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Impact of a Carvacrol Treatment on Post-Harvest Ripening Quality and Cell Wall Metabolism in the Kiwifruit

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Abstract: The objective of this study is to investigate the effects of carvacrol on the postharvest storage quality and cell wall metabolism of the 'Guichang' kiwifruit. For this purpose, the 'Guichang' kiwifruit is selected as the test material and treated with 0.03 mg/mL of a carvacrol solution for dipping. The storage quality and cell wall metabolism of the fruit are then evaluated under ambient conditions (20 ± 1 °C). The results indicate that treatment with carvacrol effectively reduces the postharvest respiration rate and weight loss in the kiwifruit, while preserving the levels of vitamin C (VC), soluble protein, total phenols, flavonoids, soluble solids, solid–acid ratio, and soluble pectin. Additionally, it delays the decline in the starch content and mitigates the increase in soluble sugars. Furthermore, carvacrol significantly decreases amylase activity and inhibits the elevation of polygalacturonase (PG), pectin methylesterase (PME), *β*-galactosidase (*β*-Gal), and cellulase (Cx) activity. Consequently, this treatment effectively maintains fruit firmness as well as protopectin (PP) and cellulose content at elevated levels. A correlation analysis shows that firmness is highly and negatively correlated with the cell wall degrading enzymes PG, PME, β-Gal, Cx, and α-L-Af, with R values of −0.88, −0.83, −0.98, −0.94, and −0.93, respectively. The negative correlation values for the control group are all lower than 0. In conclusion, treatment with carvacrol effectively preserves a high level of postharvest ripening quality in the kiwifruit, attenuates the metabolic processes of the cell wall, and delays fruit softening and senescence.

Keywords: kiwifruit; carvacrol; post-ripening quality; cell wall metabolism

Academic Editor: Rajko Vidrih

Received: 10 November 2024
Revised: 23 December 2024
Accepted: 23 December 2024
Published: 30 December 2024

Citation: Luo, D.; Wang, X.; Mi, T.; Chen, J.; Ba, L.; Cao, S. Impact of a Carvacrol Treatment on Post-Harvest Ripening Quality and Cell Wall Metabolism in the Kiwifruit. *Agronomy* **2025**, *15*, 79. https://doi.org/10.3390/agronomy15010079

1. Introduction

The 'Guichang' kiwifruit (*Actinidia deliciosa* cv. 'Guichang') is favored by consumers owing to its high nutritional value, being rich in vitamin C, vitamin E, folic acid, and other essential nutrients, as well as its delightful sweet and sour flavor [1]. Despite the rapid development of the kiwifruit industry in the Guizhou province, the post-harvest physiological metabolism of the kiwifruit remains highly active. This heightened metabolic activity accelerates the ripening and softening processes, a phenomenon which not only diminishes fruit quality, but also increases susceptibility to pathogenic microorganisms. These issues pose significant challenges to the sustainable development of the kiwifruit

industry [2,3]. Consequently, addressing the problem of post-harvest softening in kiwifruits is imperative to extend their storage life.

As a novel biological preservative, carvacrol offers several advantages, including low costs, a natural origin, and a minimal environmental impact. The potential of carvacrol as a biological preservative has attracted considerable attention from researchers. Its ability to delay the maturation and senescence of fruits and vegetables, regulate their physiological metabolism, mitigate post-harvest softening, and enhance the quality of stored produce has been extensively documented [4–6]. Chen et al. [7] demonstrated that treatment with carvacrol in citrus fruits inhibits cell wall disintegrating enzymes such as pectin methylesterase (PME), polygalacturonase (PG), cellulase (Cx), and β -galactosidase(β-gal). This inhibition resulted in a delayed fruit softening process. Sapper et al. [8] showed that applying a carvacrol coating to 'Golden Delicious' apples leads to a superior maintenance of weight loss prevention, respiration rate control, and fruit firmness retention while also inhibiting fruit softening. In research conducted by Wang et al. [9], the effects of a carvacrol treatment on blueberry quality were investigated. The results indicated that carvacrol not only reduces the decay rates during storage, but also significantly enhances anthocyanin levels in and the total phenolic content of blueberries. In our previous study, we found that carvacrol exhibits a pronounced inhibitory effect on the post-harvest tip rot pathogen affecting the 'Guichang' kiwifruit. Furthermore, we observed that carvacrol significantly enhances the post-harvest disease resistance of these fruits. Among all the treatments tested, the 0.03 mg/mL concentration of carvacrol was identified as the most effective at reducing the decay rate of these fruits [10]. Based on these findings, the present study aims to further investigate the effects of carvacrol on the post-harvest quality of the kiwifruit and its associated cell wall metabolism. Additionally, this research seeks to elucidate the role of carvacrol in regulating kiwifruit softening, with the objective of providing new insights for enhancing post-harvest storage and preservation techniques for the kiwifruit.

2. Materials and Methods

2.1. Materials and Reagents

The 'Guichang' kiwifruit was sourced from the kiwifruit test base located in Xiuwen County, Guizhou Province. Carvacrol (99% purity, molecular weight of 150.22) was obtained from Shanghai Aladdin Biochemical Science and Technology Co., Ltd., Guangdong, China. All other reagents used were of analytical grade.

2.2. Instruments and Equipment

The following instruments were utilized: a TA.XT. plus mass spectrometer (Stable Micro System, UK), a UV-2550 ultraviolet spectrophotometer (Shimadzu, Japan), a TGL-16A tabletop high-speed freezing centrifuge (Changsha Pingfan Instrument Co., Ltd., Hunan, China), and a Check Point II portable residual oxygen meter (Dansensor, Denmark).

2.3. Experimental Methods

2.3.1. Carvacrol Dipping Treatment for Fruit Preservation

In our previous studies, we found that a 0.03 mg/mL concentration of a carvacrol treatment was most effective at suppressing the decay rate of the kiwifruit [10]. Prior to the carvacrol treatment, the fruit surface was disinfected using 0.1% NaOCl. The dissolution method for carvacrol followed the procedure described by Wang et al. [11]. Specifically, 2 mL of a 5% Tween-80 solution was used to dissolve carvacrol, after which the kiwifruit was immersed in 0.03 mg/mL of a carvacrol solution for 5 min. For the control group, only Tween-80 was used. Subsequently, the fruits were air-dried at room temperature and stored under conditions of 20 $\pm$ 1 °C and 90 $\pm$ 5% relative humidity for a period of 20 days.

During this time, measurements of cell-wall-related parameters and post-harvest quality were conducted every 4 days.

2.3.2. Physiological Indicators

Fruit Firmness Determination

Firmness measurements were performed using a TA.XT plus texture analyzer (Stable Micro Systems, Godalming, UK) equipped with a p/2 probe set to penetrate to a programmed depth of 5 mm, featuring a probe diameter of 2 mm, a pre-testing speed of 2 mm/s, a testing speed of 2 mm/s, a post-testing speed of 5 mm/s, and a trigger force set to 5 g. Firmness values were recorded from ten replicates per test condition, with the results presented as average firmness expressed in kg/cm².

Determination of the Weight Loss Rate

Fifteen kiwifruits were randomly selected, individually numbered, and their weights measured every 4 days using a CN-LQC3002 precision balance (KERN & SOHN GmbH, Balingen, Germany). The collected data were meticulously recorded. The weight loss rate was calculated using the following formula:

$$\text{Weight loss rate (\%)} = [(\text{weight before storage} - \text{weight after storage})/\text{weight before storage}] \times 100 \quad (1)$$

Determination of the Respiration Rate

The respiration rate of the kiwifruits was measured using the GXH-3051H infrared CO_2 fruit respiration meter (Institute of Junfang Scientific Instrument, Beijing, China). The kiwifruit was placed in a 60 mm × 90 mm fruit respiration chamber and allowed to equilibrate indoors for 2 h before the respiration rate was determined using the meter. Six fruits were sampled for each measurement, and the process was repeated three times. Results are expressed as mg CO_2/(kg·h).

Determination of Ascorbic Acid (VC)

The content of ascorbic acid (VC) was determined by using the spectrophotometric method described by Staveckienė et al. [12], with results reported as mg/100 g.

Determination of Total Phenols and Flavonoids

The total phenol content was assessed according to the methodology outlined by Yang et al. [13]. A standard curve was established using gallic acid, with results expressed as mg/g. Flavonoid content was determined based on the procedure described by Duan et al. [14], utilizing a 1% HCl–CH_3OH extraction method; absorbance values were measured at 325 nm and reported in GAE/g (gallic acid equivalents/g)

Determination of the Soluble Protein Content

The soluble protein content was determined using the method described by Chen et al. [15]. Absorbance measurements were taken at 595 nm, with bovine serum protein serving as the standard for the calibration curve. The results are expressed as milligrams of soluble protein per gram of fresh weight of kiwifruit (mg/g).

Determination of the Starch Content and Amylase Activity

The determination of the starch content and amylase activity followed the methodology outlined by Turkiewicz et al. [16]. Starch content was quantified using glucose to establish a standard curve, with the results expressed in mg/g, while amylase activity was assessed using maltose as a standard curve, with the resukts reported in mg/(g·h).

Determination of Soluble Sugar

The soluble sugar content was measured according to the modified method proposed by Nam et al. [17]. Samples were ground into powder and dissolved in 50 mL of distilled water, then heated at 80 °C for 30 min. The supernatant was filtered prior to analysis, with absorbance readings taken at 760 nm; glucose served as the reference for constructing the standard curve expressed in percentage (%).

Determination of the Solid–Acid Ratio

The soluble solid content was measured utilizing an RA250-WE handheld digital saccharimeter and is presented as a percentage (%). The titratable acid content was determined through an acid–base titration method [13], also expressed in percentage (%). The formula used for calculating the solid–acid ratio is:

$$\text{Solid–acid ratio (\%)} = (\text{soluble solid content}/\text{titratable acid content}) \times 100\%. \quad (2)$$

2.3.3. Cell-Wall-Related Metabolic Enzyme Activity Assay

Determination of Pectin Methylesterase (PME) Activity

The method described by Lin et al. [18] was adapted with minor modifications. A total of 5 g of kiwifruit pulp was weighed, ground, and homogenized in an ice bath with the extraction buffer, followed by centrifugation at 10,000× *g* for 30 min to obtain the supernatant for the enzyme activity analysis. The consumption of 1 μmol NaOH required to neutralize the free carboxyl groups per gram of fresh fruit was defined as one enzyme activity unit. The results are expressed as μ/g

Determination of Polygalacturonase (PG) Activity

The protocol established by Gross [19] was modified for this assay. Five grams of fruit pulp was taken, ground, and homogenized in an ice bath with the extraction buffer before being centrifuged at 14,000 rpm for 20 min; the resulting supernatant was utilized for the enzyme activity analysis. The galacturonic acid served as the standard curve and the results are expressed in mg/(g·h).

Determination of Cellulase (CX) Activity

The methodology outlined by Li et al. [20] was slightly modified for this determination process using a glucose solution as a standard curve; results are expressed in mg/(g·h).

Determination of β-Galactosidase (β-Gal) Activity

This assay followed the approach detailed by Carington et al. [21], incorporating some modifications to enhance accuracy and reliability.

A total of 10 g of pulp was weighed and combined with the extraction buffer prior to grinding in an ice bath to achieve homogeneity; subsequent centrifugation at 10,000× *g* for 30 min yielded a supernatant that was used for the enzyme activity analysis.

A p-Nitrophenol solution acted as the standard curve, with the results reported in μmol/(g·h).

Determination of α-L-Arabinofuranosidase (α-L-Af) Activity

α-L-Af activity was assessed according to Chen et al.'s assay method [22]. A sucrose solution served as the standard curve, with the units of activity (U) expressed as μmol/(g·h).

2.3.4. Determination of Cell-Wall-Related Metabolite Content

Determination of the Protopectin and Soluble Pectin Content

The protopectin and soluble pectin content was determined using the carbazole colorimetric method [23]. A total of 5 g of pulp was weighed and homogenized, followed by the addition of 50 mL of a 95% ethanol solution. The mixture was subjected to boiling water bath heating for 40 min, after which it was filtered through a Brinell funnel, discarding the filtrate. Subsequently, 50 mL of distilled water was added to the precipitate, and this mixture was heated in a water bath at 50 °C for 30 min before being filtered again through a Brinell funnel; the volume of the filtrate was then adjusted to 100 mL to obtain the soluble pectin solution. For the protopectin extraction, the precipitate received an additional treatment with 100 mL of a 0.5 mol/L sulfuric acid solution and was heated in a boiling water bath for one hour. After cooling, filtration through a Brinell funnel yielded another filtrate that was also adjusted to a final volume of 100 mL to acquire the original pectin solution. The optical density (OD) value was measured at 530 nm with three repetitions conducted for accuracy purposes; the galacturonic acid served as the standard curve.

Determination of the Cellulose Content

The method described by Chen et al. [24] was referenced and modified accordingly for cellulose content determination. The OD value was measured at 620 nm with three repetitions performed for reliability; standard glucose solutions were utilized as reference standards.

2.4. Data Processing and Analysis

Graphs and data correlation analyses were conducted using the Origin 2021 software, while ANOVA tests for assessing the significance of differences were performed using the SPSS 20.0 software.

3. Results

3.1. Effects of the Carvacrol Treatment on Kiwifruit Firmness, Weight Loss, and Respiratory Rate

During storage, the degradation of in vivo substances such as pectin and starch in kiwifruits led to tissue softening and a reduction in firmness. The impact of the carvacrol dipping treatment on kiwifruit firmness is illustrated in Figure 1a. Throughout the storage period, fruit firmness gradually decreased in both control and carvacrol-treated groups. Notably, comparisons revealed that kiwifruits treated with carvacrol maintained significantly higher firmness compared to those in the control group during storage. A significant difference ($p < 0.05$) was observed on day 4 of the storage phase, with the treated fruits being 1.5 times firmer than their controls.

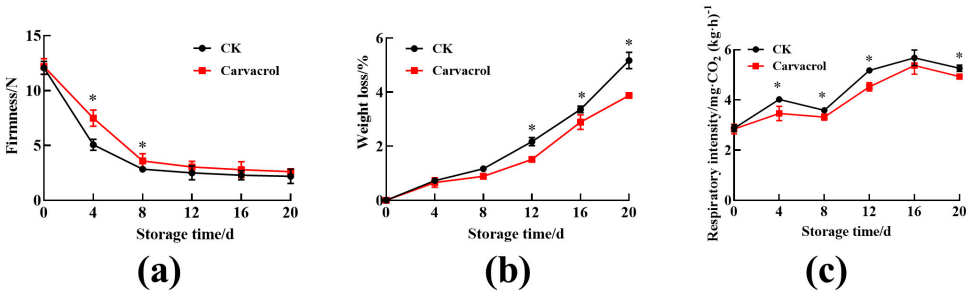

Figure 1. Effects of the carvacrol treatment on firmness (**a**), weight loss rate (**b**), and respiratory intensity (**c**). The symbol '*' means a significant difference ($p < 0.05$).

Respiration and transpiration-induced water loss contributed to a gradual increase in weight loss in the kiwifruits over time. As depicted in Figure 1b, the weight loss increased significantly throughout the storage duration in both control and carvacrol-treated groups; however, fruits subjected to the carvacrol treatment maintained significantly lower levels of weight loss compared to the controls. By the conclusion of the 20-day storage period, weight loss was markedly reduced in the carvacrol-treated fruits relative to the controls ($p < 0.05$), indicating that these treatments effectively inhibited increases in weight loss during storage.

Significant differences ($p < 0.05$) were noted between both groups from days 12 to 20 of the storage stage. After the harvest of kiwifruits, respiration leads to the consumption of fruit nutrients, resulting in a decline in both fruit quality and storability over time. As illustrated in Figure 1c, the respiratory intensity of the carvacrol-treated kiwifruits was significantly lower ($p < 0.05$) than that of the control throughout the storage period, with the exception of day 8. The respiratory intensity of the carvacrol-treated kiwifruits remained consistently and significantly lower ($p < 0.05$) than that of the control group. Notably, peak respiration rates in both control and carvacrol-treated kiwifruits were observed on day 16, measuring 5.68 mg CO_2 (kg·h^{-1}) and 5.37 mg CO_2 (kg h^{-1}), respectively. Furthermore, the peak respiration rate in the control kiwifruits was significantly higher than that observed in the carvacrol-treated group ($p < 0.05$).

An increase in respiration rates is known to accelerate fruit senescence; however, treatment with carvacrol through fruit dipping effectively delayed the increase in respiration rate in the kiwifruit, thereby postponing fruit senescence.

3.2. Effect of the Carvacrol Treatment on Ascorbic Acid, Total Phenols, Flavonoids, and Soluble Protein Content in the Kiwifruit

The impact of the carvacrol dipping treatment on the ascorbic acid content of the kiwifruit is illustrated in Figure 2a. Throughout the storage period, a consistent decline in ascorbic acid levels was observed. Notably, the treated group maintained significantly higher levels of ascorbic acid content compared to the control group and exhibited a slower rate of decline ($p < 0.05$). The carvacrol treatment effectively mitigated the loss of ascorbic acid from kiwifruits during storage. Statistically significant differences ($p < 0.05$) were noted between the two groups from day 4 to day 12, with the ascorbic acid content in the control group being approximately 1.04 to 1.05 times lower than that of the treated group.

As shown in Figure 2b, under cold storage conditions, the total phenol content of kiwifruits treated with carvacrol generally decreased over the storage period, and a significant difference was observed between the treatment and control groups. Notably, on the 8th day of storage, the total phenol content of the treatment group was significantly higher than that of the control group ($p < 0.05$).

Figure 2c demonstrates that the flavonoid content of kiwifruits generally exhibited a declining trend as storage time increased. However, throughout the post-ripening process, kiwifruit treated with carvacrol consistently maintained higher flavonoid levels compared to the control group. A statistically significant difference between these two groups was noted from day 4 through to day 20 ($p < 0.05$), indicating that the carvacrol treatment effectively enhanced the flavonoid content of the kiwifruits.

As shown in Figure 2d, the soluble protein content of the kiwifruits gradually decreased during storage. The figure indicates that the soluble protein content of the carvacrol treatment group was significantly higher than that of the control group ($p < 0.05$). By the 20th day of storage, the soluble protein content of the carvacrol treatment group remained at a high level, reaching 0.49 mg·g^{-1}, which was significantly higher than that of the control group ($p < 0.01$). These results suggest that the carvacrol treatment can effectively inhibit the degradation of soluble proteins in the kiwifruit, thereby enhancing its preservation.

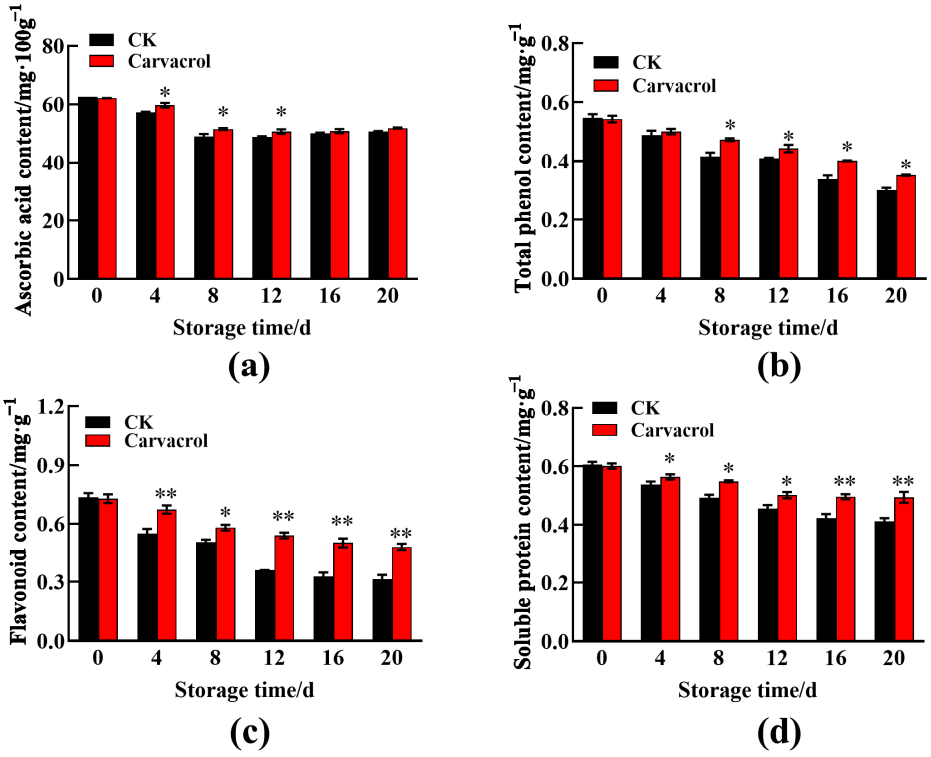

Figure 2. Effects of the carvacrol treatment on VC (**a**), total phenol (**b**), flavonoid (**c**), and soluble protein (**d**) contents. The symbol '*' means a significant difference ($p < 0.05$), while '**' means an extremely significant difference ($p < 0.01$).

3.3. Effects of the Carvacrol Treatment on Kiwifruit Starch Content, Amylase Activity, Soluble Sugar Content, and Solid–Acid Ratio

As illustrated in Figure 3a, the starch content of the kiwifruits exhibited a declining trend throughout the storage period. Notably, the starch content in the carvacrol-treated group was found to be 1.12 to 1.25 times higher than that of the control group from day 4 to day 16, representing a statistically significant difference ($p < 0.05$). This finding indicates that the carvacrol treatment effectively maintained higher levels of starch content.

Figure 3b demonstrates that the amylase activity in the kiwifruits also exhibited a decreasing trend as storage time increased. From day 4 to day 20 of storage, the amylase activity in the carvacrol-treated group was significantly lower compared to that of the control group ($p < 0.05$). Therefore, it can be concluded that the carvacrol treatment exerts an inhibitory effect on the amylase activity in post-harvest kiwifruits.

The soluble sugar content across all treatments showed a gradual increase over the storage period, as depicted in Figure 3c. After 20 days of storage, kiwifruits treated with carvacrol demonstrated a statistically significant difference ($p < 0.05$) in soluble sugar content compared to the control group. Specifically, the control group showed an increase of only 0.32% relative to the carvacrol-treated fruits. This suggests that the carvacrol treatment delays the accumulation of soluble sugars during the later stages of storage.

During storage, the soluble solid content (SSC) of the kiwifruits gradually increased over time, while the titratable acid content exhibited a gradual decrease. Consequently, the ripening index (solid–acid ratio) progressively increased with an increasing storage duration. Both control and carvacrol-treated groups showed an increase in the ripening index of the kiwifruits throughout the storage period. As illustrated in Figure 3d, treatment with carvacrol effectively delayed the increase in the kiwifruit ripening index.

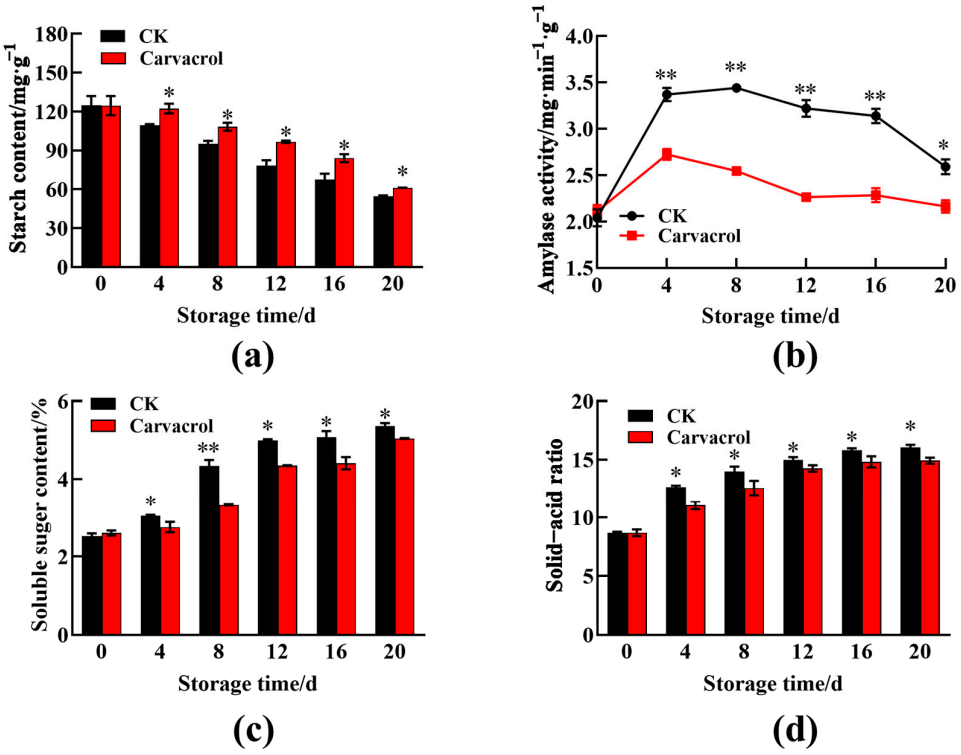

Figure 3. Effects of the carvacrol treatment on starch content (**a**), amylase activity (**b**), soluble sugar content (**c**), and solid–acid ratio (**d**). The symbol '*' indicates a significant difference ($p < 0.05$), while '**' indicates an extremely significant difference ($p < 0.01$).

Throughout the entire storage period, the carvacrol fruit dipping treatment significantly inhibited the increase in the soluble solid content and slowed down the decline of the titratable acid content. As a result, during storage, the ripening index of the carvacrol-treated fruits was significantly lower than that of the control group ($p < 0.05$). This finding indicates that the carvacrol treatment can effectively delay both fruit ripening and senescence. Notably, significant differences ($p < 0.05$) were observed between these two groups from day 4 to day 20 of the storage period.

3.4. Effect of the Carvacrol Treatment on the Activities of Cell-Wall-Related Metabolic Enzymes in the Kiwifruit

The activity of pectin methylesterase (PME) in the kiwifruits began to increase gradually after 8 days of storage, reaching maxima of 83.57 u/g and 80.68 μ/g on the 16th day before subsequently declining. The PME activity in the fruits treated with carvacrol was significantly suppressed compared to the control group ($p < 0.05$), as illustrated in Figure 4a. The PME activity in the carvacrol-treated fruits exhibited a similar trend to that observed in the control group; however, by day 8, the control group's PME activity was found to be 1.21 times higher than that of the carvacrol-treated group—a statistically significant difference ($p < 0.05$). Furthermore, a parsnip treatment also significantly inhibited PME activity during the later stages of storage ($p < 0.05$).

The post-harvest polygalacturonase (PG) activity in the kiwifruits increased gradually from 4 days post-harvest, peaking on day 16 with an increase of 1.65 mg/h/g compared to the treated group, before beginning to decline thereafter. Notably, the PG activity was highly and significantly suppressed under the carvacrol treatment ($p < 0.05$). While carvacrol influenced fruit PG activity, it did not alter its overall trend; specifically, PG levels decreased following a minor peak on day 16 (see Figure 4b).

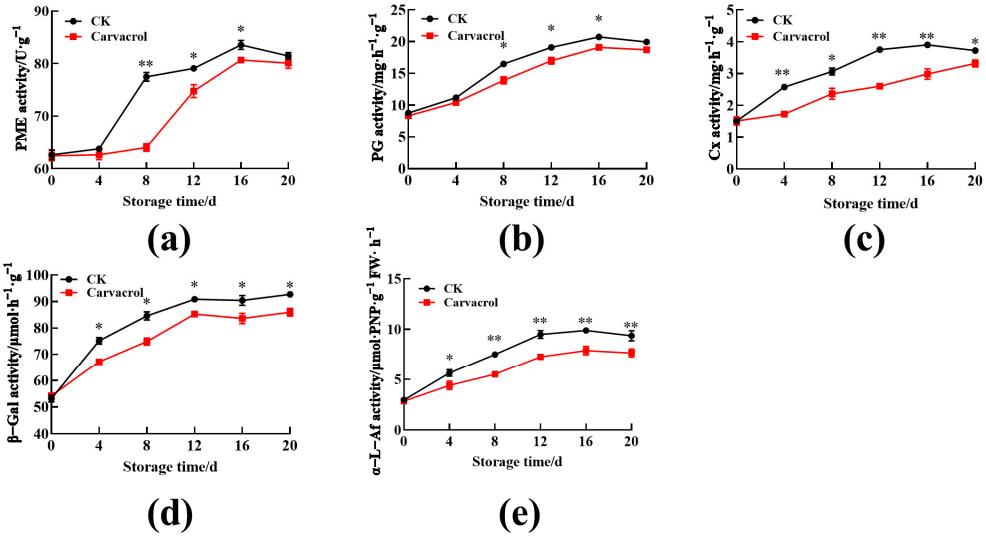

Figure 4. Effects of carvacrol on the activities of PME (**a**), PG (**b**), CX (**c**), *β*-gal (**d**), and *α*-L-Af (**e**). The symbol '*' means a significant difference ($p < 0.05$), '**' means an extremely significant difference ($p < 0.01$).

The activity of kiwifruit fruit cellulase (C_X) exhibited an increasing trend over time. Treatment with carvacrol resulted in lower fruit cellulase activity compared to the control during the storage period, although a gradual increase in activity levels was observed after day 4 (Figure 4c). The carvacrol treatment inhibited fruit cellulase activity, which initially increased in the treatment group during the pre-storage period but began to decline after day 16. The cellulase activity in the carvacrol-treated fruits followed a similar pattern to that of the control group throughout the storage period; however, it remained consistently lower than that of the control.

Kiwifruit *β*-galactosidase (*β*-Gal) activity commenced to increase from day 4 post-harvest and continued to increase thereafter. Notably, carvacrol significantly inhibited *β*-Gal activity ($p < 0.05$), which stabilized at reduced levels throughout the storage duration. In both the treated and the control groups, fruit *β*-Gal activity gradually increased up to day 12, reaching levels of 90.90 μmol h/g and 85.28 μmol h/g, respectively, before plateauing beyond this point (Figure 4d). As illustrated in Figure 4e, *α*-L-arabinofuranosidase (*α*-L-Af) activity demonstrated a pattern change analogous to that of the *β*-Gal; it began to increase on day 4 post-harvest and continued to increase alongside the fruit softening processes. The *α*-L-Af enzyme's activity was also significantly suppressed under the carvacrol treatment ($p < 0.05$), exhibiting a slight decrease following day 16 of storage. In comparison with the controls, carvacrol effectively inhibited the elevation of *α*-L-Af activity within the fruits ($p < 0.05$).

3.5. Effect of the Carvacrol Treatment on the Content of Substances Related to Cell Wall Metabolism in the Kiwifruit

As illustrated in Figure 5a, the content of protopectin in the kiwifruits exhibited a general decreasing trend throughout the storage period. Specifically, on day 12 of storage, the control group showed a protopectin content of 0.13%, while the treatment group demonstrated a higher content of 0.19%. The difference between these two groups was highly significant ($p < 0.01$). By day 20, the protopectin content in the control group had decreased by a factor of 0.4 compared to that in the treatment group, indicating that the carvacrol treatment had significantly reduced the degree of hydrolysis of protopectin ($p < 0.01$). The trend of the soluble pectin content was opposite to that of the original pectin, with its content gradually increasing (Figure 5b). On the 8th day of storage, the soluble

pectin content in the control group was 39.43% higher than that in the treatment group. Specifically, on the 8th day of storage, the soluble pectin content in the treatment group was significantly lower than that in the control group ($p < 0.01$).

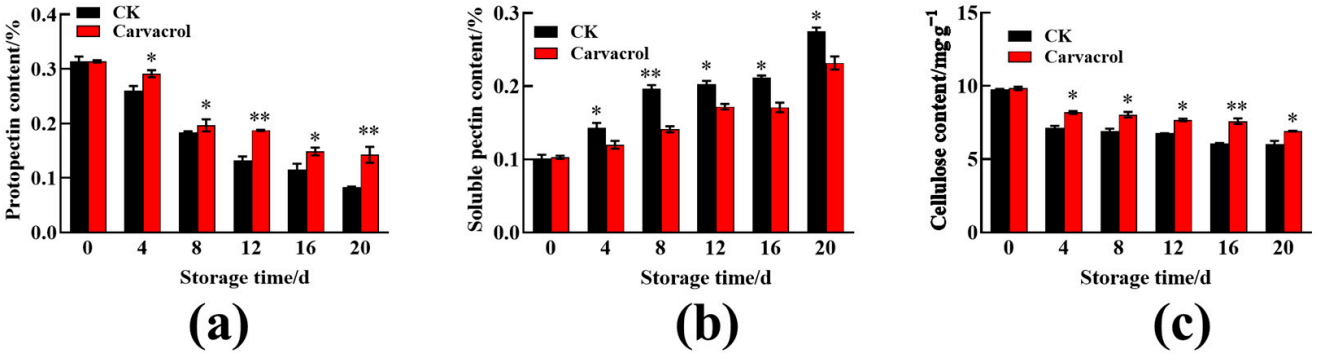

Figure 5. Effects of carvacrol on the contents of protopectin (**a**), soluble pectin (**b**), and cellulose (**c**). The symbol '*' means a significant difference ($p < 0.05$), while '**' means an extremely significant difference ($p < 0.01$).

The cellulose content in the kiwifruits gradually declined post-harvest; however, the carvacrol treatment markedly inhibited cellulose degradation within the fruit's tissues, resulting in a slower rate of decrease compared to the control group after day 4 ($p < 0.05$). As depicted in Figure 5c, there was a reduction of approximately 1.04 mg/g in cellulose content in the treated group compared to the control group; furthermore, changes in cellulose levels observed in the fruits subjected to the carvacrol treatment mirrored those seen in the control group, indicating that carvacrol significantly delayed cellulose degradation. Notable differences from the control group were statistically significant ($p < 0.05$).

3.6. Correlation Analysis

As shown in the correlation analysis graph presented in Figure 6, most indicators in the carvacrol-treated samples exhibited significant positive or negative correlations with each other ($p < 0.05$). In the control group, kiwifruit firmness demonstrated highly significant negative correlations ($p < 0.01$) with weight loss, solid–acid ratio, β-Gal, C_X, and α-L-Af; conversely, it showed highly significant positive correlations ($p < 0.01$) with VC and cellulose content. Additionally, starch content revealed highly significant negative correlations ($p < 0.01$) with amylopectin, soluble sugars, and solid–acid ratio. Furthermore, the firmness of the kiwifruits treated with carvacrol displayed a highly significant negative correlation with both the solid–acid ratio and certain cell-wall-metabolizing enzymes ($p < 0.01$), while exhibiting a highly significant positive correlation with VC and cellulose content ($p < 0.01$). Starch content also indicated robust negative correlations ($p < 0.01$) with amylase activity, soluble sugars, and solid–acid ratio, simultaneously demonstrating strong positive correlations ($p < 0.01$) with soluble protein levels, protopectin concentration, total phenols content, and flavonoids.

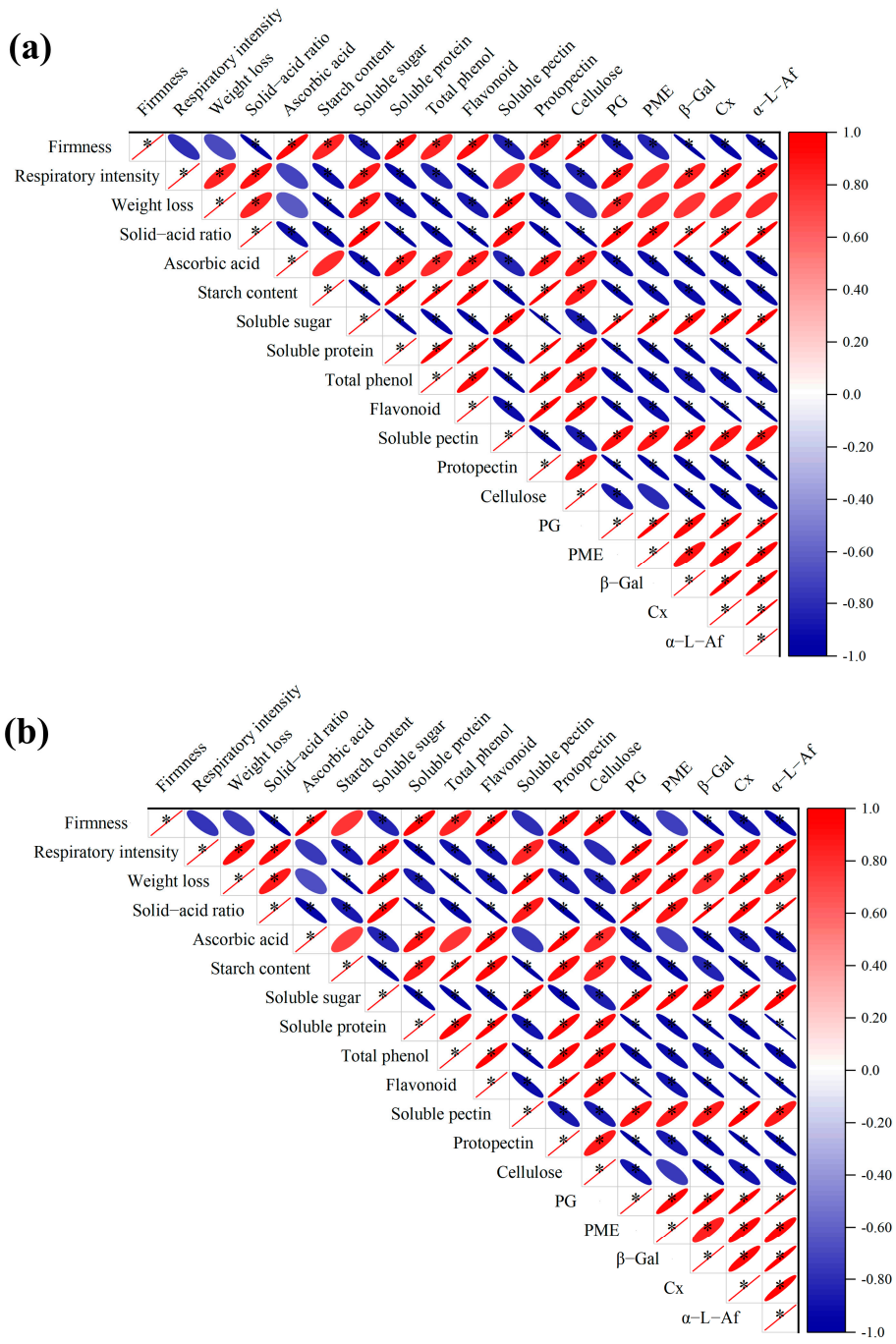

Figure 6. Correlation analyses of various indexes between the control group (**a**) and the carvacrol treatment group (**b**). Positive effects are shown in red, while negative effects are shown in blue. '*' indicates significant differences ($p < 0.05$).

4. Discussion

The kiwifruit is a typical climacteric fruit, exhibiting a pronounced respiratory climacteric during post-harvest storage. This post-ripening phase is marked by a significant peak in respiration rates, which can disrupt the physiological metabolic balance and lead to substantial consumption of sugars and organic acids. Consequently, this process alters the color, sugar content, acidity, and texture of the fruit [25]. Implementing appropriate post-harvest treatments and storage techniques can effectively mitigate the aging process of the kiwifruit while enhancing its quality after ripening. For instance, methods such as high-energy electron beam irradiation [26], methyl jasmonate application [27], and ozone

treatment [28] have been shown to delay senescence in the kiwifruit by slowing down declines in firmness, total acid levels, and total sugars, thereby preserving the ripening quality. Wang et al. [29] demonstrated that a salicylic acid treatment could modulate arginine metabolism within kiwifruits and promote endogenous polyamine accumulation, thus improving their storage resistance. In our study, we observed that parameters such as fruit firmness, ascorbic acid content, total phenols, flavonoids, and soluble protein content exhibited a gradual decline during storage; conversely, weight loss, along with the solid–acid ratio and soluble sugars, increased over time. Correlation analyses revealed significant negative relationships between these declining nutrients (firmness of kiwifruits), with increasing weight loss ratios (solid–acid ratio) throughout extended storage periods. These findings indicate that nutrient degradation occurs alongside water loss during post-ripening processes, leading to accelerated senescence; however, the carvacrol treatment effectively mitigated reductions in fruit firmness as well as levels of ascorbic acid, total phenolics, flavonoids, and soluble proteins while reducing weight loss rates. Furthermore, it delayed increases in weight loss rate while maintaining lower respiratory strength levels for the fruits, significantly preserving a better post-ripening quality.

Softening is a characteristic feature of the kiwifruit during the post-ripening phase. Changes in the starch content of the fruit significantly influence the softening process, with starch conversion primarily regulated by amylase [30]. During fruit softening, amylase facilitates the conversion of starch into soluble sugars, a phenomenon which contributes to an increase in soluble sugar content. This increase leads to a reduction in the cell expansion force and intercellular relaxation, ultimately resulting in softened fruits [31]. Previous studies have indicated that there is a negative correlation between starch and soluble solid contents throughout the post-ripening process of the kiwifruit [32]. Additionally, it has been demonstrated that melatonin [31], low temperature [33], and heat treatments [34] can effectively reduce amylase activity in the kiwifruit, thereby delaying starch degradation and enhancing the quality of kiwifruits during the post-ripening phase. The findings of this study indicate that, during the ripening process of the kiwifruit, there is a gradual decrease in the starch content, a progressive increase in the soluble sugar content, and an upward trend in the solid–acid ratio. Additionally, the amylase enzyme activity exhibited an initial increase followed by a subsequent decline. Correlation analyses revealed that the starch content was significantly and negatively correlated with both soluble sugar content and solid–acid ratio, as well as with amylase enzyme activity ($p < 0.05$). These results suggest that starch is converted into soluble sugars by amylase during the post-ripening phase in the kiwifruit, while the increasing solid–acid ratio exacerbates the extent of fruit post-ripening. Furthermore, treatment with carvacrol significantly reduced amylase activity, thereby slowing down the decline in the starch content and delaying the increase in the soluble sugar to solid–acid ratio. This indicates that the carvacrol treatment can inhibit starch degradation and mitigate fruit ripening by modulating amylase activity.

The fruit cell wall primarily comprises the intercellular layer, the primary wall, and the secondary wall. The intercellular layer is rich in pectin, while the primary and secondary walls contain polysaccharide components such as cellulose, hemicellulose, lignin, and various structural proteins that contribute to the stability of the cell wall structure [35]. The hydrolysis of cell wall polysaccharides directly influences the process of fruit softening. During the post-ripening phase, cell wall hydrolases (including PME, PG, Cx, β-Gal, and α-L-Af) depolymerize and convert these polysaccharides. This enzymatic activity results in the thinning of the cell wall, the loosening of the intercellular matrix, and the disruption of the overall cell wall integrity, ultimately leading to fruit softening and collapse. The degradation of the cell walls directly leads to fruit softening. Research has confirmed that changes in PME, PG, Cx, and β-Gal enzymes are key factors in cell wall degradation. An

increase in the activity of these key degrading enzymes accelerates the softening of the persimmon fruit [36]. PG is responsible for the increased hardness and extended post-harvest shelf life of the tomato fruit, and its enzyme activity is positively correlated with tomato fruit softening [37].

Numerous studies have demonstrated that employing exogenous factors or appropriate storage techniques can mitigate the degradation of cell wall polysaccharides. Such interventions help delay both softening and senescence processes by modulating the activity of cell wall hydrolases [38]. Carvacrol effectively maintains the firmness and cell wall components of 'Guifei' mangoes by inhibiting the activity of pectin methylesterase, cellulase, polygalacturonase, and β-galactosidase. The firmness of mango fruits is highly and negatively correlated with the activity of cell wall degrading enzymes, and the application of carvacrol can delay the post-harvest softening and senescence of mango fruits [39]. In this study, it was observed that, during the post-ripening process of kiwifruits, the contents of protopectin and cellulose gradually decreased, while the content of water-soluble pectin increased. Compared to the control group, fruits treated with carvacrol exhibited significantly higher levels of protopectin and cellulose. Additionally, the activity of soluble pectin, PME, PG, Cx, β-Gal, and α-L-Af enzymes were lower in the carvacrol-treated group than in the control group. Correlation analyses revealed that, as the storage time increased, fruit firmness was positively correlated with both original pectin and cellulose content but negatively correlated with the water-soluble pectin content. Furthermore, significant positive or negative correlations were found between these parameters and cell wall metabolism enzymes ($p < 0.05$). These findings suggest that the carvacrol treatment may slow down the degradation of cell wall polysaccharides by reducing the activity of cell wall degrading enzymes in the kiwifruit, thereby maintaining better cell wall integrity and mitigating ripening and softening processes.

5. Conclusions

Our findings demonstrate the efficacy of the carvacrol treatment at mitigating post-harvest decay and regulating cell wall metabolism in the kiwifruit. The application of carvacrol results in increased levels of ascorbic acid and soluble protein by inhibiting the respiratory intensity decline and by minimizing weight loss, while also promoting the accumulation of total phenols and flavonoids. Furthermore, the carvacrol treatment significantly reduces the enzymatic activity of PG, PME, C_X, β-Gal, and α-L-Af. The activity of these enzymes exhibits significant negative correlations ($p < 0.05$) with firmness, VC, starch content, total phenols, flavonoids, cellulose, and protopectin. Conversely, it shows significant positive correlations ($p < 0.05$) with weight loss, relative conductivity rate, solid–acid ratio, soluble sugars, soluble proteins, and soluble pectin (Figure 6). This leads to a marked delay in the degradation of protopectin and cellulose, a reduction in the soluble pectin content increase, a significant decrease in fruit amylase activity, a slowdown in the starch content decline, an extension to the time before the ratio of soluble sugar to solid acid increases, and a contribution to maintaining high firmness levels in the kiwifruit (Figure 7). These results indicate that the carvacrol treatment can substantially enhance the quality of post-harvest kiwifruits while delaying cell wall metabolism. Carvacrol, a natural plant extract, possesses several advantages, including its ecofriendly nature, environmental safety, ease of volatility, convenient acquisition, and low cost. These properties make it promising for maintaining the post-harvest quality of the kiwifruit. The outcomes of this research hold both theoretical and practical significance for kiwifruit preservation and quality control.

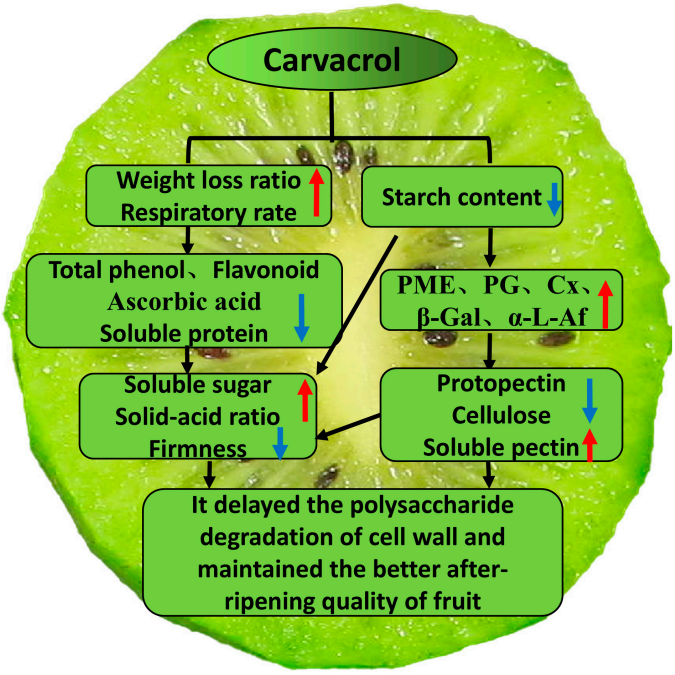

Figure 7. Inhibition mechanism of post-harvest cell wall metabolic activity induced by carvacrol in the kiwifruit. Red arrows denote an increase in substance levels, whereas blue arrows signify a decrease in substance levels.

Author Contributions: Writing—original draft preparation, D.L.; methodology, D.L.; software, X.W.; validation, X.W. and T.M.; conceptualization, T.M.; formal analysis, J.C.; writing—review and editing, J.C. and S.C.; visualization, S.C.; supervision, L.B.; project administration, L.B.; funding acquisition, L.B. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Guizhou Provincial Key Technology R&D Program (Grant No. Qiankehe Chengguo [2019] 4254).

Data Availability Statement: Data are available from the authors upon request.

Conflicts of Interest: The authors declare no conflicts of interest.

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