. In this pathway, phenylalanine ammonia-lyase (PAL) was the first critical enzyme which could catalyze the deamination of phenylalanine to produce phenolics [47]. As the precursor of lignin synthesis, the phenolic substances were synthesized at the site of injury and then converted into lignin with the catalysis of the peroxidase (POD) enzyme [48]. According to Figure 5c, the PAL activity increased sharply during the first 1 day of storage and then decreased. At 1 day, the PAL activity in the stripped, pieced, and sliced samples increased by 5.26, 7.95, and 9.44 times, respectively, compared with the initial value.

The POD activity showed an upward trend during the whole storage period (Figure 5d). At 5 day of storage, the POD activity in the stripped, pieced, and sliced samples increased by 19.15%, 23.50%, and 72.25%, respectively, compared with the whole cucumber. These results indicated that the cutting methods had a significant influence on the PAL and POD activity in the fresh-cut cucumbers ($p < 0.05$), and the PAL and POD activity was enhanced as the wounding intensity increased.

Furthermore, the result of the correlation reveals that the H_2O_2 content was positively correlated with the phenylpropanoid metabolism-related indexes including the TP content, lignin content, PAL, and POD activity, which illustrates that the H_2O_2 signal molecule plays an important role in regulating the phenylpropanoid metabolism to synthesize phenolic compounds and lignin [47]. A similar result was found in fresh-cut potatoes which showed that following mechanical wounds, ROS are overproduced, which contributes to the process of lignification [23]. Meanwhile, Han et al. [49] reported that ROS can be used as a second messenger to participate in the defense response against wounding stress and plays a role in signal transmission in the synthesis and accumulation of phenols in fresh-cut carrots.

3.7. Antioxidant Enzymes Activity

In this study, we evaluated the antioxidant-related enzyme activity including superoxide dismutase (SOD) and catalase (CAT). As shown in Figure 6a, fresh-cut processing causes an increase in the SOD activity in the first 3 day and a decrease later on. The CAT activity showed a similar variation compared with that of the SOD; the three kinds of fresh-cut tissues showed a higher enzyme activity compared with the control ($p < 0.05$, Figure 6b). Meanwhile, the cutting methods have an obvious effect on the SOD and CAT activity during the whole storage period.

The results of the correlation analysis between the antioxidant enzyme activity (SOD, CAT) and AsA–GSH cycle-related indexes (AsA, GSH, APX, and GR) showed a significant correlation between them. Moreover, there was also a significant positive correlation between the antioxidant enzyme activity and ROS level; these results demonstrate that the SOD, CAT complexed with the AsA–GSH cycle could finely modulate the ROS level [4]. It is worth mentioning that the resistance mechanisms of antioxidant enzymes in fruits and vegetables, with different biological characteristic responses to oxidative damage induced by wounding stress, are different. For instance, the SOD and CAT activity was enhanced sharply in fresh-cut tomatoes at the beginning of storage [50], while in a study of fresh-cut pitaya fruit and fresh-cut broccoli, it was found that the fresh-cutting treatment inhibited the

APX activity [9,51]. In our experiment, we found that the fresh-cutting treatment induces the activity in SOD, CAT, APX, and GR and further enhances the ability to scavenge free radicals in cucumbers.

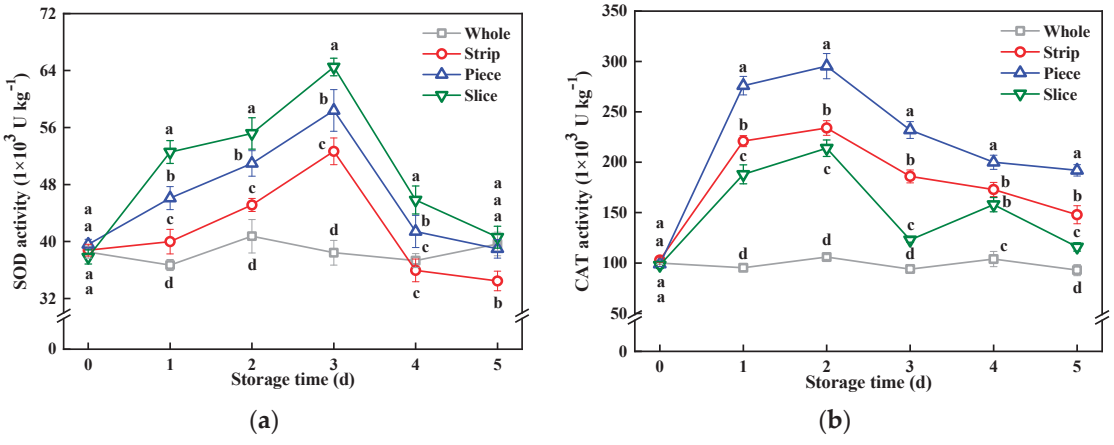

Figure 6. Effect of cutting methods on superoxide dismutase (SOD) (**a**) and catalase (CAT) activity (**b**) in fresh−cut cucumbers. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

3.8. Antioxidant Capacity

As shown in Figure 7a,b, the DPPH and ABTS$^+$ radical scavenging capacity of the sliced, pieced, and stripped samples firstly increased and then decreased slowly, reaching a maximum at 2 day and 1 day, respectively; the DPPH radical scavenging capacity of these three groups showed increases of 42.63%, 32.93%, and 14.55%, respectively; the ABTS$^+$ radical scavenging capacity of these three groups showed increases of 40.75%, 29.39%, and 23.27%, respectively, compared with the whole cucumber. Similar to the changing trend in the DPPH and ABTS$^+$ radical scavenging capacity, the OH· radical scavenging capacity of the sliced and pieced samples increased and then decreased, whereas the OH· radical scavenging capacity of the stripped samples decreased at 1 day; it was 22.05% and 9.39% lower than the levels in the sliced and pieced groups (Figure 7c). The ferric-reducing antioxidant power (FRAP) of all the treatment groups reached a maximum at 3 day; the FRAP values of the sliced, pieced, and stripped samples increased by 95.31%, 89.06%, and 61.72%, respectively, compared with the control (Figure 7d).

In this study, the correlation between the ABTS$^+$, OH· radical scavenging capacity and the SOD, CAT activity was higher, indicating that SOD and CAT play an important role in scavenging free radicals [52]. Furthermore, we found that a higher antioxidant capacity (ABTS$^+$, OH· radical scavenging capacity, and FRAP) was observed in the sliced than in the pieced and stripped samples at the later storage time, probably because the cutting method with a high wounding intensity stimulates the antioxidant system enzymes and improves the ability to resist ROS [53]. A similar result was also found in fresh-cut pitayas [9]. However, there was no significant correlation between the antioxidant capacity and POD activity; this result may be because peroxidase family proteins (POD) are composed of proteins with different biological functions corresponding to dual biological functions of scavenging ROS and catalyzing lignin synthesis in fruits and vegetables [54,55]. A previous study showed that wounding stress upregulated POD42 and downregulated POD37 in fresh-cut broccoli, which also proved that POD proteins have various functions in oxidative metabolic processes and secondary metabolic pathways [48].

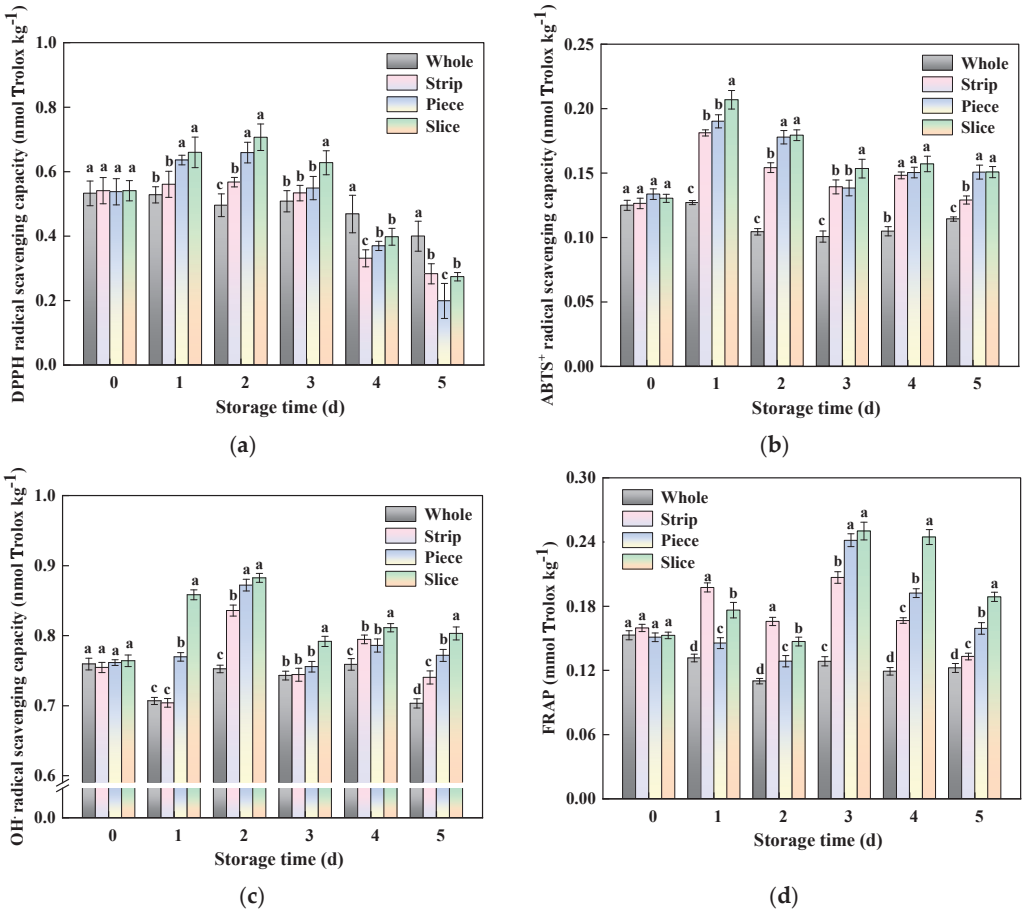

Figure 7. Effect of cutting methods on DPPH radical scavenging capacity (**a**), $ABTS^{+}$ radical scavenging capacity (**b**), OH· radical scavenging capacity (**c**), and ferric−reducing antioxidant power (FRAP) (**d**) in fresh−cut cucumbers. Vertical bars represent the standard deviation of the mean (n = 3). Different letters indicate significant differences between the groups ($p < 0.05$).

4. Conclusions

Cutting induced the enhancement in the weight loss, respiration rate, whiteness index, and total soluble solid content in fresh-cut cucumbers during storage, and there was a significant difference in cutting methods on these parameters. Meanwhile, activity in PAL and POD, which are associated with the phenylpropanoid metabolism, was also induced by the fresh-cutting treatment, and this resulted in the synthesis of phenols and lignin in the wounded cucumbers. The total phenol content increased by 1.35 times, 1.51 times, and 1.78 times in the pieced, stripped, and sliced samples, respectively. Furthermore, the cutting operation induced the production of ROS ($O_2^{-}\cdot$ and H_2O_2), then motivated the activity in antioxidant-related enzymes, including SOD, CAT, APX, and GR, leading to a 1.14–1.95 times increase in the FRAP, DPPH, $ABTS^{+\cdot}$ and OH· radical scavenging capacity. These phenomena are more obvious in the cutting method with the enhancement in the wounding intensity degree. The collected results reveal that different cutting methods have an obvious influence on the ROS metabolism, AsA–GSH cycle, and phenylpropanoid metabolism, which further affect the quality of fresh-cut cucumbers.

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Transcriptomic Analysis Reveals the Mechanism of Lignin Biosynthesis in Fresh-Cut Cucumber

Yi Wang [1], Ning Yuan [1], Yuge Guan [2], Chen Chen [1,*] and Wenzhong Hu [1,*]

[1] College of Life Science, Dalian Minzu University, Dalian 116600, China; w13936057663@163.com (Y.W.); yn2822852617@163.com (N.Y.)
[2] School of Food and Health, Zhejiang Agricultural and Forestry University, Hangzhou 311300, China; gyg@zafu.edu.cn
* Correspondence: chenchen@dlnu.edu.cn (C.C.); huwenzhongtd@sina.com (W.H.)

Abstract: When subjected to a certain degree of mechanical damages, a systematic responsive mechanism of fresh-cut cucumber is activated. Among them, the lignin produced in the secondary metabolism will make the fresh-cut cucumber lignified, which will increase the hardness and whiten the cutting surface of the fresh-cut cucumber, seriously affecting the taste and appearance quality. In order to further understand the mechanism of lignin synthesis, transcriptome analysis was carried out on two cutting types of fruit samples from the slices treatment (P) and shreds treatment (S) stored for 24 h. Compared with the whole fruit (CK), 2281 and 2259 differentially expressed genes (EDGs) were identified in the slices and shreds treatments, respectively; 1442 up-regulated genes and 839 down-regulated genes were expressed as 2281 in the slices treatment; 1475 significantly up-regulated genes and 784 significantly down-regulated genes were expressed as 2259 in the shreds treatment; and 1582 DEGs were commonly detected between the slices treatment and shreds treatment, indicating that these DEGs were related to lignin synthesis. Gene Ontology (GO) enrichment and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis showed that compared with the whole fruit, the metabolic pathways of amino acid metabolism, lipid metabolism, and secondary metabolism were affected by mechanical damages. This study revealed that JA biosynthesis was activated by mechanical damages, and the up-regulation of phenylalanine metabolism and phenylalanine, tyrosine, and tryptophan metabolism affected phenylpropanoid biosynthesis, which may promote lignin synthesis. Fifteen DEGs were selected for qRT-PCR validation, and the reliability and accuracy of transcriptome data were confirmed.

Keywords: fresh-cut cucumber; transcriptomic; lignin; amino acid metabolism; lipid metabolism; phenylpropanoid biosynthesis

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1. Introduction

Globally, cucumber (*Cucumis sativus* L.) is one of the most popular Cucurbitaceae crops, with a global volume of production of more than 75.22 million tonnes [1]. Cucumbers are widely consumed because of their high nutritional values and beneficial secondary metabolites, including sugar, vitamins, carotene, and polyphenols, which can increase the antioxidant ability of vegetables [2]. Consumer demand for fresh-cut cucumber has substantially increased in recent years due to its freshness, good flavor, and convenience. However, fresh-cut cucumber is prone to problems such as accelerated physiological and biochemical reactions, water loss, microbial pollution, and lignification after mechanical damages, which seriously affect its taste and shelf life [3,4]. After the cucumber is cut, it activates its own defense system, such as the production of secondary metabolites, to resist mechanical damages. Among them, the lignin produced by the secondary metabolism will make the fresh-cut cucumber lignified, increasing its hardness, and whitening its cutting surface, thus seriously affecting its taste and appearance quality. Therefore, understanding

the biosynthesis mechanism of lignin, as well as delaying the occurrence of lignin, has become the focus of research in the fresh-cut fruit and vegetable industry.

As an important secondary metabolite, lignin is one of the components of plant cell walls, which can promote cell connection, maintain cell osmotic balance, and membrane integrity and reduce water loss, and the lignification of fresh-cut fruits and vegetables is the result of the reverse accumulation of lignin in the cell wall [5,6]. Research has shown that the suberin formed by the lignification of potato wounds can effectively avoid the infection of microorganisms and reduce the rate of transpiration and water loss, but the quality fission of many fruits and vegetables is also the result of the reverse accumulation of lignification in the cell wall, such as loquat, bamboo shoots, and carrots [7–9]. The lignin synthesis pathway is mainly divided into three stages: the shikimate metabolism pathway, the phenylpropanoid biosynthesis, and the lignin-specific synthesis pathway [10]. The synthesis of lignin is closely related to phenylalanine ammonia-lyase (PAL), cinnamyl-alcohol dehydrogenase (CAD), peroxidase (POD), and other enzymes.

At present, the research on cucumbers mainly focuses on the fresh-keeping mechanism, microorganisms in the tissue, and the safety of fresh-cut cucumber, while the impact of mechanical damage treatments on the quality of fresh-cut cucumber is not clear, especially because the lignification of fresh-cut cucumber caused by mechanical damages needs further research [11,12]. However, lignin synthesis is a complex process, including changes in gene levels. In recent years, with the development of genome sequencing and genomics technology, transcriptome technology has been applied more and more widely in fruit biology research. For example, it has been used to reveal the aging and preservation mechanism of fruits and vegetables such as apples, carrots, and potatoes [13–15]. Based on all transcripts of organisms, transcriptomics screens key differential genes generated in biological changes and uses bioinformatics to analyze rich differential metabolic pathways. In this study, transcriptome analysis based on RNA-seq was used to screen the EDGs between the treated and untreated cucumbers, and we analyzed the KEGG metabolic pathway to find out the key DEGs and key metabolic pathways related to the lignin synthesis of fresh-cut cucumber and clarify its lignin biosynthesis mechanism. It provides a new perspective and theoretical basis for improving the potential nutritional value of cucumbers and developing effective preservation methods to maintain cucumber quality.

2. Materials and Methods

2.1. Plant Materials, Treatments and Storage Conditions

Cucumber (*Cucumis sativus* L.) variety "Mici" was harvested in July 2022 and transported to the laboratory within 2 h, then selected in uniform size for the study. The selected cucumber fruits were dipped in 0.02% sodium hypochlorite for 5 min, washed with distilled water, and then placed on a ventilator to dry naturally. The fruits were cut into slices with a 3.5 cm diameter and 0.5 cm thickness (P); shreds in the shape of a cuboid with 4 cm length, 0.5 cm width, and 0.5 cm height (S); and with whole fruits as the control (CK). Based on a previous method [16], the calculated wounding intensities were 1.8 and 4.0 $cm^2\ g^{-1}$ for slices and shreds, respectively. All the plants were then packed in 15 cm × 10 cm × 4 cm polyethylene films and stored at 4 °C and for 3 d with 95% relative humidity. Fruit samples were collected daily, frozen with liquid nitrogen, and smashed with a frozen crusher to obtain plant powder.

2.2. Measurement of Enzyme Activity

Approximately 1 g frozen sample was used for the PAL enzyme assay according to the protocol of the phenylamine ammonia-lyse measurement kit (PAL, Suzhou Comin Biotechnology Co., Ltd., Suzhou, China). One unit of PAL activity was defined as the amount of enzyme required to increase the absorbance of 0.1 at 290 nm × g^{-1} × min^{-1}. Approximately 1 g of each frozen sample was used for the CAD enzyme assay according to the protocol of the cinnamyl-alcohol dehydrogenase measurement kit (CAD, Suzhou Comin Biotechnology Co., Ltd., Suzhou, China). One unit of CAD activity was defined

as the amount of enzyme required to increase the absorbance of 1 nmol NADPH at 340 nm $\times$ g^{-1} $\times$ min^{-1}. The POD activity was determined by a modified method. Cucumber powder was added to 10 mL of phosphate-buffered saline (PBS, pH 6.4) containing 0.5% PVPP to determine POD activity. POD activity was measured with 0.9 mL of 0.2% guaiacol and 1 mL of 0.3 H_2O_2. The absorbance of POD was measured at 470 nm for 1 min. The enzyme required to cause an increase in absorbance of 0.01 in one minute is defined as one unit of activity [17]. Three independent replicates were used for each treatment.

2.3. Determination of Lignin Contents

Cucumber samples were taken as the material, and the samples were dried at 80 °C to constant mass, crushed and passed through a 40-mesh sieve, and weighed as about 2 mg (recorded as W), and the lignin content was determined by the acetyl bromide method [18,19], referring to the instructions of the lignin content kit (MZS, Suzhou Comin Biotechnology Co., Ltd., China), calculated according to the following equation:

$$\text{Lignin (mg/g)} = (A - A' - 0.0068) \div 0.0347 \times V \times 10^{-3} \div W \times T \quad (1)$$

where A is the measurement tube OD280 reading, A′ is the blank tube OD280 reading, V is the total reaction volume, T is the dilution multiple, and W is the sample weight.

2.4. Transcriptomic Analysis

2.4.1. RNA Extraction

Three cucumber samples from each treatment were randomly taken, namely, CK-1, CK-2, CK-3, P-1, P-2, P-3, S-1, S2, and S-3, for transcriptome analysis. TRIzol (Thermo Fisher, 15596018, CA, USA) was used to isolate and purify the RNA of cucumber samples according to the operation scheme provided by the manufacturer. Then, the quantity and purity of total RNA were controlled by the micro-nucleic acid protein quantitative instrument Nano Drop ND-1000 (Nano Drop, Wilmington, DE, USA).

2.4.2. Construction of Library

Oligo (dT) magnetic beads were used to specifically capture the mRNA containing polyadenylate (Poly-A) through two rounds of purification. The captured mRNA was fragmented under high temperature using a magnesium ion interruption kit. Fragmented RNA was synthesized into cDNA under the action of reverse transcriptase. The second chain was digested with UDG enzyme, pre-denatured at 95 °C for 3 min by PCR, denatured at 98 °C for a total of 8 cycles for 15 s, annealed at 60 °C for 15 s, extended at 72 °C for 30 s, and finally extended at 72 °C for 5 min to form a library with a fragment size of 300 bp $\pm$ 50 bp.

2.4.3. Sequencing Method

Illumina Novaseq TM 6000 (LC Bio Technology Co., Ltd., Hangzhou, China) was used to carry out two-terminal sequencing according to the standard operation, and the sequencing mode was PE150.

2.4.4. Verification of DEGs by qRT-PCR

The extraction of RNA in the sample was carried out according to the instructions of the plant RNA extraction kit. The reverse transcription steps of RNA refer to the instructions of the reverse transcription kit to reverse transcribe the RNA extracted from cucumbers into cDNA. The real-time fluorescence quantitative polymerase chain reaction (qRT-PCR) reaction system consisted of 10 μL 2X SYBR® Green Pro Taq HS Premix II, 0.8 μL positive primer, 0.8 μL reverse primer, 2 μL cDNA solution, and 6.4 μL RNase free water, with a total volume of 20 μL. The final concentration was 1X SYBR® Green Pro Taq HS Premix II, and 0.4 μM positive and reverse primers. The reaction procedure of qRT-PCR was 95 °C for 30 s; 40 cycles of 95 °C denaturation for 5 s, and 60 °C annealing for 30 s. The relative

quantification of genes was carried out using the $2^{-\Delta\Delta CT}$ method. The experiment was repeated three times.

2.5. Statistical Analysis

Microsoft Excel 2019 was used for data statistics and standard deviation calculation. SPSS 22.0 software was used for the Duncan difference significance test, and $p < 0.05$ indicated a significant difference. Figures were drawn with the omicstudio drawing module.

3. Results

3.1. Lignin Contents and Enzyme Activity of PAL, CAD, and POD

At earlier storage times, the lignin content increased in both cutting types and was the highest in the P treatment, indicating the P treatment had a lower wounding intensity than the S treatment (Figure 1a). Phenylpropane metabolism is the main pathway of lignin synthesis, and in order to illuminate the synthesis of lignin, the activities of critical enzymes PAL, CAD, and POD in the phenylpropane metabolic pathway were determined. PAL activity of the tissues increased slowly in the two cutting types and the control group (Figure 1b). The CAD activity decreased sharply during the first two days. At 2 d, CAD activity decreased by 46.0% and 53.6% in the P treatment and the S treatment, respectively, compared with the initial value. However, during the whole storage period, the CAD activity of the slices and shreds treatment was higher than that of the whole cucumbers (Figure 1c). POD activity increased during the 0–3 d of storage in all treatments. At 3 d, the POD activity in the P treatment and the S treatment was increased by 1.62 and 1.53 times, respectively, compared with the whole cucumbers (Figure 1d).

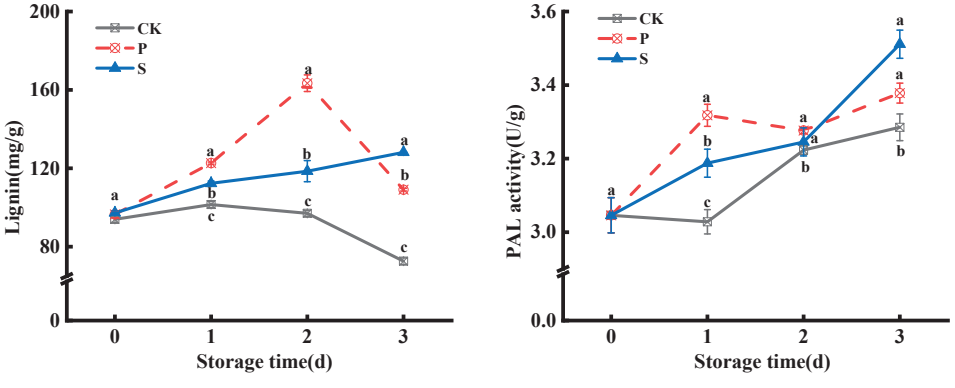
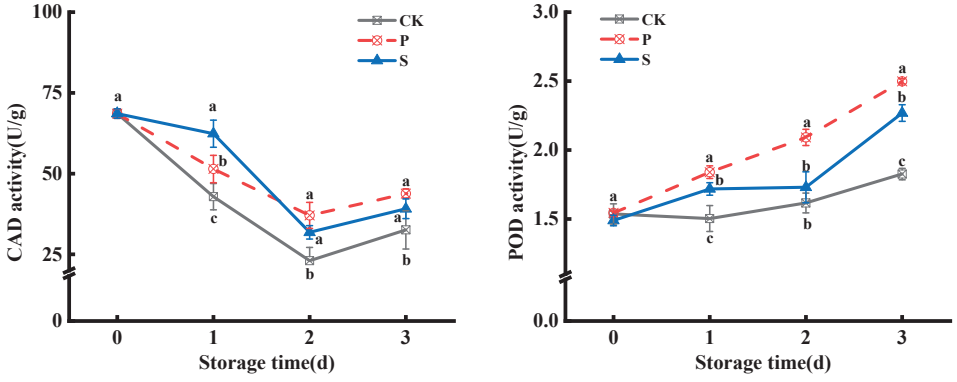


Figure 1. *Cont.*



(**c**) (**d**)

Figure 1. Effect of cutting types on lignin content (**a**), PAL activity (**b**), CAD activity (**c**), and POD activity (**d**) in cucumbers. Columns with vertical bars represent the mean ± SD (n = 3). Different letters indicate significant differences between samples of different treatment groups for the same storage time ($p < 0.05$). CK represents the samples in the control treatment, P represents the samples in the slices treatment, and S represents the samples in the shreds treatment.

3.2. Transcriptomic Analysis

In order to ensure accurate and reliable analysis results, it is generally necessary to preprocess the original data (Table 1). After removing the sequencing connector (introduced during database construction) and low-quality sequencing data (caused by the error of the sequencer itself), we obtained 54.79 GB of effective data in total. The proportion of effective reads of each sample was more than 93.02%. The range distribution of Q20 (sequencing error rate is less than 0.01) was 99.95~99.96%, and that of Q30 (sequencing error rate is less than 0.001) was 96.58~96.94%. The GC content was 42.50%. The quality of sequencing data was good and suitable for further analysis.

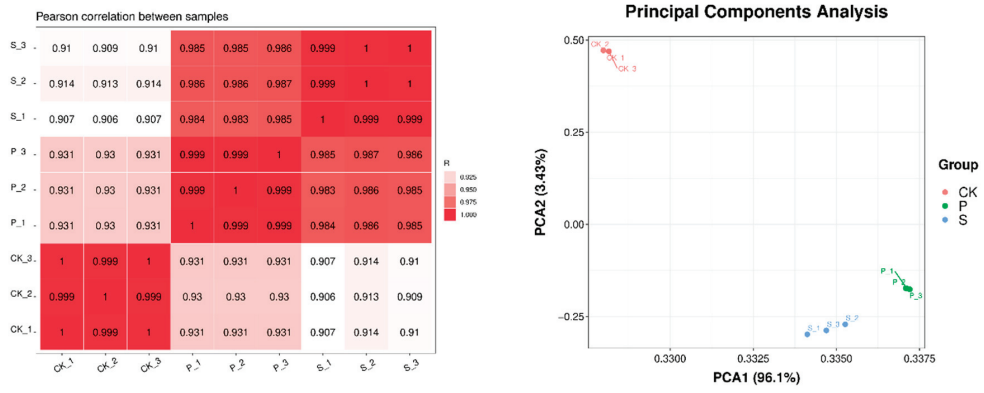
Table 1. Statistical analysis of RNA-seq.

Sample	Raw Data		Valid Data		Valid Ratio (%)	Q20%	Q30%	GC%
	Read	Base	Read	Base				
CK	40,771,589	6.11 G	37,754,237	5.68 G	93.02	99.96	96.80	42.50
P	39,870,298	5.98 G	39,146,899	5.54 G	93.11	99.96	96.74	42.50
S	41,079,656	6.03 G	38,250,758	5.73 G	93.11	99.96	96.67	42.50

CK represents the samples stored for the first day in the control group, P represents the samples stored for the first day in the slices treatment, and S represents the samples stored for the first day in the shreds treatment. Each group has three biological parallels.

Based on the FPKM value, Pearson correlation analysis was performed. The larger the correlation coefficient between samples, the better the clustering effects of samples (Figure 2a). The Pearson correlation coefficient R^2 of the three biological repeats in each sample group was greater than 0.999, indicating that the three biological repeats in the sequencing data of this transcriptome were reliable. The R^2 value between CK and S was the lowest, ranging from 0.906 to 0.914. The R^2 value between CK and P took second place, namely 0.93 to 0.931. The R^2 value between P and S was the largest, ranging from 0.983 to 0.987. Further, the principal component analysis (PCA) was carried out for the gene expression of all samples (Figure 2b), the three biological replicates of each group of samples were gathered together in space and there was a significant spatial distance between the sample groups, indicating that the samples in the same group had strong consistency and there were differences between groups, that is, there were differences

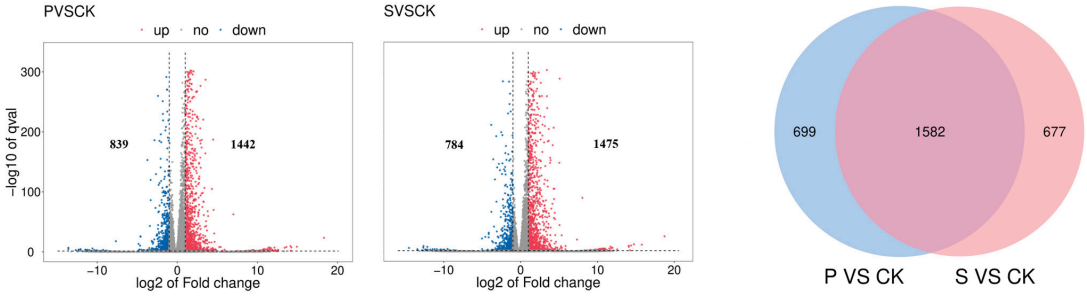
between samples processed by different cutting types. Therefore, it can be used for DEGs analysis.



(**a**) (**b**)

Figure 2. Pearson correlation analysis (**a**) and principal component analysis (**b**) were performed on the gene expression of all samples.

FC > 2 (up-regulated) or FC < 0.5 (down-regulated) and $p < 0.05$ are used as the threshold for screening DEGs. Volcanic maps of all genes in the differential expression analysis. With log2 (FC) as the abscissa and log10 (q value) as the ordinate, the DEGs among the CK, S, and P treatments after 24 h storage were shown (Figure 3a). Compared with CK, there were 2281 DEGs (1442 up-regulated genes and 839 down-regulated genes) in the P treatment, and a total of 2259 DEGs (1475 up-regulated genes and 784 down-regulated genes) were found in the S treatment. Moreover, there were 1582 common DEGs between P vs. CK and S vs. CK, of which the number of up-regulated genes was greater than that of down-regulated ones, indicating that more up-regulated genes were induced by mechanical damages in cucumbers, and they accelerated the expression of more genes involved in lignin biosynthesis in cucumbers (Figure 3b).



(**a**) (**b**)

Figure 3. Volcanic map (**a**) and Venn diagram (**b**) of differentially expressed genes between P vs. CK and S vs. CK. The abscissa represents the change of differentially expressed genes multiple in different samples, the ordinate represents the statistical significance of the difference in gene expression.

3.3. GO Annotation of Differentially Expressed Genes

In P vs. CK and S vs. CK, 1348 DEGs (920 up-regulated and 518 down-regulated) and 694 DEGs (472 up-regulated and 222 down-regulated) were annotated, respectively. The GO functions are mainly divided into three categories: biological process (BP), cellular component (CC), and molecular function (MF). In P vs. CK, DEGs were enriched in 584 GO entries, of which 64 were significantly enriched ($p < 0.05$). In S vs. CK, the DEGs were enriched in 561 GO entries, of which 75 were significantly enriched ($p < 0.05$). When comparing the GO term in P vs. CK and S vs. CK, 59 out of 197 GO terms were found in common (Figure 4). DEGs involved in BP were mainly concentrated in DNA-templated, protein phosphorylation, oxidation–reduction process, transmembrane process, carbohydrate metabolic process, and lipid metabolic process. DEGs involved in CC were transcription factor complex, an integral component of the membrane and nucleus, and DEGs involved in MF were mainly ATP binding, protein binding, DNA binding, protein kinase activity, DNA-binding transcription factor activity, heme binding, and sequence-specific DNA binding.

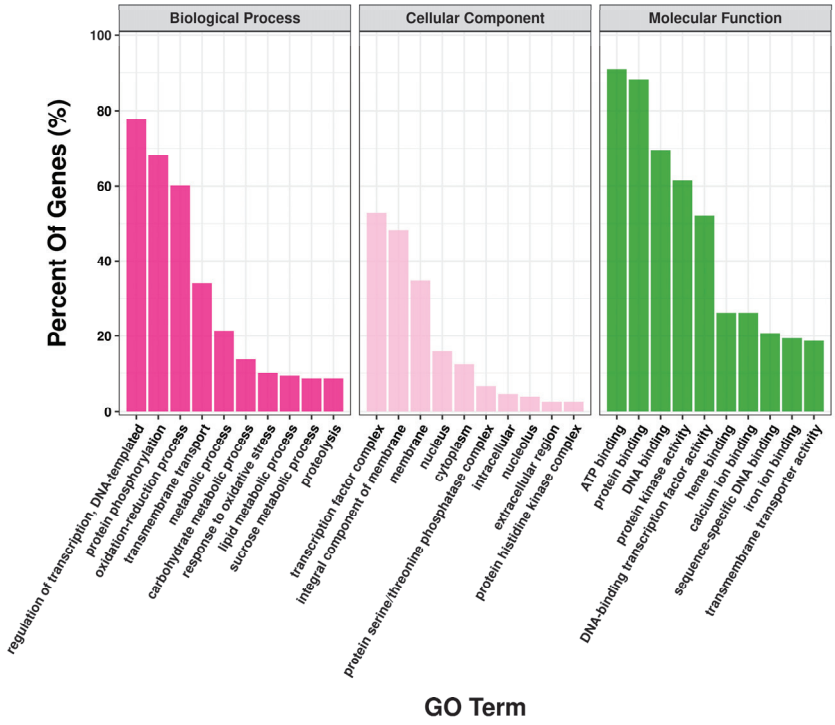


Figure 4. Main class of GO term annotated to differentially expressed genes in P vs. CK and S vs. CK. This analysis presented the top 10 pathways for each of BP, CC and MF.

3.4. KEGG Pathway Analysis

In order to characterize the pathways that were related to lignin biosynthesis by mechanical damages, we performed the metabolic pathways analysis on DEGs based on the KEGG database with $p < 0.05$ as the threshold. We found that 116 and 125 KEGG pathways were annotated by the DEGs of P vs. CK and S vs. CK, respectively. When comparing the KEGG pathway in P vs. CK and S vs. CK, 15 out of 94 KEGG pathways were found in common (Figure 5). The result showed that there were 10 pathways of metabolism, mainly including carbohydrate metabolism, energy metabolism, amino acid metabolism, biosynthesis of other secondary metabolites, lipid metabolism, nucleotide metabolism, metabolism

of terpenoids and polyketides, metabolism of cofactors and vitamins, metabolism of other amino acids, and glycan biosynthesis and metabolism.

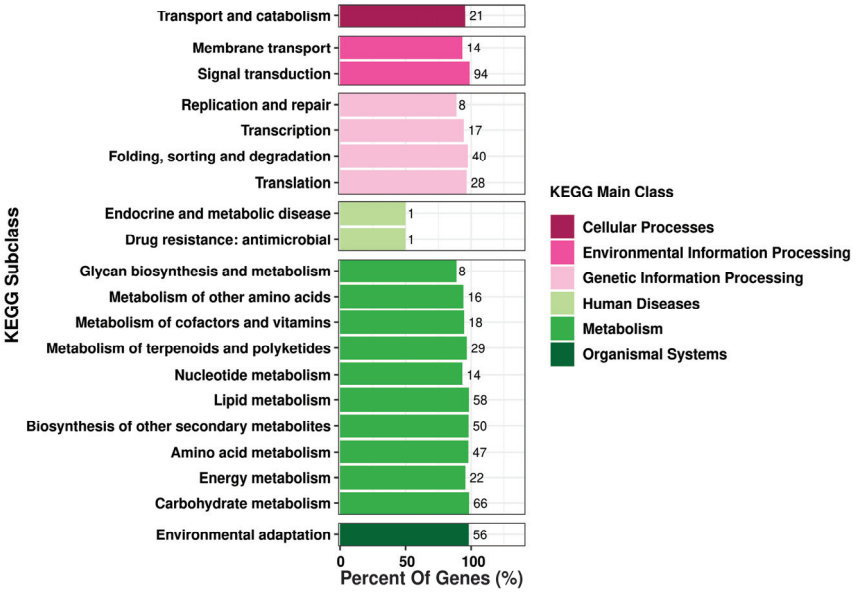


Figure 5. Main class of KEGG pathways annotated to differentially expressed genes in P vs. CK and S vs. CK. This analysis presented the top 20 pathways.

3.5. Critical Metabolic Pathways Induced in Response to Mechanical Damages in Cucumbers

Based on previous studies, we focused on three metabolism pathways, specifically lipid metabolism, amino acid metabolism, and biosynthesis of other secondary metabolites, which could be involved in mechanical damages [20,21]. It is very necessary to explore the interrelation and regulation of these metabolic pathways and find the key connection points between them for analyzing the mechanism of lignin biosynthesis in fresh-cut cucumber.

3.5.1. Lipid Metabolism

In order to analyze the effect of the mechanical damages on the metabolism of cucumber lipid compounds, the statistical analysis of the DEGs in the P treatment and the S treatment is shown (Table 2). There were 100 DEGs (88 were significantly up-regulated and 12 were significantly down-regulated) and 98 DEGs (84 were significantly up-regulated and 14 were significantly down-regulated) involved in lipid metabolism in the slices and shreds cucumbers. Among the lipid metabolism pathways annotated by DEGs in the two cutting type groups, linoleic acid metabolism (p = 0.0023) was significantly enriched in the slices group and fatty acid elongation (p = 0.0350) in the shreds one. However, the pathways with significant differences were alpha-linolenic acid metabolism (p = 0.0001), and the number of DEGs was 20 in both of the cutting types, in which there were 19 up-regulated and 1 down-regulated DEGs in the slices group and 18 up-regulated and 2 down-regulated in the shreds one, respectively (Figure 6). There were four *PLA1*, six *13S LOX2*, three *AOS*, two *AOC*, and one *JOMJ* up-regulated both in P vs. CK and S vs. CK. Compared with CK, the *HPL1* was down-regulated in the P treatment and not significantly expressed in the S treatment. One *OPR* was down-regulated in the S treatment and not significantly expressed in the P treatment.

Table 2. Lipid metabolism in fresh-cut cucumber.

Lipid Metabolism	P vs. CK		S vs. CK	
	DEGs	*p*	DEGs	*p*
Alpha-linolenic acid metabolism	20	0.0001	20	0.0001
Fatty acid elongation	5	0.2169	7	0.0350
Linoleic acid metabolism	10	0.0023	7	0.0615
Ether lipid metabolism	7	0.1773	8	0.0821
Secondary bile acid biosynthesis	1	0.1918	1	0.1894
Glycerophospholipid metabolism	14	0.2693	14	0.2536
Arachidonic acid metabolism	5	0.1801	4	0.3503
Glycerolipid metabolism	13	0.3768	13	0.3589
Biosynthesis of unsaturated fatty acids	4	0.7434	5	0.5479
Cutin, suberine, and wax biosynthesis	7	0.1255	4	0.6144
Sphingolipid metabolism	9	0.4929	8	0.6222
Steroid biosynthesis	1	0.9634	2	0.8297
Fatty acid degradation	3	0.9573	4	0.8786
Fatty acid biosynthesis	1	0.9961	1	0.9958

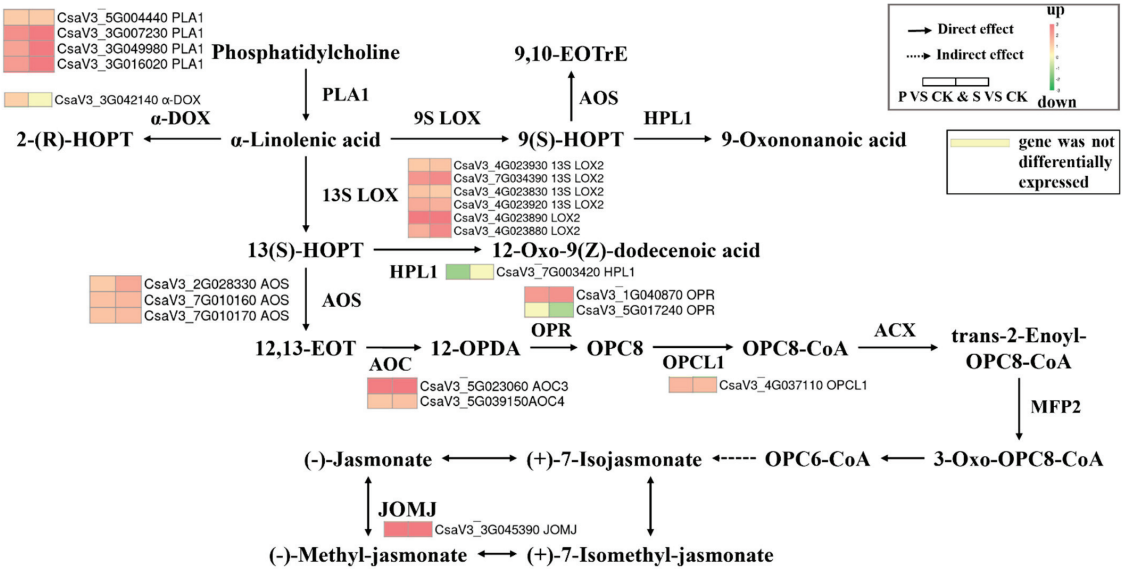


Figure 6. Changes in the alpha-linolenic acid metabolism pathway in fresh-cut cucumber. Genes with differential abundance involved in alpha-linolenic acid metabolism are mapped to the corresponding pathway. Red indicates up-regulation, and green indicates down-regulation. PLA: peroxisomal acyl-coenzyme A oxidase; 13S LOX: linoleate 13S-lipoxygenase; AOS: allene oxide synthase; AOC: allene oxide cyclase; a-DOX: alpha-dioxygenase; HPL: linolenate hydroperoxide lyase; OPR: 12-oxophytodienoate reductase; OPCL: OPC-8:0 CoA ligase; JOMJ: jasmonate O-methyltransferase.

3.5.2. Amino Acid Metabolism

In order to analyze the effect of mechanical damages of cucumbers on amino acid metabolism, the DEGs of P vs. CK and S vs. CK were statistically analyzed (Table 3). There are 78 DEGs in P vs. CK, of which 62 are up-regulated and 16 are down-regulated. These DEGs participate in 11 amino acid metabolism pathways, of which three pathways are significantly different ($p < 0.05$), namely phenylalanine metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, and arginine and proline metabolism. There are 80 DEGs in S vs. CK, of which 59 are up-regulated and 21 are down-regulated. These DEGs are mainly distributed in 14 amino acid metabolic pathways, of which three pathways are

significantly different ($p < 0.05$), namely phenylalanine metabolism, phenylalanine, tyrosine, and tryptophan biosynthesis as well as glycine, serine, and threonine metabolism. The significantly different pathways shared by DEGs in P vs. CK and S vs. CK are phenylalanine metabolism, phenylalanine, tyrosine, and tryptophan biosynthesis. Among them, the 16 DEGs in phenylalanine metabolism are all up-regulated in the two cutting types; 11 DEGs of slice treatment are all up-regulated, followed by 10 DEGs (8 up-regulated and 2 down-regulated) in the shreds treatment of phenylalanine, tyrosine and tryptophan biosynthesis (Figure 7). By analyzing the genes in these two metabolic pathways, *SK*, *ADT*, *POA*, and *PAL* were found significantly up-regulated in the two cutting type treatments: *TSase A* and *ADH* were significantly down-regulated in the shreds cucumbers, but different from the slices treatment group that *CS*, *CM*, and *PPA-AT* in the shreds one did not change significantly.

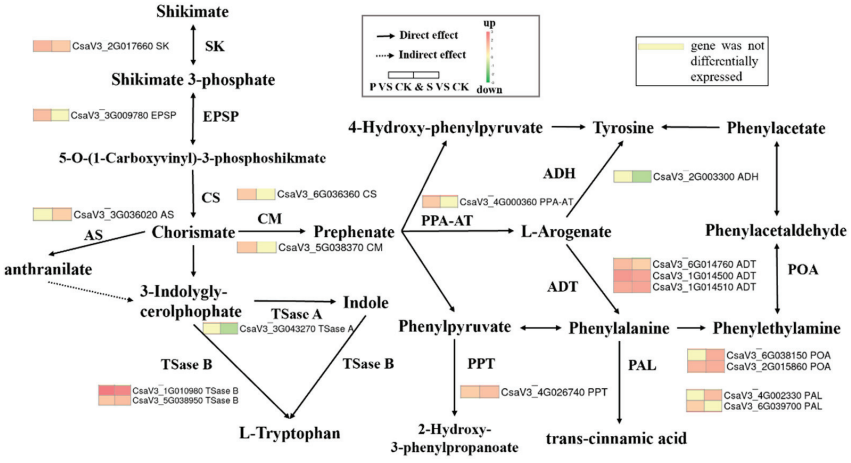


Figure 7. Changes in the phenylalanine, tyrosine, and tryptophan biosynthesis and phenylalanine metabolism pathway in fresh-cut cucumber. Genes with differential abundance involved in phenylalanine, tyrosine and tryptophan biosynthesis and phenylalanine metabolism are mapped to the corresponding pathway. Red indicates up-regulation, and green indicates down-regulation. SK: Shikimate kinase; EPSP: 3-phosphoshikimate 1-carboxyvinyltransferase; CS: chorismate synthase; CM: chorismate mutase; TSase A: tryptophan synthase alpha chain; TSase B: tryptophan synthase beta chain; ADH: arogenate dehydrogenase; ADT: arogenate dehydratase; PPA-AT: prephenate aminotransferase; PPT: phenylpyruvate tautomerase; POA: primary amine oxidase; PAL: phenylalanine ammonia-lyase; AS: anthranilate synthase.

Table 3. Amino acids metabolism in fresh-cut cucumber.

Amino Acid Metabolism	P vs. CK		S vs. CK	
	DEGs	*p*	DEGs	*p*
Phenylalanine metabolism	16	0.0028	16	0.0025
Phenylalanine, tyrosine, and tryptophan biosynthesis	11	0.0098	10	0.0236
Glycine, serine, and threonine metabolism	13	0.1175	15	0.0316
Alanine, aspartate and glutamate metabolism	7	0.1186	6	0.5433
Arginine biosynthesis	7	0.1773	4	0.6864
Valine, leucine, and isoleucine biosynthesis	2	0.7133	2	0.7063
Lysine biosynthesis	1	0.7497	1	0.7450
Tryptophan metabolism	3	0.9007	4	0.7623
Tyrosine metabolism	4	0.797	4	0.7884
Cysteine and methionine metabolism	11	0.7487	10	0.8269

Table 3. *Cont.*

Amino Acid Metabolism	P vs. CK		S vs. CK	
	DEGs	*p*	DEGs	*p*
Histidine metabolism	-	-	1	0.8493
Valine, leucine, and isoleucine degradation	-	-	3	0.9717
Arginine and proline metabolism	3	0.0423	3	0.9777
Lysine degradation	-	-	1	0.9852

- indicates that it is not checked out.

3.5.3. Phenylpropanoid Biosynthesis

There were 55 DEGs involved in the phenylpropanoid biosynthesis pathway in P vs. CK (40 up-regulated genes and 15 down-regulated genes, and 53 DEGs (37 up-regulated genes and 16 down-regulated genes) were found in S vs. CK. Most genes were up-regulated in the phenylpropanoid biosynthesis pathway, indicating that mechanical damages could promote lignin biosynthesis (Figure 8). The mechanical damages induced seven *PAL*, two *CYP37A*, three *4CL*, and one *CAD* up-regulation in the two cutting types of cucumbers. Among the DEGs involved in the phenylpropanoid biosynthesis pathway in cucumbers subjected to mechanical damages, the changes of *CCR* and *POD* family genes were the most complex. Compared with the control group, three *CCR2* were up-regulated in the slices and shreds groups, five *CCR1* (three *CCR1* up-regulated, two *CCR1* down-regulated) in slices, and three *CCR1* (two *CCR1* up-regulated, one *CCR1* down-regulated) in shreds. Three lignin-forming *POD* were up-regulated and three *POD17* were down-regulated both in the slices and shreds cucumbers, two *POD5* in the slices cucumbers were significantly up-regulated, while no significant changes in the shreds treatment, one *POD49* was only up-regulated in the shreds cucumbers, and one *POD4* was only up-regulated in the slices one.

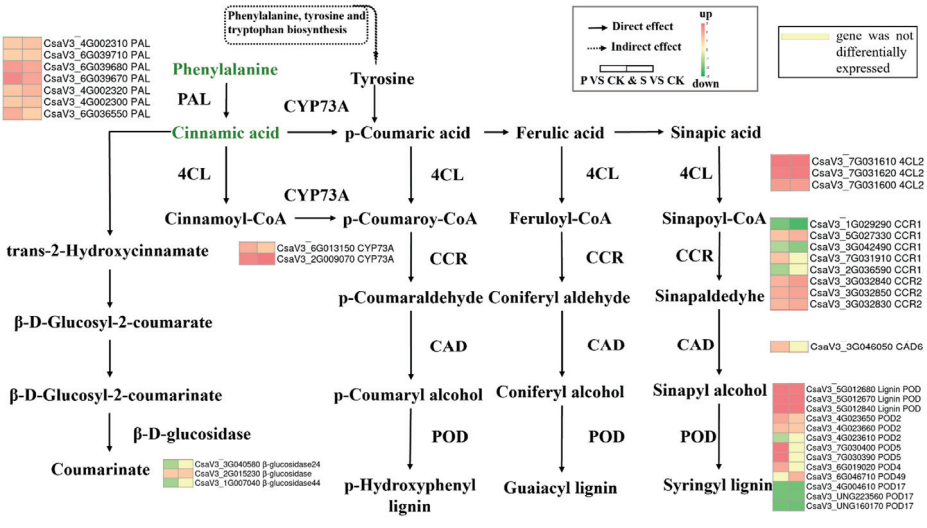
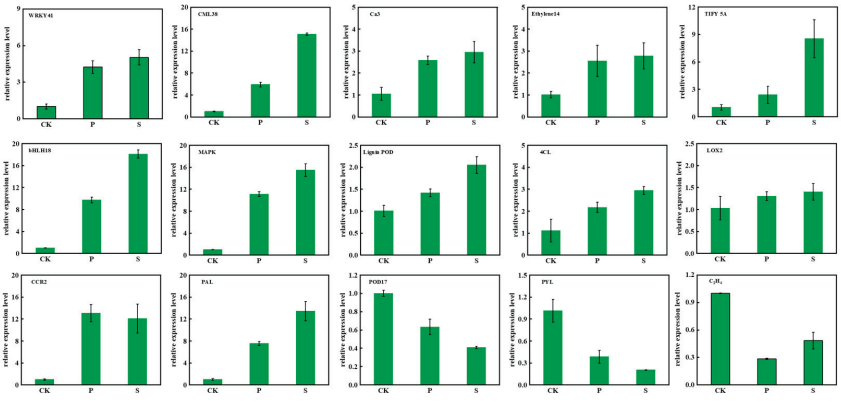


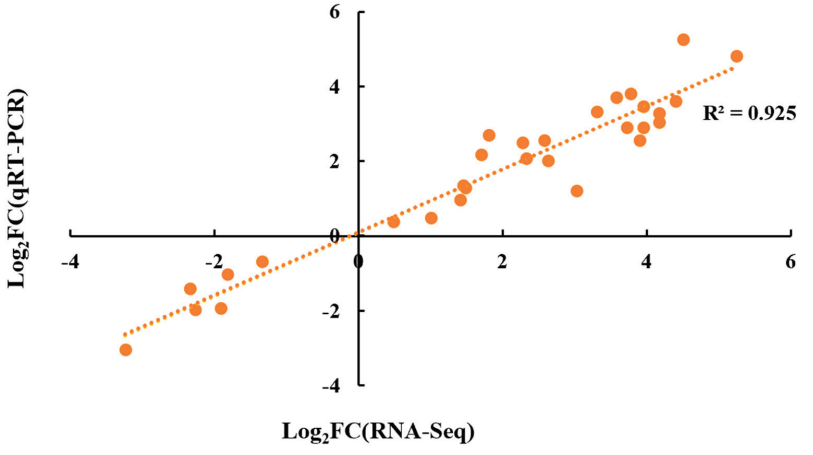
Figure 8. Changes in the Phenylpropanoid biosynthesis pathway in fresh-cut cucumber. Genes with differential abundance involved in phenylpropanoid biosynthesis metabolism are mapped to the corresponding pathway. Red indicates up-regulation, and green indicates down-regulation. PAL: phenylalanine ammonia-lyase; CYP73A: trans-cinnamate 4-monooxygenase; Lignin-forming POD: lignin-forming anionic peroxidase; POD: peroxidase; CCR: cinnamoyl-CoA reductase; 4CL: 4-coumarate-CoA ligase; β-glucosidase: beta-glucosidase; CAD: cinnamyl-alcohol dehydrogenase.

3.6. Verification of DEGs by qRT-PCR

A total of 15 DEGs were selected to validate the RNA-seq data by qRT-PCR evaluation using specific primers (Table S1). Relative expression levels of 15 selected genes were analyzed by qRT-PCR (Figure 9a), and the square value of the correlation coefficient R in the linear regression analysis was 0.925 (Figure 9b), indicating that the transcriptome results were reliable in our study.



(**a**)



(**b**)

Figure 9. Validation of RNA-seq results. Relative expression levels of 15 selected genes were analyzed by qRT-PCR (**a**). Regression analysis of gene expression levels based on log2 FC (RNA-seq) (*x*-axis) and log2 FC (qRT-PCR) (*y*-axis) (**b**).

4. Discussion

The lignification of fruits will inevitably affect the quality of vegetables and fruits, and fresh-cut cucumber is prone to lignification during storage, which increases its lignin content and hardness, leading to a reduction in consumer acceptance. The physiological study found that the two cutting types could make lignin accumulate in a large amount, and mechanical damages could promote the activities of PAL and POD. Therefore, we further analyzed the mechanism of lignin biosynthesis in transcriptomics. The amino acid metabolism, the lipid acid metabolism, and the biosynthesis of other secondary metabolites

were the enriched pathways, regardless of the cutting styles, indicating that these processes were involved in the response of the lignin biosynthesis of cucumbers to mechanical damages. Thus, the analysis of the possible functions of DEGs by comparison between them would help to reveal the mechanism of lignin biosynthesis in fresh-cut cucumber. The DEGs were significantly enriched in alpha-linoleic acid metabolism, phenylpropane metabolism, phenylalanine, tyrosine and tryptophan biosynthesis, and phenylpropanoid biosynthesis by the analysis of KEGG enrichment. The results suggested that these pathways might be closely related to lignin synthesis induced by mechanical damages.

Mechanical damages can make fruits and vegetables produce JA signaling, which triggers a series of reactions and secondary metabolites that are synthesized for repairing the damages of tissue [22,23]. Previous reports have shown that JA are important plant hormones with multiple roles in mechanical damages, which can activate secondary metabolite biosynthesis pathways in the defense response, especially in lignin biosynthesis or development pathways [24]. Transcriptomic analysis showed that the *LOX* in cucumbers was significantly up-regulated by mechanical damage treatment. The main function of LOX was to oxidize linolenic acid by inserting oxygen at the C-13 position, providing a material precursor for JA synthesis. Secondly, *AOS* and *AOC* were significantly up-regulated, both of which could catalyze the conversion of alkoxides to OPDA, and finally, JA was generated under the catalysis of JOMJ [25]. Therefore, mechanical damages can induce JA synthesis, which may be an important reason for accelerating the lignin synthesis of fresh-cut cucumber (Figure 6).

The common function of amino acids is used as raw materials for protein synthesis. They can also be used as precursors for the synthesis of vitamins, nucleotides, terpenes, alkaloids, phenolic acids, flavonoids, lignin, purines, and pyrimidines [26,27]. Phenylalanine (Phe), tyrosine (Tyr), and tryptophan (Try) are aromatic amino acids in plants, which are synthesized mainly through the shikimate pathway. Phosphoenolpyruvate (PEP) produced by the glycolysis pathway and erythrose 4-phosphate (E4P) produced by the pentose phosphate pathway enter the shikimate metabolism pathway under the catalysis of enzymes [28,29]. SK can convert shikimate into shikimate 3-phosphate, which can be further converted into chorismate [30]. Under the catalysis of CM, prephenate is generated and aromatic amino acids are further synthesized. The reaction is divided into two ways, one of which is converted to Phe and Tyr, and the other way is to generate Try (Figure 7). It can be seen that although the different cutting type treatments have a different impact on the phenylalanine, tyrosine, and tryptophan biosynthesis in cucumbers, they all accelerate the conversion of shikimate to Phe, which can provide more carbon sources for lignin synthesis and then accelerate the synthesis of lignin. Phe is an essential amino acid for plants, which can be used as a carbon source and affect the growth and development of plants by participating in protein synthesis [31]. At the same time, in the secondary metabolism of plant cells, Phe is an important precursor for the synthesis of lignin, alkaloids, flavonoids, and other secondary substances, so it has an important physiological significance [32]. Phenylalanine metabolism is the main way to determine the fate of phenylalanine, and also an important intermediate way to connect primary metabolism (such as TCA cycle and glycolysis) and secondary metabolism (such as phenylpropanoid biosynthesis). Therefore, mechanical damages can promote the accumulation of Phe and provide more precursors for lignin synthesis.

Lignin is an important final metabolite of cucumbers, which is mainly synthesized through the phenylpropane metabolic pathway [33,34]. Cutting treatment has significant effects on genes in the phenylpropane metabolism pathway of cucumbers. The mechanical damages induced PAL up-regulation both in P vs. CK and S vs. CK (Figure 9). As the first key enzyme in lignin synthesis, PAL could catalyze phenylalanine to produce cinnamic acid, providing more precursors for the lignin synthesis pathway of phenylpropanoid biosynthesis. This result was similar to previous reports in fresh-cut broccoli and carrot [35,36]. In this study, some novel information related to phenylpropanoid biosynthesis was found in fresh-cut cucumbers, and *4CL*, *CCR*, *CAD*, and *POD* were identified as

DEGs in both P and S treatments. These genes were the critical genes of the lignin synthesis pathway. *CCR*, *CAD*, and *POD* are the key rate-limiting enzymes for lignin synthesis. It is worth mentioning that the effect of mechanical damages on CCR family genes was complicated. The *CCR* family genes were simply divided into *CCR1* and *CCR2*, in which *CCR2* was all up-regulated in the two cutting types of cucumbers; however, the changes of *CCR1* were complex, and some were up-regulated while others were down-regulated. The main reason may be that *CCR* catalyzes the reduction of hydroxycinnamoyl-CoA to corresponding cinnamaldehydes, thereby further regulating the synthesis process of precursor substances for lignin. *CAD* can catalyze the synthesis of mono-lignin in the process of lignin synthesis, reduce cinnamaldehyde to cinnamyl-alcohol, and provide precursors for lignin synthesis. The results showed that the fresh-cut treatment promoted the lignin synthesis pathway, and the previous research results of potato and kiwifruit [37,38] also showed that the fresh-cut treatment accelerated the lignin synthesis, which was consistent with the results of this study. However, the *POD* family has multiple functions in the process of plant metabolism. On the one hand, POD, with the dual functions of clearing active oxygen and accelerating browning, can catalyze hydrogen peroxide and oxidize phenols to form brown products in the presence of H_2O_2. On the other hand, *POD*, which can catalyze the polymerization of lignin monomers to produce lignin, participates in the final catalytic reaction of lignin formation in the downstream pathway of phenylpropanoid biosynthesis. In this study, the mechanical damage treatment increased the expression of three lignin-forming *POD* all up-regulated in the two cutting types of cucumbers, which can catalyze the polymerization of lignin monomers to complete the process of lignin, the last step of lignin biosynthesis. Taken together, the results suggested that the lignin biosynthesis genes may be up-regulated by mechanical damages according to the phenylpropanoid metabolism, resulting in a promotion of lignification in fresh-cut cucumber.

5. Conclusions

The gene profile of fresh-cut cucumber subjected to mechanical damages was obtained through the transcriptome method, P vs. CK involved 2281 (1442 up-regulated and 839 down-regulated genes) differentially expressed genes, and S vs. CK involved 2259 (1475 up-regulated and 784 down-regulated genes) differentially expressed genes. Bioinformatics analyses revealed that DEGs are mainly involved in alpha-linolenic acid metabolism and phenylalanine metabolism, as well as phenylalanine, tyrosine, and tryptophan biosynthesis. Meanwhile, phenylpropanoid biosynthesis was activated by mechanical damages by regulating some critical enzymes such as PAL, CAD, and POD. Moreover, this study revealed that JA signals were activated by inducing the up-regulated expression of genes such as *LOX*, *AOS*, and *AOC* by mechanical damages, and the JA signals significantly up-regulated the expression of genes such as *PAL*, *C4H*, *4CL*, *CCR*, and *CAD*, which accelerated the synthesis of lignin monomers and finally up-regulated the expression of lignin-forming *POD*, which accelerated the synthesis of lignin. This investigation provides useful information to better understand the molecular mechanisms of mechanical damages in post-harvest cucumbers and then further enhance the potential nutritional values in the processing of harvested cucumbers.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae9040500/s1, Table S1: Primers used in qRT-PCR.

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Review

Bibliometrics and Visual Analysis of Non-Destructive Testing Technology for Fruit Quality

Peng Ni [1,2], Hao Niu [1,2,*], Yurong Tang [1,2], Yabo Zhang [1,2], Wenyang Zhang [1,2], Yang Liu [1,2] and Haipeng Lan [1,2]

[1] Modern Agricultural Engineering Key Laboratory at Universities of Education Department of Xinjiang Uygur Autonomous Region, Tarim University, Alaer 843300, China; 10757223132@stumail.taru.edu.cn (P.N.); 120150010@taru.edu.cn (Y.T.); zhangyabo0320@163.com (Y.Z.); zhangwenyang1106@163.com (W.Z.); hxtxylove@126.com (Y.L.); 120110045@taru.edu.cn (H.L.)

[2] College of Mechanical Electrification Engineering, Tarim University, Alaer 843300, China

* Correspondence: 120200013@taru.edu.cn; Tel.: +86-157-7005-1435

Abstract: This study examined the development and trends in non-destructive testing technology for fruit quality. The status of the research field and the application hotspots were investigated to provide a reference for future research in this field. Relevant studies on the non-destructive testing of fruit quality published between 1993 and 2022 were identified in the core database Web of Science. The temporal distribution, spatial distribution, literature features, research progress, and leading research hotspots were quantified and visualised using bibliometrics. The findings revealed that there continues to be active research and publications on non-destructive testing technology for fruit quality, with a good development trend. China and the USA are the major contributors to research on non-destructive testing technology for fruit quality. The major research institutions include Zhejiang University and the United States Department of Agriculture. The major papers are published in Postharvest Biology and Technology and Acta Horticulturae, among others. These studies mainly focus on agriculture, food, and gardening, among other topics. The detection indices mainly concern internal quality, such as sugar degree and soluble solids, and apparent quality, such as hardness. The detection technologies mainly include electronic nose (E-nose) technology, machine vision technology, and spectral detection technology. In the future, technological developments in artificial intelligence and deep learning will further promote the maturation and application of non-destructive testing technologies for fruit quality.

Keywords: fruit quality; non-destructive testing; bibliometrics; clustering mapping; CiteSpace

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1. Introduction

Fruits are an indispensable part of the human diet due to their rich nutrients and sweet taste. The increasing consumer demand for fruits, and their central role in a healthy diet, have contributed to the societal appreciation of high-quality fruits, which has led to increased research interest in the detection of fruit quality [1]. At present, fruit quality detection is restricted by the low efficiency of the available detection devices, the inapplicability of destructive testing technologies, and the low generalisation rate of non-destructive testing technologies. As a result, fruit quality grades are mixed, and insignificant price differences exist among different levels of fruits, resulting in damage to brand value [2]. Hence, there is a critical need to develop fruit quality detection technology within the forest and fruit industry to increase the economic value and market competitiveness of fruits.

Traditionally, fruit quality detection methods have mainly included the subjective experience-based judgement of orchard workers or the detection of the physiochemical properties of fruits based on local samples. However, the former approach is time-consuming and requires a large labour force for large-scale fruit detection; it also lacks uniform standards for accuracy. The latter approach can damage fruits and is limited by its detection cost and time requirements. As such, it is not suitable for large-scale fruit

detection. To solve the above problems and meet the demands for fruit quality detection, non-destructive testing technologies are currently being developed. Non-destructive testing technology involves the application of external stimuli to test samples and the examination of the transmitted and reflected physical indices of the test objects [3]. The chemical and physical properties of the test samples can be evaluated without damaging the original state of the samples. Since non-destructive testing technology is simple, fast, highly efficient, and non-destructive, it has been extensively applied to fruit quality testing [4]. Common non-destructive testing methods for fruit quality include optical property detection [5–7], the machine vision test [8,9], magnetic resonance imaging (MRI) [10,11], the acoustic feature test [12,13], the mechanical property test [14,15], the dielectric property test [16,17], the e-nose test [18,19], and so on. Examining the research status and leading hotspots in the field of non-destructive testing technology can provide a reference for the future development of non-destructive testing methods for fruit quality. Bibliometrics refers to the science of quantitative analysis of the literature in a certain research field, which has the characteristics of objectivity, quantification, and modeling. It plays an important role in uncovering hotspots in research fields and identifying future research directions. Bibliometrics has been widely applied to many fields, including agriculture, economy, and ecology, among others [20,21].

In this study, a quantitative analysis of the research progress and development trend in the non-destructive testing of fruit quality was performed. Studies related to the non-destructive testing of fruit quality published between 1993 and 2022 were identified and analysed using bibliometrics. High-frequency keywords related to the non-destructive testing of fruit quality were analysed using the CiteSpace and Hiplot Pro visual literature analysis tools. The research progress and leading hotspots in the non-destructive testing of fruits were analysed [22].

2. Data Sources and Research Methods

2.1. Data Sources

All data for this study were derived from the core database Web of Science. This database has groundbreaking content, high-quality data, and a long history. It covers the most important and influential studies in the relevant research field [23].

Relevant non-destructive testing technologies for common fruits (e.g., peach, pear, and apple) were employed as the search terms. Specifically, the search terms were TI = ("apple*" OR "pear*" OR "peach*" OR "fruit*") AND TI = ("Nondestructive*" OR "optical*" OR "visible*" OR "infrared*" OR "spectra*" OR "spectroscopy*" OR "vision*" OR "acoustic*" OR "scattering*" OR "mechanical*" OR "vibration*" OR "nuclear magnetic resonance*" OR "NMR*" OR "hardness*" OR "elasticity*" OR "impulse*" OR "dielectric*" OR "microwave drying*" OR "electronic noses*") NOT TS = ("tree*" OR "leaf*" OR "root*" OR "pest*" OR "disease*" OR "fruit fly*" OR " juice*" OR "pearl*" OR "Pearson*" OR " cell*" OR "slice*"). Studies not relevant to the research topic, for example, where "peach" referred to "peach blossom" or "impulse" referred to "a sudden strong and unreflective urge or desire to act", were deleted.

More than 1400 studies published between 1 January 1993 and 31 December 2022 were collected for bibliometric analysis. Subsequent search results may differ slightly due to updating of the database. Quantity changes may have a slight influence on the final research conclusions. Data searching was terminated on 8 May 2023.

2.2. Research Methods

The quantitative analysis of the identified studies was carried out using bibliometrics. The number of relevant studies and the development trends in non-destructive testing technology for fruit quality were analysed using Excel statistics. The statistical analysis of high-publication institutions, high-publication journals, frequently cited studies, and high-frequency keywords was carried out. A keyword clustering map, emergence map, time map, common creation country/region field Chordal graph, and keyword–journal

double mode matrix were plotted using the knowledge mapping visualisation software CiteSpace (CiteSpace. 6.2.R2. March 2023. Chaomei Chen, Professor, Institute of Computer and Information Science, Drexel University, USA, https://citespace.podia.com/download accessed on 27 March 2023) and the visual analysis platform Hiplot Pro (https://hiplot.com.cn/home/index.html accessed on 27 March 2023). The temporal and spatial developments in the non-destructive testing of fruit quality were reviewed and visualised.

3. Descriptive Statistical Analysis of Identified Studies

The distributions of the identified studies in terms of time, space, and source were examined. The development timeline was investigated, and the major research countries/regions and institutions were analysed. The key publications and most influential studies in the field of non-destructive testing of fruit quality were identified.

3.1. Temporal Distribution

The quantity of publications in different years reflects the attention of researchers to the topic of non-destructive testing of fruit quality, to some extent. It also reflects the development process of the field and subjects [24]. The annual quantity of published papers on the non-destructive testing of fruit quality in the top 10 countries, and the publication growth trend, are shown in Figure 1.

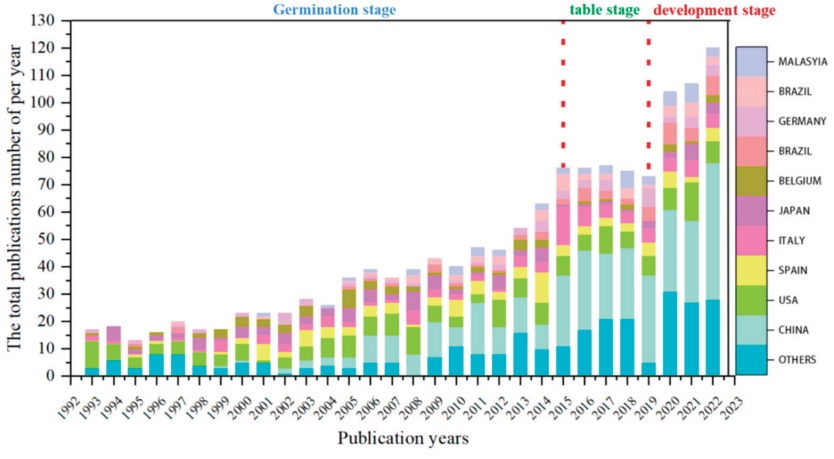

Figure 1. Cumulative histogram of the annual change in the number of publications for the top 10 countries in the field of non-destructive testing technology for fruit quality.

It can be seen from Figure 1 that the number of publications generally exhibited an increasing trend over the study period. The germination stage was evident from 1993 to 2014. At this time, few scholars studied the non-destructive testing of fruit quality, and these scholars were from a limited number of countries. However, there were many high-quality studies. For instance, Peris A et al. (2001) conducted a non-destructive test of the internal quality and optimal harvest period of apples using visible–near-infrared (Vis–NIR) spectral technology [25]. Li et al. (2002), from China Agriculture University, developed a new automatic screening experimental system for apple surface defects based on computer image technology. This system was able to simultaneously test and screen the four side surfaces of each apple online and was found to have relatively high practicability and feasibility [26]. Bart et al. (2007) reviewed the application of new technologies for fruit quality detection, such as spectral resolution spectra, near-infrared multispectral technology, and hyperspectral imaging technology. These technologies are highly relevant to the application of spectral technologies to the non-destructive testing of fruit quality [27].

Over time, the number of relevant papers increased slowly, year by year. An increasing number of scholars published papers in this field.

The stable stage was evident from 2014 to 2019. The number of research papers from China, Malaysia, and Belgium markedly increased, but the total quantity of relevant research papers exhibited little change. Cen et al. (2016) detected cold damage in cucumbers through hyperspectral imaging technology by combining feature selection and a supervised classification algorithm. They demonstrated the potential of hyperspectral imaging technology for cold damage detection in fruits [28]. Arendse et al. (2018) demonstrated that near-infrared spectra are feasible for studying the internal quality of fruits with thick pericarps [29]. Bhargava et al. (2018) carried out a critical comparison of several detection algorithms for fruit and vegetable quality proposed by researchers in recent years [30]. Zhang et al. (2014) described the latest development of a computer vision system for the external quality detection of fruits and vegetables and discussed its applications [31]. This particular study offers important guidance for the application of machine vision technology to the non-destructive testing of fruit quality.

Finally, the research field was observed to be in a fast development stage from 2019 to 2022. The number of publications increased significantly. More than 100 papers were published in 2020. The integrated application of multiple technologies was a prominent characteristic of the published research on non-destructive testing technology for fruit quality during this stage. For example, Kasampalis Dimitrios et al. (2021) evaluated the nutritional quality of pepper using colour, chlorophyll fluorescence, visible–near-infrared (Vis–NIR) spectra, red–green–blue (R–G–B) and red–green–near-infrared (R–G–NIR) digital imaging technology [32]. Tang et al. (2020) reviewed the application and research progress of harvest robots and vision technology in the harvest of fruits and described the fruit recognition and positioning technology based on digital image processing technology and deep learning algorithms. Moreover, they proposed future development trends in machine vision [33]. Wan et al. (2020) proposed a deep learning framework for the detection of different fruits based on an improved Faster R-CNN and evaluated its performance [34]. The development of digital image technology and deep learning technology has injected new vitality into studies on the non-destructive testing of fruit quality. It is expected that the number of publications will significantly increase in future years.

3.2. Spatial Distribution

3.2.1. Countries/Region

To determine the influential countries/regions in the field of non-destructive testing technology for fruit quality, and evaluate their cooperative relations, a chordal graph of countries/regions was plotted with Hiplot Pro. The node size expresses the quantity of studies, and the connected lines between two nodes express the cooperative relations. It can be seen from Figure 2 that more than 70 countries/regions have participated in this research field. Asia, Europe, and North America were the major contributors. This might be related to national policy orientations, economic development levels, agricultural development levels, and the climatic and geographical conditions being appropriate for the local development of the forest and fruit industry [35]. There was relatively frequent cooperation between China and the USA, between the USA and Canada, and between Spain and Iran. The top five countries/regions in terms of the number of publications were China, the USA, Spain, Italy, and Japan, which published 361 (25.79%), 199 (14.21%), 99 (7.07%), 98 (7%), and 91 (6.5%) papers, respectively. China accounted for 25.79% of papers, more than one-quarter of the total studies. This is mainly attributed to China's attention to agricultural development and the formulation and implementation of multiple supporting policies in recent years. In the "14th Five-Year Plan" of promoting agricultural and rural modernisation, China proposed the promotion of "diversified development of fruits, vegetables and tea, developing facility agriculture, and developing unique industries like forest and fruit industry, traditional Chinese medicines and edible mushrooms according to local conditions" [36]. Since 2020, the Ministry of Agriculture and Rural Affairs and the Ministry

of Finance have been constructing dominant and unique industrial clusters, and have included 16 fruit industrial clusters in the construction range [37]. In 2021, the Ministry of Agriculture and Rural Affairs printed the "14th Five-Year Plan" on the Implementation Scheme of "Three Quality Indices and One Standard" in the planting industry, which aims to improve the development quality benefits and competitiveness of industries such as the fruit industry [38]. Scientific research funds for the forest and fruit industry have also continued to increase, bringing annual growth in the contribution of forest and fruit studies to academic development. There is no doubt that other countries have also made considerable contributions to the research progress on the non-destructive testing of fruit quality. It is likely that further developments in the non-destructive testing of fruit quality in the future will rely on collaborative endeavours between multiple countries and regions.

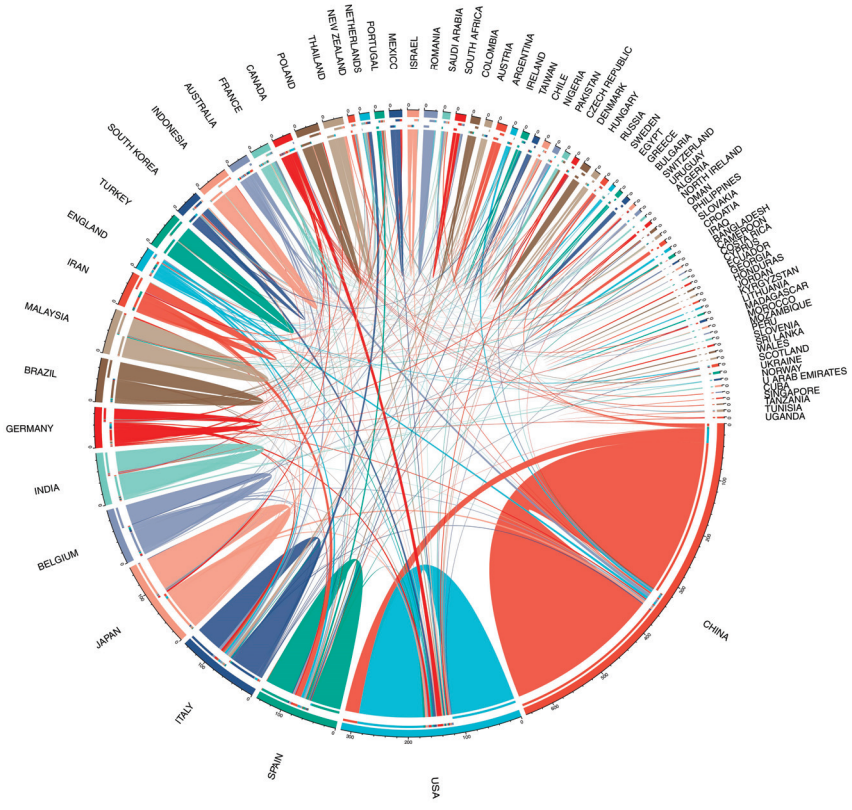

Figure 2. Mapping knowledge domains of co-authoring countries/regions.

In summary, a country's economic level is the basis for the development of non-destructive testing of fruit quality. Compared with economically underdeveloped countries/regions, economically developed ones have published more in-depth studies on the non-destructive testing of fruit quality and have achieved more research progress. Nevertheless, a high economic development level alone cannot bring considerable development to the field. This is because policy orientation determines the continuous economic inputs of the country/region to the field. Hence, development in the non-destructive testing of fruit quality cannot occur without economic support and policy guidance. Moreover, transbackground and transnational cooperation is conducive to the cooperation between different teams, facilitating leaps and diversified development in the field.

3.2.2. Institutions and Units

According to the analysis, more than 300 institutions were involved in studies on the non-destructive testing of fruit quality between 1993 and 2022. These institutions have made considerable contributions to the development of non-destructive testing technologies for fruit quality. The top 10 institutions in terms of the number of publications on the non-destructive testing of fruit quality in the Web of Science are listed in Table 1. Chinese and American institutions accounted for 7 of these 10 institutions, reflecting these countries' attention to and continuous efforts in this field. It is important to note that Zhejiang University achieved the most relevant scientific publications, with 71 papers published during the study period, accounting for 5.07% of the total publications. The United States Department of Agriculture published 63 papers, accounting for 4.5% of the total, ranking second.

Table 1. Top 10 institutions with a high number of publications in the Web of Science.

High-Volume Institutions Top 10	Number	Ratio (%)
Zhejiang University	71	5.07
United States Department of Agriculture	63	4.50
China Agricultural University	36	2.57
Ministry of Agriculture Rural Affairs	36	2.57
Ku Leuven	34	2.43
Consiglio Nazionale Delle Ricerche	32	2.29
Michigan State University	27	1.93
Washington State University	26	1.86
Northwest A&F University, China	24	1.71
National Agriculture and Food Research Organization, Japan	24	1.71

Among the global research institutions, Zhejiang University had the highest publication number in the Web of Science. This implies that the contribution of Zhejiang University to the non-destructive testing of fruit quality in China has been the most significant. This institution has strong scientific research power and an extremely strong influence on the field. The top 10 institutions comprised 6 universities and 4 research institutions. Based on the international scientific and technological development trend, the continued strengthening of scientific research cooperation among universities, research institutions, and enterprises is needed. This will facilitate the transformation of scientific research achievements.

3.3. Source Distribution

3.3.1. High-Publication Journals

Journals are the most important source of academic developments and scientific reports. Hot journals in the field can be identified by analysing the distribution of publications in journals. This is also applicable to the tracking of information in the most recently published studies. Journal distribution analysis is highly relevant to the information acquisition of researchers [39]. The top 10 journals in terms of the number of publications on the non-destructive testing of fruit quality in the Web of Science database are listed in Table 2. All journals published more than 20 papers. Specifically, *Postharvest Biology and Technology*, a journal from the Netherlands, achieved the top rank, with 85 papers (6%); this publication number is far higher than those of the other journals. Thus, this journal is the most influential journal in the field.

The above journals encompass several research fields, such as agriculture, food, optics, computer science, biology, gardening, etc. This means that the non-destructive testing of fruit quality is a multidisciplinary research field, and its development requires strengthened cooperation, exploration, and common progress across multiple disciplines.

Table 2. Top 10 high-publication journals in the Web of Science.

High-Published Journals Top 10	Number	Ratio (%)
Postharvest Biology and Technology	85	6.07
Acta Horticulturae	55	3.93
Horticulturae	46	3.29
Journal of Food Engineering	42	3.00
Computers and Electronics in Agriculture	34	2.43
Spectroscopy and Spectral Analysis	32	2.29
Transactions of the Asae	29	2.07
Journal of Agricultural and Food Chemistry	26	1.86
Food Chemistry	23	1.64
Biosystems Engineering	22	1.57

3.3.2. High-Citation Studies

The frequency of citation is an important evaluation index that provides an objective measure of the research influence, citation value, and degree of attention a paper has received in the field. A higher frequency of citations indicates higher attention to the paper and greater influence of the paper within scientific communication. High-citation studies are often those that focus on hotspot themes in the field, providing a guide on the academic frontier of the field. Analysing the high-citation studies on the non-destructive testing of fruit quality can provide researchers with a reference for the future research direction of the field. The top 10 high-citation studies on the non-destructive testing of fruit quality are listed in Table 3; all papers were cited more than 240 times.

Table 3. Top 10 high-citation studies in the Web of Science.

Top 10	Document	Frequency
1	DeepFruits: A Fruit Detection System Using Deep Neural Networks	482
2	Apple Detection during Different Growth Stages in Orchards Using the Improved YOLO-V3 Model	372
3	Advances in Machine Vision Applications for Automatic Inspection and Quality Evaluation of Fruits and Vegetables	329
4	NIR Spectroscopy Applications for Internal and External Quality Analysis of Citrus Fruit—A Review	300
5	Principles and Applications of Hyperspectral Imaging in Quality Evaluation of Agro-Food Products: A Review	284
6	Reflectance Spectral Features and Non-Destructive Estimation of Chlorophyll, Carotenoid and Anthocyanin Content in Apple Fruit	264
7	Measurement of the Optical Properties of Fruits and Vegetables Using Spatially Resolved Hyperspectral Diffuse Reflectance Imaging Technique	257
8	Non-destructive Measurement of Acidity, Soluble Solids, and Firmness of Jonagold Apples Using NIR-Spectroscopy	243
9	Principles, Developments and Applications of Computer Vision for External Quality Inspection of Fruits and Vegetables: A Review	240
10	A New Index Based on Vis Spectroscopy To Characterize the Progression of Ripening in Peach Fruit	240

In the Web of Science, "DeepFruits: A Fruit Detection System Using Deep Neural Networks", a study published by Sa, Inkyu et al. in 2016, was cited the most. This study proposed an accurate, fast, and reliable real-time fruit detection method based on deep convolutional neural networks [40]. According to the research results, the proposed detection method not only improves detection accuracy but can also be deployed to new

fruit detection systems quickly. It began to attract significant attention after 2019, and the annual number of citations exceeded 100. The second most cited paper, "Apple Detection During Different Growth Stages in Orchards Using the Improved YOLO-V3 Model", was published by Tian et al. in 2019. In this study, an improved YOLO-V3 model was proposed for fruit quality detection under complicated conditions, such as illumination fluctuations, disordered backgrounds, overlapping apples, and different maturity stages [41]. This study also attracted extensive attention after 2019. This indicates that neural network technology became popular and began to attract significant attention in the field of non-destructive testing of fruit quality around 2019. Furthermore, "Advances in Machine Vision Applications for Automatic Inspection and Quality Evaluation of Fruits and Vegetables", a study published by Sergio Cubero in 2010, reviewed the development trend in machine vision technology for the internal and external quality inspection of fruits and vegetables [42]. This study has a high guiding role and reference value for the future study of non-destructive testing technologies for fruit quality.

These 10 studies have had significant influences on this research field. Their research directions and methods are representative. It is important to note that 5 of these 10 studies were published in the top 10 journals, and 3 were published in *Postharvest Biology and Technology*. This indicates that journals with a high number of publications on non-destructive testing technology for fruit quality are associated with high-citation publications, to some extent. This also verifies that *Postharvest Biology and Technology* is the most influential journal in the field. Moreover, the top 10 high-citation research hotspots in the field of non-destructive testing of fruit quality included spectral detection technology, machine vision technology, and neural networks. Common research indices included physical properties (e.g., the degree of maturity and hardness) and internal fruit quality (e.g., soluble solid content).

4. Research Progress and Leading Hotspots

Keywords in academic papers are tags indicating the research core and subject information. They provide a high-level summary of the major contents of a paper and can be used to identify the content characteristics and academic research direction of a paper. Here, clustering analysis and emergency analysis of the keywords, terms, and subject terms of studies on non-destructive testing technology for fruit quality were carried out using CiteSpace. Based on this analysis, the overall research progress in the field of non-destructive testing of fruit quality was analysed, the current research hotspots were identified, and the future development trend was predicted.

4.1. Statistical Analysis of Keywords

A statistical analysis of the frequency of keywords in high-publication journals was carried out. A high-frequency keyword–journal clustering tree was plotted by combining the high-frequency keywords with the high-publication journals (Figure 3). Furthermore, a hierarchical clustering analysis of high-frequency keywords and high-frequency journals was carried out. The hierarchical clustering analysis based on the bimodule matrix effectively improves the single-dimensional analysis of the traditional system clustering algorithm, achieving simultaneous clustering of topic keywords and journals [43]. The vertical clustering tree reveals the clustering results of high-frequency keywords, with 15 high-frequency keywords listed at the bottom of the graph. The horizontal clustering tree illustrates the clustering results of high-publication journals, with 12 high-publication journals listed on the right of the graph. Each box represents the high-frequency keyword–journal corresponding to the column and row. The colour depth reflects the frequency of co-occurrence in publications. The clustering results were interpreted, and hotspot research directions and popular journal groups in relation to the non-destructive testing of fruit quality were summarised.

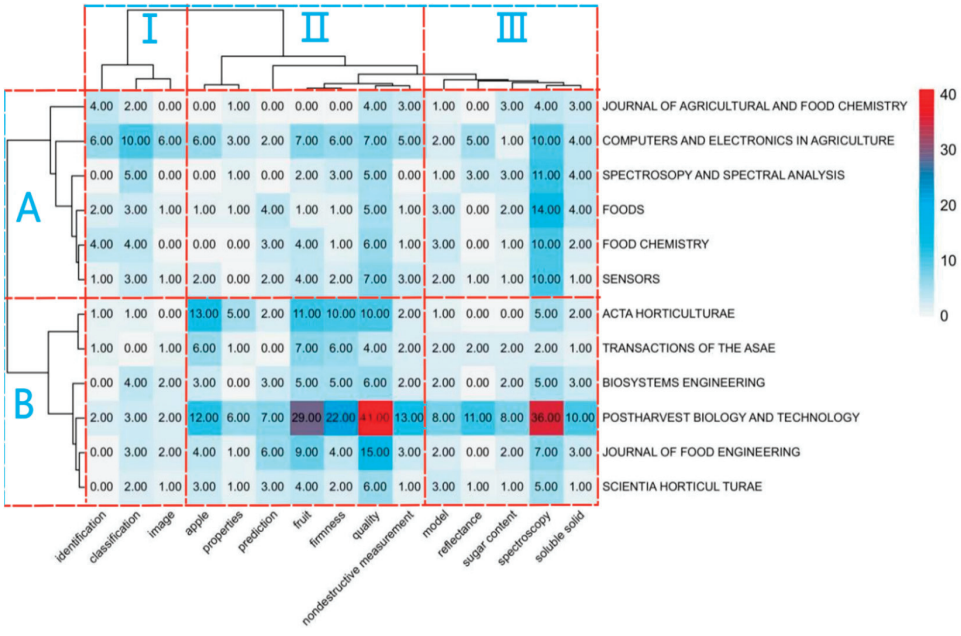

Figure 3. Knowledge domain mapping of hierarchical clustering trees based on keywords and bimodule matrix of journals.

Keywords that occurred frequently included apple, fruit, classification, quality, properties, prediction, and non-destructive measurement. This agrees well with the research topic of this study. Keywords like image, identification, spectroscopy, reflectance, and model also occurred relatively frequently. This demonstrates that these are currently popular research methods in the field of non-destructive testing of fruit quality. Moreover, words like firmness, sugar content, and soluble solids also occurred frequently, indicating that these are quality indices of interest in the field of non-destructive testing of fruit quality.

The research hotspots in this field can be divided into three main categories: (I) image recognition technology (image, classification, and identification); (II) general research objects and contents, including fruit, apple, non-destructive measurement, quality, properties, prediction, and firmness; and (III) traditional research methods and detection indices, including model, reflectance, spectroscopy, sugar content, and soluble solids. The journals can be divided into two major types according to the disciplines: (A) agriculture, food, and electronics; and (B) biology and gardening.

4.2. Clustering Maps of Keywords

A range of research topics were observed in the literature regarding the non-destructive testing of fruit quality. In this study, a clustering analysis of high-frequency keywords in relevant papers was carried out to identify the key research problems.

Clustering analysis of keywords is performed to investigate the contributions of keywords in relevant papers and the close relations among keywords. It is used to identify the core contents and structure of a specific field. The clustering analysis of keywords in relevant studies was carried out using CiteSpace. Nine colour-distinguished clustering maps were generated, as shown in Figure 4. The font size of the tags of the nodes represents the frequency. The quantity and width of the connecting lines represent the co-occurrence intensity among keywords. A smaller clustering number indicates that more keywords are included, and content closer to the centre of the cluster is more important.

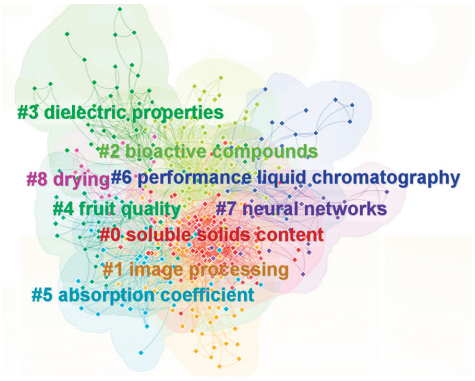

Figure 4. Clustering maps of keywords.

Clustering tags were divided into two types according to meaning: research object (#0 soluble solid content; #2 bioactive compounds; #3 dielectric properties; #4 fruit quality; and #5 absorption coefficient) and research method (#1 image processing; #6 performance liquid chromatography; #7 neural networks; and #8 drying).

4.3. Emergency Analysis of Keywords

An emergency word is one where the value of the keyword increases sharply into a hotspot over a short period. The emergency intensity of a keyword is proportional to the activity of the represented topic in the corresponding period. Here, an emergency analysis of keywords in relevant studies was carried out using CiteSpace. The results are shown in Figure 5. Deep learning, soluble solids, sugar content, internal quality, phenolic compounds, machine vision, and system were identified as emergency keywords with widespread interest and significant influence in this academic field. Moreover, the activity of the keywords corresponds with the growth trend of publications in Figure 1.

Keywords	Year	Strength	Begin	End	1993 - 2022
apples	1993	4.5	1993	2008	
sugar content	1998	4.88	1998	2014	
machine vision	1998	4.1	1998	2008	
soluble solids	1999	5.27	1999	2008	
nondestructive determination	2002	4.67	2002	2014	
fruits	2001	4.64	2001	2006	
firmness	2001	4.58	2001	2010	
tissue	2001	4.05	2001	2010	
internal quality	2005	4.51	2005	2012	
physical properties	2007	3.93	2007	2014	
phenolic compounds	2011	5.1	2011	2014	
image analysis	2011	3.57	2011	2014	
storage	2007	3.43	2011	2014	
system	2007	4.73	2015	2016	
wavelength range	2015	3.65	2015	2016	
moisture content	2010	3.62	2015	2020	
models	2007	3.49	2017	2020	
deep learning	2020	6.63	2020	2022	
parameters	2019	3.71	2019	2022	
scattering property	2019	3.31	2019	2022	

Figure 5. Evolution of emergency hotspots of keywords.

4.4. Temporal Analysis of Keywords

To further clearly and intuitively examine the development history of research on the non-destructive testing of fruit quality, a temporal map of keywords was plotted using

CiteSpace (Figure 6). The temporal map depicts keywords included in the clustering according to time. Each box in Figure 6 represents a keyword and the year of its first occurrence in the dataset. The box size represents the frequency of occurrence of the keyword, and the lines among keywords represent their connections. The development veins within the field of non-destructive testing technology for fruit quality were analysed. In combination with the number of publications and occurrence of keywords, the veins and leading points were further studied according to the time nodes and keywords.

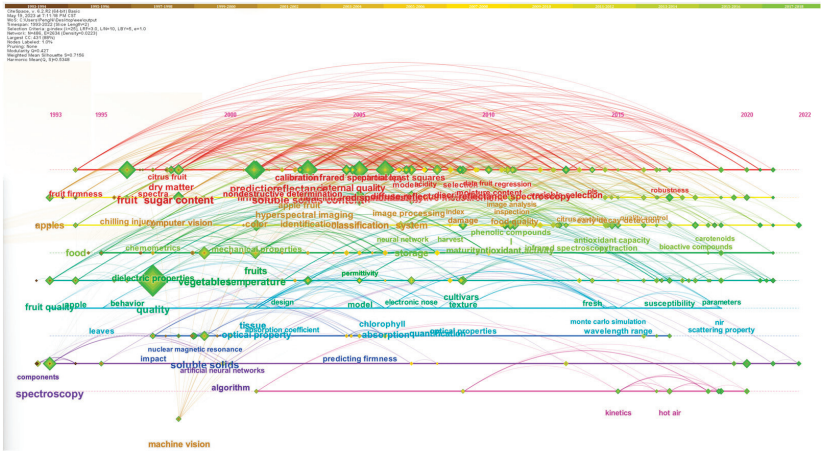

Figure 6. Temporal map of keywords.

The hotspot evolution of emergency keywords in Figure 5 and the growth trend of publications in Figure 1 were combined. The co-occurrence map of keywords was divided into three stages according to the timeline: the germination stage (1993–2014), the stable stage (2014–2019), and the development stage (since 2019). In the germination stage, texture analysis [44], sugar refractometry [45], analysis methods using optical instruments [46], and other traditional methods were used to test the physical properties (e.g., hardness) [47] and internal quality (e.g., sugar degree [48], soluble solids [49], and phenolic compound content [50]) of fruits. It is important to note that machine vision technology [51] began to be used for the inspection of the external quality of fruits in 1998. In the stable stage, most studies applied e-nose [52], diffuse reflection [53], and near-infrared spectra [54] technologies. Machine vision technology [55] was further developed. In the development stage, neural networks and deep learning technologies were developed and integrated with traditional technologies like machine vision and spectra [56–58]. This injected new vitality into the field of non-destructive testing technologies for fruit quality.

5. Conclusions

Journal papers on the non-destructive testing of fruit quality published in the Web of Science database between 1993 and 2022 were identified in this study. Using bibliometrics, the quantitative distribution and sources of the relevant studies were analysed. The research contents, content progress, leading hotspots, and development trends in the field of non-destructive testing of fruit quality were summarised based on knowledge maps. Moreover, the research dynamics in the field were investigated. The major conclusions are outlined below:

(1) Studies on the non-destructive testing of fruit quality were in the germination stage from 1993 to 2014. There were few published papers from a limited number of countries in 1993. Over time, the number of related papers gradually increased, year by year. More countries began to investigate and publish studies on the non-destructive testing of fruit quality. The field was in the stable stage from 2014 to

2019. During this time, there was continuous publication of relevant research papers. Finally, there was sharp growth in the number of studies from 2019 to 2022. Significant growth in the field is expected over the next few years.

(2) Research on non-destructive testing technology for fruit quality has mainly concentrated in Asia, North America, and Europe. China and the USA are the most active countries in this field. Furthermore, there has been close cooperation between China and the USA, the USA and Canada, and Spain and Iran. Continuous capital funding and policy support from these countries in addition to good transnational cooperation will further facilitate the diversified development of non-destructive testing technology for fruit quality.

(3) Major research institutions that have published in this field include Zhejiang University, the United States Department of Agriculture, China Agricultural University, the Ministry of Agriculture Rural Affairs, and so on. Zhejiang University has made a remarkable contribution and has a significant influence on the field.

(4) Relevant studies in the field have mainly been published in *Postharvest Biology and Technology*, *Acta Horticulture*, *Horticulturae*, *Computers and Electronics in Agriculture*, *Spectroscopy and Spectral Analysis*, and so on. They have mainly been focused on agriculture, food, electronics, biology, gardening, etc.

(5) The primary evaluation indices in the published studies include internal quality (e.g., sugar degree and soluble solids) and physical properties (e.g., hardness). The research methods mainly include e-nose technology, machine vision technology, and spectral detection technology (including hyperspectra and visible/near-infrared spectra), etc. Recently, neural networks and deep learning have undergone significant development. They have been combined with spectral technology and machine vision technology. As a result, non-destructive testing technology for fruit quality has entered a new development stage.

This paper provides an overview of the data recorded in the Web of Science on non-destructive testing of fruit quality from 1993 to 2022 for a total of 30 years, mainly relying on the pioneering content of the Web of Science, high-quality data, long history, and authority, covering the most important and influential research in related research fields [59]. However, a variety of disciplines may also be included in other databases, resulting in data omissions [60]. Therefore, even if the use of multiple databases will bring problems such as overlapping samples, it should also be considered so as to expand the research scope, which is an aspect that should be considered in future bibliometric research [61].

There is currently significant research and publication activity in the field of non-destructive testing technology for fruit quality. There remains a good development trend. Studies on the application of non-destructive testing technology for fruit quality to agricultural production and agricultural product processing are becoming increasingly mature. In particular, non-destructive testing technology for fruit quality has promising application prospects in agricultural product processing, and its application is booming. Moreover, technological developments, such as sensors, the Internet of Things (IoT), big data, machine vision, artificial intelligence, and deep learning will promote the maturity and application of non-destructive testing technology for fruit quality. This technology can also be applied to the whole process of fruit production, transportation, and marketing, meeting the market needs for fruit quality detection.

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Review

Physiology and Application of Gibberellins in Postharvest Horticultural Crops

Jingyu Zhang [1,†], Yuhao Cao [1,†], Jia Tang [1], Xujie He [1], Ming Li [2], Chen Li [3], Xiaolin Ren [1,*] and Yuduan Ding [1,*]

[1] College of Horticulture, Northwest A & F University, Yangling 712100, China; zhangjingyu@nwafu.edu.cn (J.Z.); caoyvhao@nwafu.edu.cn (Y.C.); 2021055234@nwafu.edu.cn (J.T.); hexujie@nwafu.edu.cn (X.H.)
[2] College of Politics and Public Administration, Huaqiao University, Quanzhou 362000, China; liming106@126.com
[3] College of Bioengineering, Yangling Vocational and Technical College, Yangling 712100, China; lc17792903032@163.com
* Correspondence: tjw689@126.com (X.R.); dingyuduan@nwafu.edu.cn (Y.D.)
† These authors contributed equally to this work.

Abstract: Gibberellins (GAs) are plant hormones indispensable in regulating the growth and development of fruits. Recent studies have shown that GAs play important roles in delaying horticultural crop ripening and senescence, enhancing the internal and external quality of horticultural crops and resistance to stress and disease. We reviewed the role of GAs in the postharvest physiology of fruits in recent years. GAs are closely related to their ability to retard fruit senescence. GAs could effectively improve fruit storage quality and significantly increase flesh hardness, reduce respiration intensity, inhibit the release of endogenous ethylene, and effectively inhibit fruit softening and ripening. It can also improve the intrinsic and extrinsic quality of fruit storage by improving fruit shape, regulating color, delaying the reduction of soluble solids, promoting sugar accumulation, and delaying vitamin loss. GAs also play a role in postharvest biotic and abiotic stress resistance. The GA treatment effectively reduces the cold damage index, reduces the production and accumulation of superoxide anion(O_2^-), improves the antioxidant capacity of fruits, and maintains the integrity of cell membranes during low-temperature storage. Moreover, GAs could effectively control some postharvest fruit diseases. In conclusion, GAs play an important role in the physiological regulation of postharvest fruits and have important application prospects in postharvest fruits.

Keywords: gibberellins; postharvest; physiology; ripening

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1. Introduction

Bioactive gibberellin (GA) is one kind of phytohormone that regulates plant growth and development. GAs are involved in plant reproductive development, promoting organ growth (enhancing cell elongation and cell division), and activating developmental processes such as seed germination, maturation, and induction of flowering [1]. In recent years, the regulation mechanism of GAs and the application of exogenous plant hormones in the postharvest storage quality of horticultural crops has been studied. GAs were first found in the metabolites of the fungus *Gibberella fujikuroi* and later reclassified as *Fusarium fujikuroi*. GA was discovered as a natural plant hormone in the late 1950s that promoted growth in higherplants [2]. GAs are in a class of phytohormones with kaurene as their backbone. GAs contain a large group of diterpenoid carboxylic acids, which are classified based on their structure.

The biologically active GAs mainly include GA_1, GA_3, GA_4, and GA_7, which play direct or relative roles in plants. Among them, GA_1 is the most widespread and is present in most plants [3], while GA_4 is mainly found in *Arabidopsis thaliana* and some cucurbits. As for the relative roles of GA_1 and GA_4 or GA_3 and GA_7, further studies are needed to

clarify them. At the same time, other types of GAs that are not biologically active in plants can often be converted into biologically active GAs by certain means. For example, GA_{12} is converted to GA_4 by GA 20-oxidase (GA20ox) and GA3ox, and it can also be converted to intermediate GA_{53} by GA13ox and then to biologically active GA_1, GA_3, and GA_6 by different pathways in the presence of GA20ox and GA3ox [4].

The current understanding of the physiological roles of gibberellins in postharvest horticulture crops was outlined and discussed. The main content is as follows: (1) GAs are closely related to their ability to retard fruit senescence. GA treatment could improve fruit storage quality and significantly increase flesh hardness, reduce respiration intensity, inhibit the release of endogenous ethylene, and effectively inhibit fruit softening and ripening. (2) They can also improve the intrinsic and extrinsic quality of fruit storage by improving fruit shape, regulating color, delaying the reduction of soluble solids, promoting sugar accumulation, and delaying vitamin C loss. (3) GAs improve fruit chilling resistance through effective control of postharvest chilling damage. The treatment of GA_S effectively reduces the cold damage index, reducing the production and accumulation of superoxide anion (O_2^-), improving the antioxidant capacity of fruits, and maintaining the integrity of cell membranes during low-temperature storage. (4) GAs also a play role in postharvest disease control. Overall, GAs, as an important class of plant hormone, play an essential role in the postharvest physiological regulation of fruits. Their application in postharvest could provide valid biotechnology for extending the storage period of fruits.

2. Gibberellins for Regulating the Ripening and Senescence Process

Gibberellins could influence the ripening of fruit by affecting their size, color, flavor, and nutritional value (Table 1). Ripening is generally accomplished when fruits' internal and external components reach optimal states. It has been found that GA_3 affected the size, metabolic contents, and color of fruits during tomato softening [5,6]. Meanwhile, exogenous GA treatment could improve the nutritional traits of bulbs in most garlic cultivars, and it regulates starch synthesis and degradation by affecting related enzyme activities, promoting sucrose accumulation and inhibiting starch regeneration to accumulate soluble sugars in potato tubers [7,8]. Moreover, it has been found that applying exogenous GA_3 and paclobutrazol (PAC, an inhibiter of gibberellin biosynthesis) could prolong and shorten fruit ripening time, respectively [9]. Those results suggested that GAs may exert a delayed influence on fruit ripening.

Table 1. Effect of GAs for regulating the ripening and senescence of horticultural crops.

Horticultural Varieties	Main Points	Concentration	References
Cabbage (*Brassica rapa* var. *glabra* Regel)	GA_3 spraying retarded fruit senescence.	100 μM GA_3	Fan et al., 2021 [10]
Garlic (*Allium sativum* L.)	GA_3 injection improves the nutritional traits of fruits.	1 mM GA_3	Liu et al., 2019 [7]
Potato (*Solanum tuberosum* L.)	GA spraying accumulates tuber sugar nutrients.	0.015 g L^{-1} GA	Xie et al., 2018 [8]
Tomato (*Solanum lycopersicum* L.)	GA_3 spraying affects the size of fruits.	50 μM GA_3	Zhu et al.,2019 [5]
Tomato (*Solanum lycopersicum* L.)	GA_3 injection delays color change in fruit at ripening stage.	0.1 mM GA_3	Li et al., 2019 [7]
Toona *(Toona sinensis* (A. Juss.) Roem.)	GA_3 soaking retards fruit senescence.	100 mg L^{-1} GA_3	Zhao et al., 2018 [11]

Regarding the molecular mechanisms of exogenous gibberellins in fruit ripening, studies have identified the effects of GAs on ripening characteristics and the expression of ethylene biosynthesis and signaling genes in tomato fruit (Figure 1). It was found that the exogenous GA treatment could effectively increase the expression level of gibberellin-stimulated transcript 1 (*GAST1*), slow down (or inhibit) the decrease of endogenous GA

concentration, inhibit the expression of fruit ripening regulators *RIPENING INHIBITOR (RIN)*, *NON-RIPENING (NOR)*, and *COLORLESS NON-RIPENING (CNR)*, attenuate the expression levels of crucial genes in ethylene synthesis (*ACS2*, *ACS4*, and *ACO1*), and also reduce the expression of key ethylene receptor genes *EYR3* (NR) and *ETR4*. The altered expression of these maturation regulators also suppressed the expression of the major ethylene signaling gene *EIN2* to a certain extent [6]. GAs were demonstrated to play a negative role in tomato fruit ripening by regulating the ethylene-related pathways.

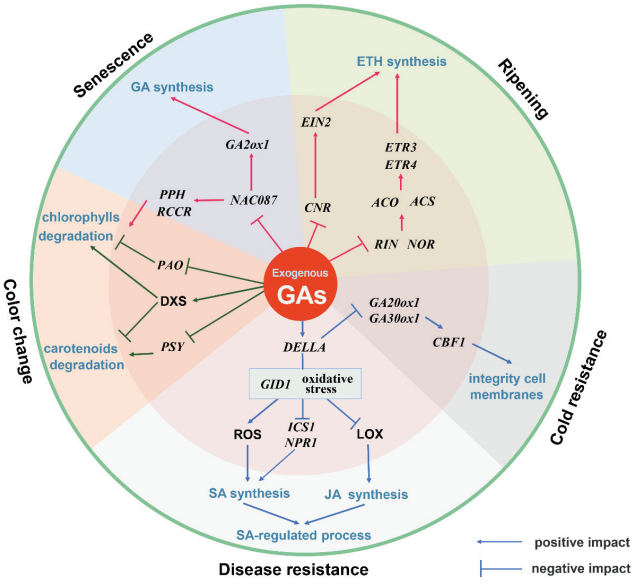

Figure 1. Molecular mechanisms of gibberellins related to regulating postharvest physiology. (1) GAs regulated the ripening process (modeling based on tomato): GAs affect the expression of *EYR3*, *ETR4*, and *EIN2* by regulating *RIN*, *NOR*, and *CNR*. (2) GAs regulated the senescence process (modeling based on cabbage): GAs affect the expression levels of *PPH*, *RCCR*, and *GA2ox1* by regulating the expression of *BrNAC087*. (3) GAs regulated the color change of fruit (modeling based on citrus): the use of GAs can regulate DXS activity and gene expression of *PAO* and *PSY*. (4) GAs regulated the cold resistance of fruit (modeling based on tomato): GAs regulate *GA20ox1* and *GA3ox1* expression by affecting the DELLA gene expression, which in turn incrementally affects *CFB1* expression. (5) GAs regulated the disease resistance of fruit (modeling based on tomato): GAs can affect DELLA gene expression through both binding to GID1 and induced oxidative stress, which in turn regulates the levels of ROS, LOX, and *ICS1*, *NPR1* to affect SA and JA biosynthesis and thus the SAR process. Abbreviations: *ACO* (*1-aminocyclopropane-1-carboxylic acid oxidase*), *ACS* (*1-aminocyclopropane-1-carboxylic acid synthase*), *CBF1* (C-repeat binding transcription factor 1), *CNR* (*COLORLESS NON-RIPENING*), DXS (1-deoxy-D-xylulose 5-phosphate synthase), *EIN2* (*ETHYLENE INSENSITIVE 2*), ETH(ethylene content), *ETR* (*ETHYLENE RESPONSE*), GA (gibberellin), *GID1* (*GIBBERELLIN INSENSITIVE DWARF 1*), *ICS1* (isochorismate synthase 1), JA (jasmonate), LOX (lysyl oxidase), *NOR* (*NON-RIPENING*), *NPR1* (nonexpressor of *pathogenesis-related* genes 1), *PAO* (*pheophorbidea oxygenase*), *PPH* (*pheophytinase*), *PSY* (*phytoene synthase*), *RIN* (*RIPENING INHIBITOR*), SA (salicylic acid), *RCCR* (*red chlorophyll catabolite reductase*), ROS (reactive oxygen species).

Gibberellins had important roles in regulating fruit senescence. Aging fruit refers to the gradual loss of water, flavor, and nutrition; it also reduces enzyme activity, causes cell aging, and occurs with decay and spoilage. The physiological indicators of fruit aging include enzyme activities of superoxide dismutase (SOD), the function of scavenging reactive oxygen species and is a protective enzyme for plant cells [12], peroxidase (POD), and

catalase (CAT), the function of decomposing H_2O_2 [13], polyphenol oxidase (PPO), trigger fruit browning phenolic substances [14], and malonaldehyde (MDA), and are considered to measure the rate of lipid peroxidation in the cell membrane and be an indicator of the amount of senescence and stress [15]. It was found that GA_3 treatment could significantly reduce the accumulation of MDA and H_2O_2, enhance the activity of CAT and SOD, and reduce the activity of POD and PPO in the shoots of *Toona sinensis*, which showed that GA treatment could effectively delay the aging process of Toona [11].

Molecular mechanisms related to gibberellin delaying plant aging have also been reported. A study identified *BrNAC087* (a homolog of *ANAC087*, senescence-associated NAC transcription factor) as an important regulator of postharvest Chinese flowering cabbage leaf senescence (Figure 1). It was demonstrated that *BrNAC087* acted as a direct activator of two chlorophyll catabolic genes (*BrPPH* and *BrRCCR)* and one GAs inactivation gene *BrGA2ox1*, by specifically binding to the promoters of *BrPPH*, *BrRCCR*, and *BrGA2ox1*. Exogenous GA_3 treatment could effectively inhibit the expression of *BrNAC087* and reduce the expression levels of *BrPPH*, *BrRCCR*, and *BrGA2ox1*, thus reducing chlorophyll degradation and GAs catabolism and achieving the purpose of delaying leaf senescence [10].

3. Gibberellins for Exterior Quality Control of Horticultural Crops

Gibberellins could influence fruit appearance by regulating cell expansion and enlargement, enhancing fruit stalk length, controlling fruit shape, adjusting pigment content, and modulating fruit color (Table 2). It has been shown that the fruit shape index (the ratio of the longitudinal diameter to the transverse diameter of the fruit and a quality indicator for commercial fruits) was influenced by GAs [16]. Cruz-Castillo et al. showed that GA_3 supplied to the pedicel of 'Hayward' kiwifruit increased the length of the terminal pedicel and affected the fruit shape index [17]. Liu et al. found that GA_3 treatment could significantly increase apple fruit stalk length and improve fruit shape [18]. Another study on 'Chandler' strawberry showed similar experimental results, which found that GA_3 and cytokinin (substances with cell division activity) could promote cell elongation and thus achieve an elongation of fruit stalk length to improve the fruit shape index [19]. It has been found that GA_3 treatment in tomato fruit can promote cell expansion and enlargement, thus improving the shape index [9]. GA_3 spraying on self-pollinated apple plants increased fruit weight, reduced the proportion of asymmetric fruits, and restored the shape and quality of external fruits to the level of cross-pollinated fruits [18]. It is worth noting that, at the molecular level, GA-related genes could regulate fruit exterior shape during fruit development. It was claimed that, in cucumber, the expression pattern of the GA receptor gene *CsGID1a* was closely related to the formation of fruit form. Silencing the *CsGID1a* gene resulted in fruits exhibiting an abnormal carpel and ovary phenotype, while overexpression of *CsGID1a* in the Arabidopsis double mutant (*gid1a* and *gid1c*) exhibited 'cucumber ovary-like' fruits [20].

Table 2. Effect of GAs on regulating the exterior quality of horticultural crops.

Horticultural Varieties	Main Points	Concentration of GAs	References
Apple (*Malus pumila* Mill.)	GA_3 spraying increases fruit stem length and improves fruit shape index.	100 mg L^{-1} GA_3	Liu et al., 2022 [18]
Apple (*Malus pumila* Mill.)	GA_3 spraying increases fruit weight and improves fruit shape index.	100 mg L^{-1} GA_3	Liu et al., 2022 [18]
Broccoli (*Brassica capitata* var. *italica*)	GA_3 soaking retards the degradation of chlorophyll in fruit peel and slows down the color change of fruit skin.	10 mg L^{-1} GA_3	Wang et al., 2023 [21]
Citrus (*Citrus reticulata* Blanco)	ProCa spraying reduces chlorophyll and increases carotenoid concentrations in fruits.	400 mg·L^{-1} ProCa	Barry et al., 2010 [22]
Citrus (*Citrus reticulata* Blanco)	PBZ spraying affects fruit color.	1500 mg L^{-1} PBZ	Rehman et al., 2018 [23]

Table 2. *Cont.*

Horticultural Varieties	Main Points	Concentration of GAs	References
Citrus (*Citrus reticulata* Blanco)	GA_3 spraying affects fruit color by influencing enzyme activity.	60 mg L^{-1} GA_3	Alos et al., 2006 [24]
Cucumber (*Cucumis sativus* Linn.)	GA_3 spraying regulates fruit shape.	50 μM GA_3	Liu et al., 2016 [20]
Grape (*Vitis vinifera* L.)	GA_3 spraying reduces flavanol and anthocyanin content and slows down the color change of fruit skin.	20 ppm GA_3	Tyagi et al., 2022 [25]
'Hayward' kiwifruit (*Actinidia chinensis* Planch.)	GA_3 soaking increases fruit stem length and improves fruit shape index.	100 mg L^{-1} GA_3	Cruz-Castillo et al., 2006 [17]
Lychee (*Litchi chinensis* Sonn.)	GA_3 soaking retards the degradation of anthocyanins in fruit peel and slows down the color change of fruit skin.	0.05 g L^{-1} GA_3	Qu et al., 2021 [26]
'Valencia' orange (*Citrus sinensis* (Linn.) Osbeck)	GA_3 spraying retards the degradation of chlorophyll in fruit peel and reduces carotenoid content.	500 μM GA_3	Keawmanee et al., 2022 [27]
'Chandler' strawberry (*Fragaria chiloensis* (L.) Duchesne)	GA_3 spraying increases fruit stem length and improves fruit shape index.	75 ppm GA_3	Sharma et al., 2009 [19]
Tomato (*Solanum lycopersicum* L.)	GA_3 spraying improves fruit shape index.	0.1 mM GA_3	Chen et al., 2020 [9]

Gibberellins could modulate fruit color changes by influencing the content of pigments such as chlorophylls, carotenoids, and anthocyanins. It was also found that GA_3 treatment on broccoli could control chlorophyll metabolism and delay yellowing [21]. GA_3 treatment on 'Valencia' orange induced chlorophyll accumulation and reduced carotenoid contents (β-astaxanthin, all-trans-violaxanthin, and 9-cis-violaxanthin) [27]. It has also been shown that GA_3 treatment could inhibit the degradation of anthocyanins to suppress lychee browning [26]. The treatment of GA_3 on grapes reduced the flavonol and anthocyanin content and also slowed down the color change of the skin [25]. In addition, Prohexadion-calcium (ProCa, an inhibitor of GA biosynthesis with growth retarding activity) was shown to reduce chlorophyll and increase carotenoid concentrations in the yellow matter of citrus fruits [22]. Similarly, GA biosynthesis inhibitor polycarbazole (PBZ) was found to exert a similar effect in citrus fruit color conversion [23].

It was further suggested that gibberellins successfully affect the accumulation of various pigments in the fruit by regulating the activities of various enzymes in the fruit. Exogenous GA_3 delayed strawberry fruit color change by inhibiting the increase in phenylalanine ammonia-lyase (PAL) activity and delaying the decrease in chlorophyllase and peroxidase (enzymes that may be involved in chlorophyll metabolism) activity (Figure 1). Similar results have been found in studies on the mechanism of color break regulation in citrus fruits, where the use of exogenous GAs delayed the reduction of 1-deoxy-D-xylulose 5-phosphate synthase (DXS) activity, while the increasing gene expression of *pheophorbidea oxygenase* (*PAO*) and *phytoene synthase* (*PSY*) increased the content of their corresponding transcripts, and delayed the consumption of pericarp chlorophyll and the accumulation of total carotenoids and retarded fruit color change [24].

4. Gibberellins for Improving the Internal Quality of Horticultural Crops

Gibberellins could influence taste and texture quality, such as fruit hardness, water loss, soluble solids, acids, and nutrient contents (Table 3). It was demonstrated that the GA_3 treatment of kiwifruit could effectively delay the decrease of fruit hardness, maintain the total acid content, and accumulation of soluble solids [28]. Moreover, several studies have also shown that GA_3 treatment effectively delays postharvest fruit weight loss and decay of the 'Angelino' plum [29] and Japanese plum varieties of 'Obilnaja' and 'BlackStar' [30]. Meanwhile, Ozturk et al. also found that the application of GA_3 with $CaCl_2$ significantly

delayed the weight loss of sweet cherry [31]. These findings further support the fact that GAs are effective in delaying the aging of fruits. GA_3 treatment of plum fruit increased the soluble solids content and reduced the titratable acidity at harvest, which could effectively maintain the fruit quality during storage [32]. Zang et al. found that the soluble solids content increased in GA_3-treated 'rabbit eye' blueberry, thus improving the fresh eating quality of 'rabbit eye' blueberry [33]. A similar phenomenon in sweet cherry cultivars was that GA_3 treatment significantly increased acidity levels [34]. Meanwhile, it was also found that treating the Japanese plum with GA_3 effectively controlled the degradation of soluble solids and titratable acids [35]. Similar results have been found in apples and mangoes [16]. In addition, the water content in fruits treated with GAs has been varied, and Forchlorfenuron (CPPU) and GA_3 have been reported to significantly reduce water loss in banana and broccoli [36].

Gibberellins could also influence the nutrient contents of vitamins, phenols, and soluble proteins. 'Barhee' dates were sprayed with different rates of GAs which effectively controlled the degradation of the vitamin C (Vc) content in the fruit [37]. Li et al. dipped the fruits of plum varieties into GA_3 solution after harvest and found that the application of GA_3 to plums significantly delayed the decrease in ascorbic acid concentration and the increase in total phenolic content [38]. Meanwhile, studies on fruits such as 'rabbiteye' blueberry, Japanese plum, and 'nanguo' pear fruit have shown that GA_3 treatment can delay the reduction of ascorbic acid concentration and total phenolic content in the fruits, maintaining the postharvest fruit quality [33,35,39]. Besides, the results of some studies showed that the spraying of different concentrations of GA_3 significantly improved the quality of garlic and Welsh onion; their soluble protein content was significantly higher [7,40]. It was also found that the phenolic content of fruit was significantly higher after GA_3 spraying on 'Cabernet Sauvignon' grapes [41].

Table 3. Effect of GAs on regulating the internal quality of horticultural crops.

Horticultural Varieties	Main Points	Concentration of GAs	References
Apple (*Malus pumila* Mill.)	GA_3 spraying retards the decrease in soluble solids content in fruits.	100 mg L^{-1} GA_3	Liu et al., 2022 [18]
Banana (*Musa nana* Lour.)	GA_3 and CPPU treatments reduce water loss in fruits.	50 mg L^{-1} GA_3 + 10 mg L^{-1} CPPU	Huang et al., 2012 [36]
'rabbit eye' blueberry (*Vaccinium virgatum* Ait.)	GA_3 spraying retards the decrease in soluble solids content of fruits.	500 mg L^{-1} GA_3	Zang et al., 2016 [33]
'rabbit eye' blueberry (*Vaccinium virgatum* Ait.)	GA_3 spraying retards the decline of ascorbic acid concentration and the increase of total phenolic content of fruits.	500 mg L^{-1} GA_3	Zang et al., 2016 [33]
Broccoli (*Brassica capitata* var. *italica*)	GA_3 and CPPU treatments reduce water loss.	50 mg L^{-1} GA_3 + 10 mg L^{-1} CPPU	Huang et al., 2012 [36]
Sweet cherry (*Cerasus pseudocerasus* (Lindl.) G. Don)	GA_3 spraying retards fruit weight loss.	30 mg L^{-1} GA_3	Burhan et al., 2022 [31]
Sweet cherry (*Cerasus pseudocerasus* (Lindl.) G. Don)	GA_3 spraying raises fruit acidity levels.	60 mg L^{-1} GA_3	Ozkan et al., 2016 [34]
'Barhee' date (*Ziziphus jujuba* Mill.)	GA_3 spraying retards the decline of Vc content in fruits.	50 ppm GA_3	Awad et al., 2012 [37]
Garlic (*Allium sativum* L.)	GA_3 injection increases soluble protein content of fruits.	1 mM GA_3	Liu et al., 2019 [7]
'Cabernet Sauvignon' grape (*Vitis vinifera* L.)	GA_3 spraying increases phenol content of fruits.	15 mg L^{-1} GA_3	Song et al., 2023 [41]
Kiwifruit (*Actinidia chinensis* Planch.)	GA_3 spraying retards the decline of fruit hardness and soluble solids content.	0.3 g L^{-1} GA_3	Yang et al., 2023 [28]
Welsh onion (*Allium cepa* L.)	GA_3 spraying increases soluble protein content of fruits.	40 ppm GA_3	Yamazaki et al., 2015 [40]
'Nanguo' pear (*Pyrus* spp)	GA_3 soaking retards the decline of ascorbic acid concentration and the increase of total phenolic content of fruits.	100 mg L^{-1} GA_3	Martínez-Romero et al., 2000 [39]
Plum (*Prunus salicina* Lindl.)	GA_3 spraying retards the decrease in soluble solids content of fruits and reduces titratable acidity at harvest.	200 mg L^{-1} GA_3	Barac et al., 2022 [32]

Table 3. *Cont.*

Horticultural Varieties	Main Points	Concentration of GAs	References
Plum (*Prunus salicina* Lindl.)	GA_3 spraying retards the reduction of soluble solids and titratable acid content in fruits.	50 mg L^{-1} GA_3	Erogul et al., 2015 [35]
Plum (*Prunus salicina* Lindl.)	GA_3 soaking retards the decline of ascorbic acid concentration and the increase of total phenolic content of fruits.	200 μL L^{-1} GA_3	Li et al., 2006 [38]
Plum (*Prunus salicina* Lindl.)	GA_3 spraying retards the decline of ascorbic acid concentration and the increase of total phenolic content of fruits.	50 mg L^{-1} GA_3	Erogul et al., 2015 [35]
'Angelino' plum (*Prunus salicina* Lindl.)	GA_3 spraying retards fruit weight loss and decay.	50 mg L^{-1} GA_3	Erogul et al., 2016 [29]
'BlackStar' plum (*Prunus salicina* Lindl.)	GA_3 spraying retards fruit weight loss and decay.	50 ppm GA_3	Harman et al., 2016 [30]
'Obilnaja' plum (*Prunus salicina* Lindl.)	GA_3 spraying retards fruit weight loss and decay.	50 ppm GA_3	Harman et al., 2016 [30]

5. Gibberellins for Improving Cold Resistance in Horticultural Crops

Gibberellins participate in plant responses to biotic and abiotic stresses. When fruits are subjected to postharvest stress, the exogenous application of GAs can effectively regulate biotic and abiotic stress resistance. Cold damage is one of the more important abiotic stresses in the postharvest aspect of fruits, and it is a major constraint to the low-temperature storage of cold-sensitive fruits, resulting in sunken skins, watery spots, dry scars, flesh flocculation or lignification, reduced flavor, failure to properly ripen, and accelerated decay. It was found that elevated GA_3 levels were accompanied by the down-regulation of DELLA genes (members of the GRAS protein family that repress GA responses, degraded by the 26S-proteasome upon interaction with the GID1-GA complex) and the expression of key GA biosynthetic genes, *GA20ox1* and *GA3ox1*, was down-regulated in the application of exogenous GA_3 (Figure 1). Zhu et al. found that GA_3 treatment reduced the cold damage index, maintained the integrity of cell membranes during low-temperature storage, and activated the feedback mechanism of GA anabolism and the expression of C-repeat binding transcription factor 1 (*CBF1*), an important regulator of cold resistance, thus effectively improving the cold resistance of tomato fruits [42]. Ding et al. showed that, during low-temperature storage of the GA-deficient mutant *gib-3* tomato fruit, the cold damage index was significantly higher than that of normal fruit, and the breakage of microstructures such as cell membranes and cell walls was accelerated and more severe. GA deficiency also led to an up-regulation of *GA3ox1* and a significant down-regulation of *CFB1*, indicating that GA deficiency reduced the cold resistance of the fruit [43]. Ding et al. treated cherry tomato with GA_3 and stored them at low temperatures; the fruit cold damage index decreased significantly, effectively maintaining the stability of the cell membrane during storage, reducing the production and accumulation of O_2^- and improving the antioxidant capacity of the fruit. The results indicated that GA treatment effectively controlled postharvest cold damage in the fruit [44] (Table 4).

Table 4. Effect of GAs on regulating cold resistance of horticultural crops.

Horticultural Varieties	Main Points	Concentration of GAs	References
Tomato (*Solanum lycopersicum* L.)	GA_3 spraying maintains the integrity of fruit cell membranes during low-temperature storage and activates cold resistance regulation.	0.5 mM GA_3	Zhu et al., 2016 [42]
Cherry tomato (*Solanum lycopersicum* L.)	GA_3 spraying maintains the integrity of fruit cell membranes during low-temperature storage and enhances antioxidant capacity.	0.2 mmol L^{-1} GA_3	Ding et al., 2015 [44]
gib-3 tomato (*Solanum lycopersicum* L.)	GA_3 deficiency leads to accelerated destruction of fruit cell membranes during low-temperature storage and reduces cold resistance.	GA mutant	Ding et al., 2016 [43]

6. Gibberellins Regulate Disease Resistance in Horticultural Crops

Research has indicated that gibberellin treatment effectively controlled the incidence of disease during storage. Disease is a series of morphological, physiological, and biochemical pathological changes in fruits under the influence of biotic or abiotic factors. In the horticultural postharvest sector, this mainly manifests as the phenomenon of fruit that are healthy at harvest but infected with disease afterward, and as fruits that are apparently intact but diseased at harvest and only show traces of disease after harvest. *Trichothecium roseum* [45] and *Fusarium* [46] are susceptible to postharvest fruit infestation, causing pink mold and white mold, which cause extensive fruit rot. Ma et al. found that GA_3 did not inhibit the growth of *Trichothecium roseum* and *Fusarium*, but when mixed with azoxystrobin (AZX), the spot area of both diseases was reduced to different degrees compared to the two alone, thus inferring that GA_3 improved fruit resistance by regulating the physiological activity of postharvest fruits [47]. Black skin disease is a major physiological disease that occurs on pear fruit after harvesting, producing irregular black-brown spots of varying sizes on the surface of the fruit skin, which seriously reduces the quality of the appearance of the fruit [48]. Ma et al. treated 'apple pear' fruit with GA_3 and found that POD, CAT, APX, and SOD activities increased, while PPO activity decreased and the thickness of surface protective tissue increased; the results indicated that GA treatment effectively controlled 'apple pear' black skin disease [49]. In addition, in studies on persimmon's black spots, GAs were found to reduce rot by inhibiting the extracellular enzyme activity of *Alternaria alternata* (*A. alternate*) to maintain fruit cell wall integrity and limit pathogen expansion and access to host nutrients [50]. Similarly, one study found that GAs use reduced fruit cuticle dehiscence and susceptibility to persimmon fruit black spot disease during late fruit growth and storage. The present results suggest that GAs cause delayed fruit ripening and are a major factor in enhancing resistance to persimmon fruit black spots during harvest and storage [51] (Table 5).

Table 5. Effect of GAs on regulating disease resistance of horticultural crops.

Horticultural Varieties	Main Points	Concentration	References
Melon (*Citrus limon* (L.) Osbeck)	GA_3 and AZX spraying inhibits the growth of *Trichothecium roseum* and *Fusarium.*	100 mg L^{-1} GA_3 + 100 mg L^{-1} AZX	Ma et al., 2005 [47]
'apple pear' pear (*Pyrus* spp.)	GA_3 spraying increases the thickness of protective tissue on the fruit surface and controls black skin disease.	50 mg L^{-1} GA_3	Ma et al., 2018 [49]
Persimmon (*Diospyros kaki* Thunb.)	GA_3 spraying reduces fruit cuticle cracking and controls black spot.	200 μg mL^{-1} GA_3	Biton et al., 2014 [51]
gib-3 tomato (*Solanum lycopersicum* L.)	GA regulates early blight resistance by regulating hormone content.	GA mutant	Wu et al., 2018 [52]

The molecular mechanisms of gibberellin to regulate disease resistance in fruits were studied. DELLA regulates the balance of salicylic acid (SA)/jasmonate (JA) signaling during plant immunity [53]. SA and JA play key roles in the induction of the systemic acquired resistance (SAR) process for defense against pathogenic bacteria infestation, while isochorismate synthase 1 (*ICS1*) transcription activates SA biosynthesis; nonexpressor of *pathogenesis-related* genes 1 (*NPR1*) is an important regulator of the SA-regulated SAR process [54] (Figure 1). Early blight caused by *Streptomyces aegypti* infestation is one of the most common postharvest fungal diseases [55]. A study indicated that *ICS1* and *NPR1* were up-regulated, PAL, PPO, CHI, and GLU activities were significantly enhanced, brassinolide (BR) content was increased, fruit incidence was significantly reduced, and spot area was significantly decreased in the GA-deficient mutant strain *gib-3* tomato fruit, indicating that GAs negatively regulated postharvest tomato fruit resistance to early blight [52].

7. Conclusions

In conclusion, as important plant hormones, gibberellins play a significant role in the postharvest physiological regulation of fruits. During the storage of fruits, GAs can effectively delay the respiratory leap in some fruits and can delay the weight loss and decay of fruits by inhibiting ethylene production, reducing respiration intensity, scavenging free radicals in the body to maintain cell membrane integrity, and delaying the senescence of fruit stalks to prevent them from falling off. They can also improve the intrinsic and extrinsic quality of fruit storage by improving fruit shape, regulating color, and delaying the reduction of soluble solids. GAs also play an important role in enhancing postharvest stress resistance in fruits. This review systematically described the role of GAs in the physiological regulation of postharvest fruits and their existing applications, providing a more comprehensive perspective to further enhance the scope of their application in postharvest fruits and to clarify their usage in practice. At the same time, this paper lays a certain foundation for accelerating and improving the research on the physiological aspects of the regulation of GAs in postharvest fruits, and also points to a certain direction for in-depth molecular-level mechanism research.

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Grosspeteranlage 5
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