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Effects of Different Foliar Fertilizer Treatments on Fruit Quality of the Korla Fragrant Pear

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Abstract: Persistent calyx affects the fruit quality of the Korla fragrant pear including increasing fruit hardness. In order to reduce fruit hardness and improve fruit quality, in this study, we used the Korla fragrant pear which has persistent calyx. Korla fragrant pear fruit at different development stages were treated with foliar fertilizers of 101 and calcium–magnesium (Ca–Mg) solutions, and the hardness and pectase, cellulose, lipoxygenase, and amylase activities of persistent calyx fruit were determined and analyzed. We found that the fruit hardness of two foliar fertilizers increased compared with the control after treatment. The fruit hardness of calcium–magnesium foliar fertilizer and 101 foliar fertilizer treatments were increased by 11.7% and 6.8% compared with the control, respectively. Furthermore, the activities of cellulase, pectinase, and lipoxygenase were increased by 30%, 12.7%, and 42% after treatment with calcium–magnesium foliar fertilizer, respectively. The 101 foliar fertilizer inhibited the contents of cellulose and starch. In summary, the internal quality of Korla fragrant pear fruit treated with calcium–magnesium foliar fertilizer was better than 101 foliar fertilizer. It was confirmed that calcium and magnesium foliar fertilizer had a good control effect on the hardness of Korla fragrant pear persistent calyx fruit, and its fruit quality was also improved. This study has great application value in production practice.

Keywords: Korla fragrant pear; fruit hardness; foliar fertilizer; enzyme activity

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1. Introduction

The Korla fragrant pear is one of the geographical indication products recognized by China and Europe. It is a highly regional variety [1]. It has a cultivation history of more than 1400 years in Korla City, Xinjiang [2]. In addition to the core planting area, Korla City, it is planted in its surrounding areas such as Luntai County, Yuli County, Aksu City, and Awati County [3]. Korla City has a warm, temperate, continental, arid, desert climate, with scarce rainfall, less snow in winter, and strong surface evaporation.

The Korla fragrant pear is crispy, thin-skinned, and juicy. However, it often suffers from physiological diseases during growth and cultivation. For example, fruit sclerosis, which often occurs on persistent calyx pears, hardens the flesh and reduces the quality of fruit [4]. The hardened fruit has thick peel, coarse flesh, poor sweetness and crispness, low vitamin content, high stone cell content, large stone cell, large fruit core, and poor flavor.

Persistent calyx fruit of the Korla fragrant pear is spindle-shaped, with the end of the calyx protruding. The persistent calyx fruit surface of the pear's rough peel is often uneven. Mild symptoms occur only in the calyx end, which can be heavy throughout most of the fruit surface. After the fruit matures, the calyx end of the diseased fruit slows down from green to yellow to form "green head fruit" or "green top fruit". The fruit flesh becomes harder.

Fruit hardness is influenced by genetics, the external environment, and cultivation techniques [5]. The texture is an important index of fruit quality and is related to the freshness and edible quality of fruit [6].

Fruit hardness is related to the activities of cell wall degradation enzymes [7]. Cellulase is involved in the hydrolysis of cell wall polymers and plays an important role in fruit softening [8]. During fruit development, amylase activity varies with starch content and can affect the fruit ripening and quality. The degradation of pectin by pectinase breaks down pectin into soluble pectin, thus reducing the fruit hardness [9]. At the same time, the ripening and softening of fruit are also regulated by lipoxygenase [10].

The changes in fruit hardness and related enzyme activities are usually affected by foliar fertilizer during the development of the Korla fragrant pear [11]. The application of calcium-containing foliar fertilizer affects the activities of cell wall degradation enzymes, increases cell hardness, and affects fruit quality [12]. Boron is a common trace element in agricultural production and its deficiency can cause poor fruit quality [13]. Fertilizer has a great influence on fruit enlargement and quality. The application of boron can promote the growth of new shoots, increase the soluble solids and hardness of pear fruit, and decrease the content of fruit acid [14]. The application of boron, copper, calcium, and other trace element fertilizers on foliar spraying is beneficial to plant growth and development [15]. Calcium plays a role in maintaining fruit hardness and reducing the soft fruit rate [16]. The common foliar fertilizers are mainly boron, iron, zinc, and other organic compounds [17]. A reasonable regulation of mineral nutrient supply is an important measure to improve fruit quality [18].

The Korla fragrant pear is a unique fruit in Xinjiang, but fruit sclerosis of persistent calyx fruit affects its quality. At present, there are few studies on the prevention and management of fruit sclerosis. To study the effects of different foliar fertilizer treatments on the fruit quality and related enzyme activities of the Korla fragrant pear, the pear was used as the experimental material and water treatment was used as the control. The effects of the same volume of calcium–magnesium foliar fertilizer and 101 foliar fertilizer on the quality and enzyme activities of the Korla fragrant pear in different periods were compared. The correlation between texture parameters and enzyme activity was analyzed by drawing relevant heat maps. The purpose was to reveal the change rule of texture during the fruit development of the Korla fragrant pear and the effect of foliar fertilizer on its fruit quality, to provide a theoretical basis for preventing fruit hardening.

2. Materials and Methods

2.1. Plant Materials

A 24-year-old Korla fragrant pear tree was collected at an orchard in Alar City, Xinjiang Province. The irrigation method used for fruit trees is flood irrigation, with watering five to seven times a year, the field water holding capacity controlled at 60–80%, and many times of watering and fertilization in the early stages to promote seedling growth; by mid-July, when there is an appropriate control of watering to control the growth rate, the soil type is sandy loam. The following materials will be used: organic matter 18.35 $g \cdot kg^{-1}$, alkali-hydrolyzable nitrogen 29.25 $mg \cdot kg^{-1}$, available phosphorus 14.21 $mg \cdot kg^{-1}$, and available potassium 113.7 $mg \cdot kg^{-1}$. The orchard is managed according to conventional management and the row spacing is 3 m $\times$ 4 m. Thirty trees with relatively uniform growth were selected and divided into three groups. The buds on the short fruit branches in the middle of the west side of the row were sprayed with water (control), 101 foliar fertilizers (Guoguang produced Ca $\geq$ 100 g/L, B $\geq$ 10 g/L), and calcium–magnesium foliar fertilizers (Guoguang produced medium element water-soluble fertilizer Ca $\geq$ 160 g/L, Mg $\geq$ 10 g/L). A 2 L solution was sprayed on each tree.

From 15 May, it was sprayed once every 30 days, for a total of four times. The sample object was the persistent calyx fruit of the Korla fragrant pear. The fruit on the short fruit branch outside the canopy was collected on 15 June, and the samples were collected between 9:00 and 12:00 over intervals of 15 days. The sampling periods were S1 (15 June), S2 (1 July), S3 (15 July), S4 (1 August), S5 (15 August), and S6 (1 September). A total of six samples were collected, with 30 fruit collected from each sample. The sample collection method was random sampling. The fruit were placed in an icebox at the laboratory. Some of the fruit were used to measure the hardness and staining of sections and other fruit were frozen with liquid nitrogen for further experiments.

2.2. Test Equipment

TA.XT plus texture analyzer (Stable Micro Systems., London, UK). Label, kettle, ice bag, 2 mL PE tube, centrifuge (CENCE H1750R.; Changsha, Hunan, China), microplate reader (TECAN Infinite R 200 PRO; Männedorf, Switzerland), water bath pot (Jinyi Instruments DK-8D.; Changzhou, Jiangsu, China), electronic balance, beaker, volumetric flask, filter paper, mortar, tweezers, 10–100 pipette gun, 100–1000 pipette gun, 96-hole UV plate, and optical microscope.

2.3. Determination of Internal Material Indexes of Pulp Tissue

The fruit were sliced by hand and the pectin was stained with a 0.03% ruthenium red solution. The starch was stained with 0.1% iodine–potassium iodide solution. Cellulose was stained with 0.5% safranine solution; callose was stained with 0.05% aniline blue solution, washed with water for 2 min, and photographed by optical microscope [19].

2.4. Determination of Flesh Texture

The fruit was placed on the surface of a physical analyzer and measured 10 times with a mass analyzer, with the average value calculated. Parameters were set as follows: the force sensing element range was 25, the deformation component was 40%, the detection speed was 1.5 mm·s^{-1}, the initial force was 0.5 N, and the following flesh texture assessment parameters were obtained: hardness, adhesiveness, elasticity, chewiness, and viscosity [20].

2.5. Determination of Pectin Degrading Enzyme

An amount of 8 g of flesh pulp was added with the EDTA solution and PVP (pH = 7) to grind into homogenate. The homogenate was transferred to a 25 mL volumetric flask, while the volume was fixed with ultrapure water and was centrifuged at 4000× g for 15 min at 4 °C. The supernatant was taken out as the crude enzyme solution of pectin degrading enzyme, with specific reference to "the principle and method of biochemical experiment technology" [21]. The pectin and redistilled water were then heated in a boiling bath for 5 min. After natural cooling, 5 mL Na_2CO_3 solution, 1 mL iodine solution, and a small amount of sulfuric acid, sodium thiosulfate, and starch was added to the solution and then titrated. The number of milliliters of Na_2CO_3 consumed was recorded. The test was repeated three times.

2.6. Determination of Cellulase Activity

An amount of 8 g of flesh pulp was ground with enzyme extract (NaCl, EDTA, PVP) and homogenized, and then centrifuged at 10,000× g for 10 min at 4 °C. After 1 mL of the prepared enzyme solution was preheated at 37 °C for 3 min in a constant temperature water bath, 1% CMC-Na 2 mL was added and reacted at 40 °C for 30 min. Then, 1.5 mL of DNS reagent was continuously added to the solution and was then added to a boiled water bath for 5 min and the volume was fixed to 10 mL with redistilled water. Colorimetry was performed at 540 nm with a spectrophotometer. The standard curve was drawn with glucose as the standard sample [22] (p. 96).

2.7. Determination of Amylase Activity

An amount of 1 g flesh was added with 1 mL extraction solution, ground at low temperature, and diluted with redistilled water. The solution was centrifuged at 8000× g for 10 min at 4 °C and the supernatant was taken out and placed on ice for testing. The supernatant was added to a citric acid buffer and NaOH (pH = 7) and then bathed with water at 40 °C for 30 min. NaOH and dinitrosalicylic acid were added to the solution, mixed well, and developed in boiling water bath for 15 min. The absorbance was measured after the solution was cooled. Colorimetry was performed at 540 nm with a spectrophotometer. The standard curve was drawn with glucose as the standard sample [22] (p 83).

2.8. Determination of Lipoxygenase Activity

After 70 mg of sodium linoleic acid, 70 µL of TritonX-100, and 4 mL of anaerobic water were mixed, the solution was titrated with 0.5 mol L^{-1} sodium hydroxide until it was clear, with a constant volume of 25 mL and stored at −18 °C for standby. Then followed the extraction of crude enzyme solution: 2.0 g of flesh tissue was placed in a pre-cooled mortar, and liquid nitrogen and 10 mL of 50 mol L^{-1} phosphate buffer (pH = 7.0), pre-cooled at 4 °C, were added for grinding. The mixture was centrifuged at 15,000× *g* for 15 min at 4 °C. The supernatant was taken out for the determination of enzyme activity. Sodium linoleate mother liquor (25 µL), citric acid-phosphate buffer (pH = 6.0) (2.775 mL), and enzyme solution (0.2 mL) were added to the enzyme solution in a 30 °C water bath. The enzyme solution was added. The change of absorbance at 234 nm within 1 min was measured and recorded. The test was repeated three times. The absorbance was used to represent its activity, and the absorbance increase of 0.001 was used as an active unit.

2.9. Data Analysis and Statistics

The data were analyzed with the mean ± standard deviation of three replicates. IBM SPSS 26 was used for variance analysis and the LSD method was used for multiple comparisons ($p < 0.05$ was considered statistically significant). GraphPad Prism 9.5.0 and Origin 2021 software were used for plotting [23].

3. Results

3.1. Effects of Different Foliar Fertilizers on Fruit Hardness and Texture of the Korla Fragrant Pear

3.1.1. Effects of Different Foliar Fertilizers on Fruit Hardness during the Development of Korla Fragrant Pear Persistent Calyx Fruit

As can be seen from Figure 1a, the hardness of pear fruit first increased and then decreased with ripeness during development. The force required for the flesh to deform under the action of external force is called hardness. The hardness change trend of the Korla fragrant pear after different leaf fertilizer treatments was basically the same, but the hardness trend was significantly different in every period. There was no significant difference between S1 and S2, but significant differences existed between S2 and S3, S3 and S4, S4 and S5, and S5 and S6. There was no significant difference between S4 and S5 after leaf 101 foliar fertilizer treatment, but significant differences existed between S1 and S2, S2 and S3, S3 and S4, and S5 and S6. S2 and S3, S3 and S4, and S5 and S6 were significantly different after treatment with calcium and magnesium leaf fertilizer. Compared with the two foliar fertilizer treatments, it was found that the fruit hardness of calcium–magnesium foliar fertilizer treatment was higher than that of 101 foliar fertilizer treatment at S3, S4, and S5, and the difference was 17.9 N, 15.1 N, and 11.8 N, respectively. Comprehensive analysis of the three different treatments showed that the maximum hardness peak of the three different treatments was at S3, which first increased and then decreased with the fruit development time. The difference value of fruit hardness under the two foliar fertilizer treatments at the fruit ripening stage was 11.8 N. In summary, calcium and magnesium foliar fertilizer treatment and 101 foliar fertilizer treatment reduced the hardness of Korla fragrant pear fruit compared with the control group, and calcium and magnesium foliar fertilizer treatment had the greatest impact on the hardness of Korla fragrant pear fruit.

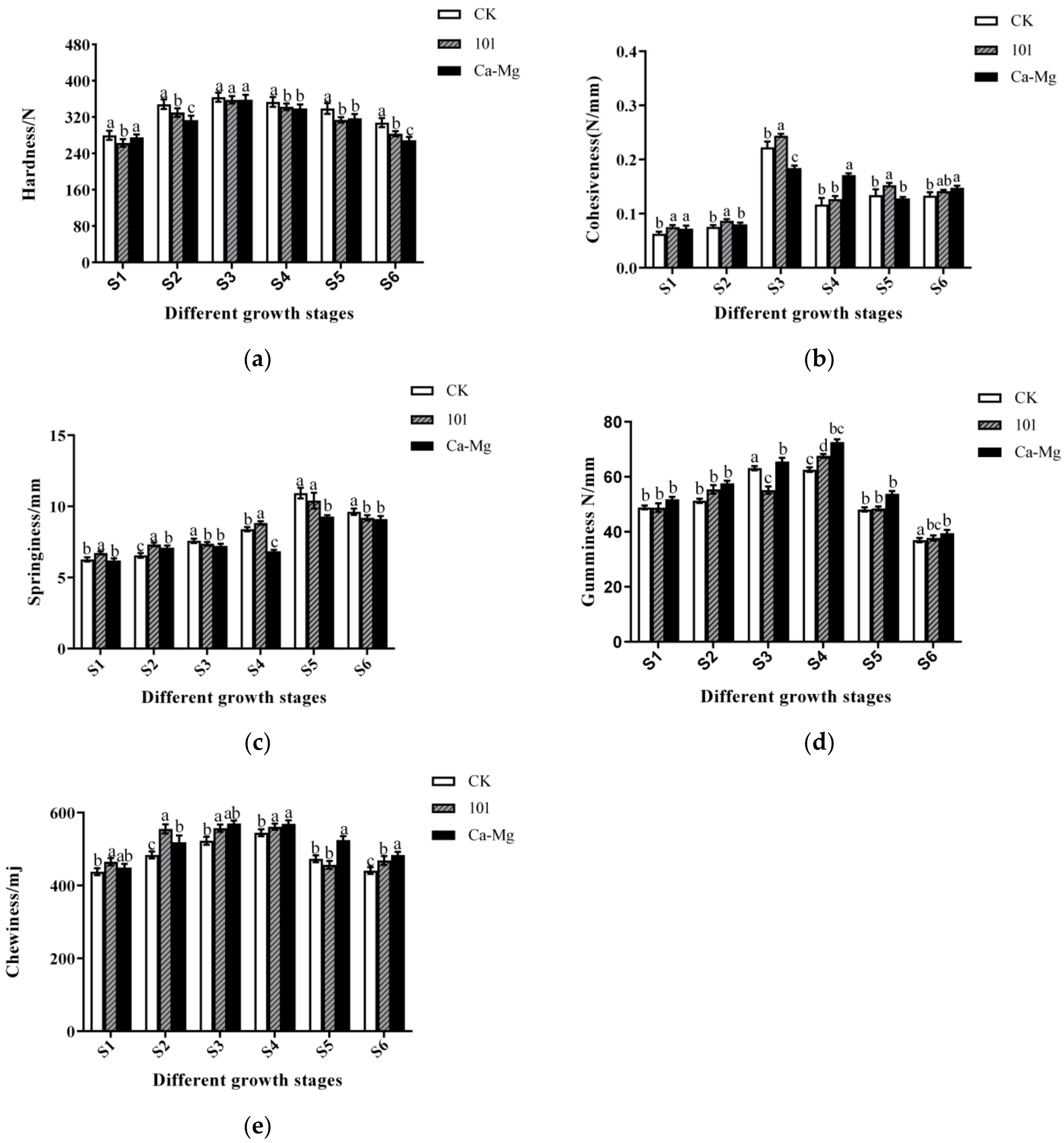

Figure 1. Effects of different foliar fertilizers on hardness, cohesiveness, springiness, gumminess, and chewiness of the Korla fragrant pear. (**a**) Effects of different foliar fertilizers on fruit hardness during the development of Korla fragrant pear persistent calyx fruit. (**b**) Effects of different foliar fertilizers on fruit cohesiveness during the development of Korla fragrant pear persistent calyx fruit. (**c**) Effects of different foliar fertilizers on the springiness during the development of Korla fragrant pear persistent calyx fruit. (**d**) Effects of different foliar fertilizers on gumminess during the development of Korla fragrant pear persistent calyx fruit. (**e**) Effects of different foliar fertilizers on chewability during the development of Korla fragrant pear persistent calyx fruit. (In the figure, abc represents the significant difference).

3.1.2. Effects of Different Foliar Fertilizers on Fruit Adhesiveness during the Development of Korla Fragrant Pear Persistent Calyx Fruit

As can be seen from Figure 1b, the adhesiveness of Korla fragrant pear persistent calyx fruit increased, then decreased, and then increased as the fruit ripeness increased. Cohesiveness reflects the size of the intercellular binding force, which is to evaluate the nature of the fruit to maintain integrity when chewing the flesh. There was a significant difference between S1 and S2 in the control group, and no significant difference between

S3, S4, S5, and S6. There were significant differences between S1 and S2, S3 and S4, and S4 and S5 in the fruit treated with 101 foliar fertilizer, but there were no significant differences between S2 and S3, and S5 and S6. There were significant differences between S1 and S2, S2 and S3, and S3 and S4, but there was no significant difference between S5 and S6. After 101 treatments of 101 foliar fertilizer, the calyx adhesiveness was highest in the Korla fragrant pear, followed by the control group. The adhesiveness of calcium–magnesium foliar fertilizer to fruit was slightly lower than that of the other two groups. According to the difference of adhesiveness change of leaf fertilizer to the Korla fragrant pear, the maximum time difference of S3 reached 0.1 N/mm. In summary, calcium and magnesium foliar fertilizer treatment and 101 foliar fertilizer treatment improved the cohesiveness of Korla fragrant pear fruit compared with the control group, among which calcium and magnesium foliar fertilizer treatment had the greatest effect on the cohesiveness of calyx fruit.

3.1.3. Effects of Different Foliar Fertilizers on Fruit Springiness during the Development of Korla Fragrant Pear Persistent Calyx Fruit

As can be seen from Figure 1c, the springiness of Korla fragrant pear fruit first increased and then decreased with ripeness after different leaf foliar fertilizer treatments. Springiness refers to the ability of the fruit to be stressed and then return to its original state when the pressure is removed. There was a significant difference between S1 and S2 in the control group and S3 and S4. There was no significant difference between S5 and S6. In the 101 foliar fertilizer treatment group, except for S5 and S6, there was no significant difference in the remaining S1, S2, S3, and S4 periods. There was no significant difference in the treatment of calcium and magnesium foliar fertilizer in the S3, S4, S5, and S6 periods except S1 and S2. The springiness of fruit treated with leaf fertilizer was higher than that of the control group. The springiness of fruit treated with calcium–magnesium foliar fertilizer was the smallest, and fruit springiness reached a maximum in the S5 period. From S1 to S6, the maximum difference of fruit springiness between 101 and calcium–magnesium foliar fertilizer was 2.0 mm and the minimum difference was 0.3 mm. After 101 foliar fertilizer was treated, the fruit springiness changed greatly. In summary, calcium and magnesium foliar fertilizer treatment and 101 foliar fertilizer treatment reduced the springiness of Korla fragrant pear fruit compared with the control group.

3.1.4. Effects of Different Foliar Fertilizers on Gumminess during the Development of Korla Fragrant Pear Persistent Calyx Fruit

It can be seen from Figure 1d that the gumminess of Korla fragrant pear fruit increased with ripeness, then decreased. In the control treatment, there were significant differences between S2 and S3, S3 and S4, S4 and S5, and S5 and S6. After 101 foliar fertilizer treatment, there were significant differences between S2 and S3, S3 and S4, and S4 and S5. There was no significant difference between each period after the treatment of calcium–magnesium foliar fertilizer. The gumminess of calcium–magnesium foliar fertilizer was higher than that of the control group. The gumminess of the control group was roughly the same as that of 101 foliar fertilizer. The difference between calcium–magnesium foliar fertilizer and 101 foliar fertilizer was 11.1 N/mm at the maximum and 0.6 N/mm at the minimum. The maximum value of the difference between calcium–magnesium foliar fertilizer and the control group was 10.6 N/mm and the minimum value was 1.9 N/mm. Among the two kinds of foliar fertilizers, calcium and magnesium foliar fertilizers changed the gumminess of Korla fragrant pear persistent calyx fruit more, while 101 foliar fertilizers changed the gumminess slightly less. In summary, 101 foliar fertilizer and calcium–magnesium foliar fertilizer treatment increased fruit gumminess, among which calcium–magnesium foliar fertilizer had the most obvious effect.

3.1.5. Effects of Different Foliar Fertilizers on Chewability during the Development of Korla Fragrant Pear Persistent Calyx Fruit

It can be seen from Figure 1e that the chewiness of Korla fragrant pear persistent calyx fruit first increased and then decreased with the increase of maturity. Chewiness is the ability to evaluate the continuous resistance of fruit to chewing. There were significant differences in chewiness between S1 and S2, S4 and S5, and S5 and S6 in the control group. The chewiness of fruit treated with 101 foliar fertilizer had a significant difference between S3 and S4, S4 and S5, and S5 and S6. After calcium and magnesium foliar fertilization, there were significant differences in fruit chewiness between S1 and S2, S3 and S4, S4 and S5, and S5 and S6. The fruit chewiness of 101 foliar fertilizer treatment was better than that of calcium and magnesium foliar fertilizer treatment in S1, S2, and S4 periods, and the difference was 11.5 mj, 31.1 mj, and 11.8 mj, respectively. The chewiness of the Korla fragrant pear was highest in the fourth period. The difference in chewiness between the fruit treated with 101 and calcium–magnesium foliar fertilizer was small, and the chewing of the fruit treated with 101 foliar fertilizer at maturity was less than that of the fruit treated with calcium–magnesium foliar fertilizer. In summary, 101 foliar fertilizer treatment and calcium–magnesium foliar fertilizer treatment significantly increased the chewiness of Korla fragrant pear fruit compared with the control group, and the effect of calcium–magnesium foliar fertilizer treatment was the greatest.

3.2. Effects of Different Foliar Fertilizers on Tissue Content of Perennial Calyx Fruit of the Korla Fragrant Pear

3.2.1. Effects of Different Foliar Fertilizers on Starch during the Development of Korla Fragrant Pear Persistent Calyx Fruit

It was found (Figure 2) that the flesh starch (C-S6) and 101 (B-S6) treated with calcium–magnesium foliar fertilizer had the characteristics of more layers of cells, smaller cells, and denser arrangement compared with the control group (A-S6) (as shown by the position indicated by the arrow in the picture). However, the starch of the flesh treated with 101 leaf fertilizer changed, and the cell morphology of the single layer could be observed after slicing and squeezing the flesh. Single-layer cells were clear in the flesh treated with 101 foliar fertilizer. From the control (A-S5), 101 foliar fertilizer treatment (B-S5), calcium and magnesium foliar fertilizer treatment (C-S5) to the control (A-S6), 101 foliar fertilizer treatment (B-S6), and calcium and magnesium foliar fertilizer treatment (C-S6), it was not difficult to find that the starch content increased with the maturity period, and calcium and magnesium foliar fertilizer had a more obvious promoting effect on the formation of starch, while 101 foliar fertilizer had an inhibitory effect on the formation of starch.

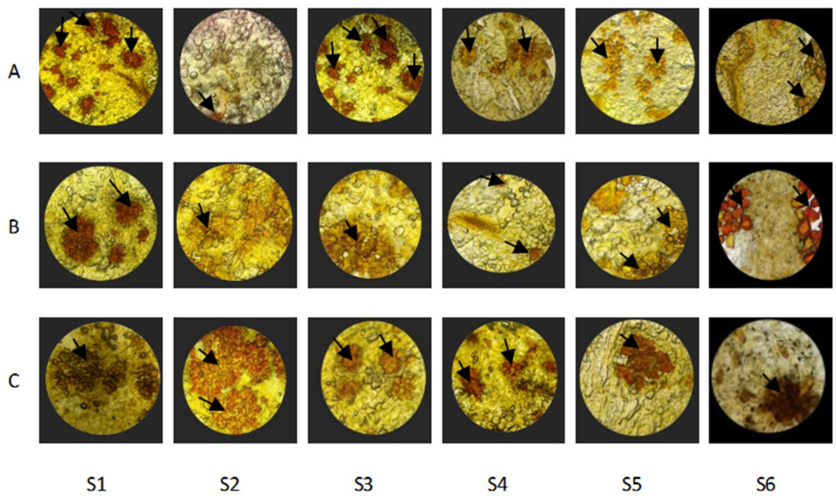

Figure 2. Effects of different foliar fertilizers on starch during the development of Korla fragrant pear persistent calyx fruit. ((**A**) is the control group, (**B**) is 101 foliar fertilizer treatment, and (**C**) is calcium–magnesium foliar fertilizer; S1, S2, S3, S4, S5, and S6 are different periods of fruit tissue. The position indicated by the arrow in the figure is shown as starch).

3.2.2. Effects of Different Foliar Fertilizers on Cellulose during the Development of Korla Fragrant Pear Persistent Calyx Fruit

As shown in Figure 3, it was found that the cellulose content of the pulp treated with calcium and magnesium foliar fertilizers was higher than that of the control group and the fruit treated with 101 foliar fertilizers (as shown by the position indicated by the arrow in the picture). The cellulose treated with calcium and magnesium is shown in Figure 3 (C-S6), which has the characteristics of cell stacking, multiple layers, large cells, and dense arrangement. The cellulose of the flesh treated with 101 foliar fertilizer is shown in Figure 3 (B-S6), which is characterized by cell dispersion, few layers, small cells, and scattered arrangement. As fruit texture is softened, the change in cell morphology will be aggravated. The effects of different leaf fertilizers on fruit characteristics are the level of arrangement of the flesh cells and the degree of deformation of the cells. We found that foliar fertilizer of calcium–magnesium can promote cellulose formation and foliar 101 fertilizer can inhibit cellulose.

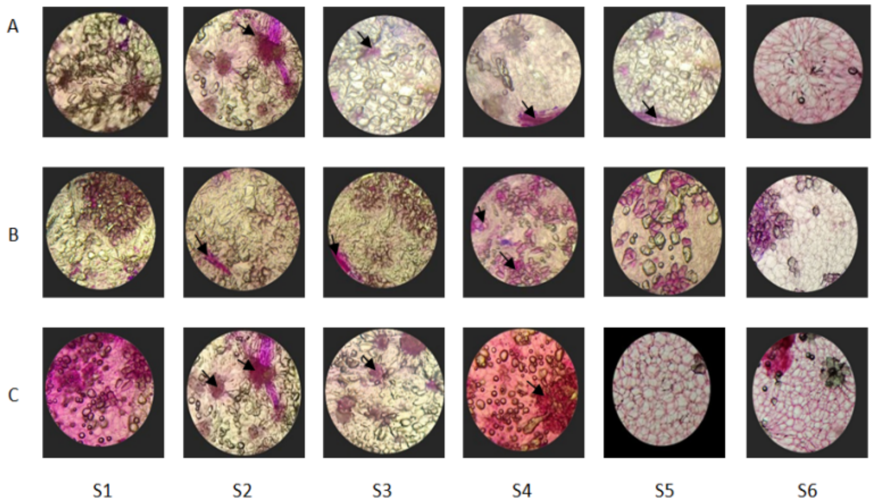

Figure 3. Effects of different foliar fertilizers on cellulose during the development of Korla fragrant pear persistent calyx fruit. ((**A**) is the control group, (**B**) is 101 foliar fertilizer treatment, and (**C**) is calcium–magnesium foliar fertilizer; S1, S2, S3, S4, S5, and S6 are different periods of fruit tissue. The position indicated by the arrow in the figure is cellulose).

3.2.3. Effects of Different Foliar Fertilizers on Pectin during the Development of Korla Fragrant Pear Persistent Calyx Fruit

From the pectin unarmed slices of fruit treated with different foliar fertilizers (Figure 4), it can be seen that as the fruit matured, the change of pectin increased to a stable state (as shown by the position indicated by the arrow in the picture). From Figure 4 (B-S6), it is not difficult to see that the pectin of this period was higher than that of the other two treatments, (A-S6) and (C-S6). Pectins of fruit treated with 101 foliar fertilizers had the characteristics of cell stack, multilevel, small cell, and dense arrangement. These phenomena indicate that the softening of fruit texture will aggravate the change of cell morphology. Features of fruit treated with different foliar fertilizers included the level of arrangement of pulp cells and the degree of deformation of the cells. The pectin content of the fruit sprayed with 101 foliar fertilizer was relatively better than that of calcium–magnesium foliar fertilizer.

3.2.4. Effects of Different Foliar Fertilizers on Callose during the Development of Korla Fragrant Pear Persistent Calyx Fruit

As shown in Figure 5, callose deposition gradually accumulates with fruit ripening (as shown by the position indicated by the arrow in the picture). There was a clear effect of 101 foliar fertilizer-treated fruit on callose formation. There was a greater cell packing area and a more significant degree of staining, while callose formation in fruit treated with calcium–magnesium leaf fertilizer was lower than that of the control. A comprehensive analysis revealed that 101 foliar fertilizers on callose deposition had a clear promoting impact.

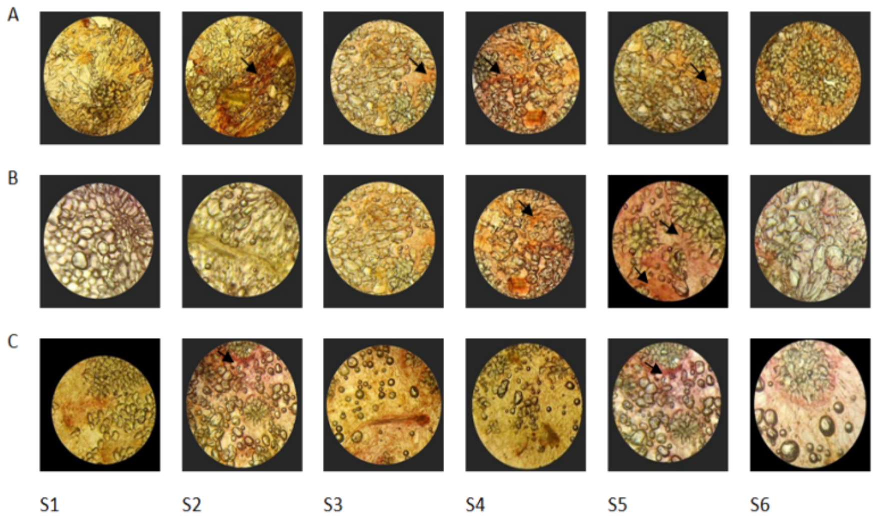

Figure 4. Effects of different foliar fertilizers on pectin during the development of Korla fragrant pear persistent calyx fruit. ((**A**) is the control group, (**B**) is 101 foliar fertilizer treatment, and (**C**) is calcium–magnesium foliar fertilizer; S1, S2, S3, S4, S5, and S6 are different periods of fruit tissue. The position indicated by the arrow in the figure is pectin).

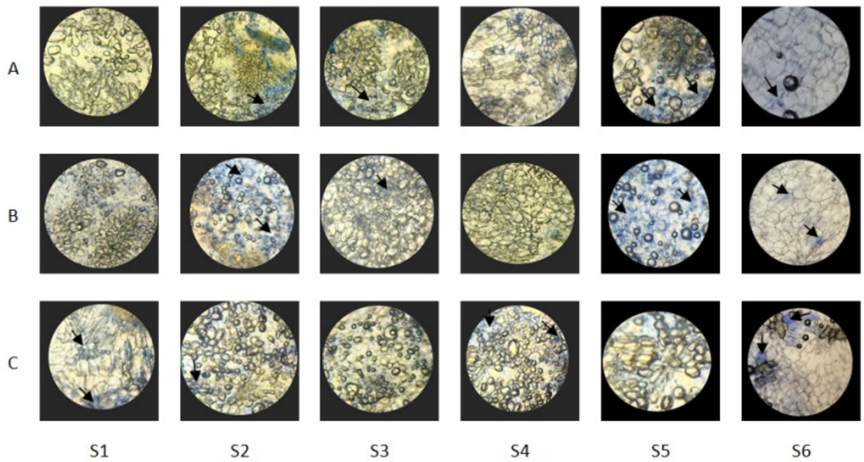

Figure 5. Effects of different foliar fertilizers on callose during the development of Korla fragrant pear persistent calyx fruit. ((**A**) is the control group, (**B**) is 101 foliar fertilizer treatment, and (**C**) is calcium–magnesium foliar fertilizer; S1, S2, S3, S4, S5, and S6 are different periods of fruit tissue. The position of the arrow in the figure is shown as callose).

3.3. Effects of Different Foliar Fertilizers on Enzyme Activity of Perennial Calyx Fruit of the Korla Fragrant Pear

3.3.1. Effects of Different Foliar Fertilizers on Pectinase Activity during the Development of Korla Fragrant Pear Persistent Calyx Fruit

As shown in Figure 6a, Korla fragrant pear fruit in the overall development process showed a bimodal trend, reaching the highest activity in the S5 period. The enzyme activity of the control group fruit was lower than that of 101 foliar fertilizer and calcium–magnesium foliar fertilizer. In the S1, S4, and S6 periods, the enzyme activity of fruit treated with 101 foliar fertilizer was lower than that of the control group. Pectinase activity of calcium–magnesium foliar fertilizer was higher than 101 and the control group. Pectinase activity of the control group was lower than that of 101 foliar fertilizer treatment. The difference of pectinase activity between calcium–magnesium foliar fertilizer and 101 foliar fertilizer was 0.0086 U/mL and 0.0012 U/mL. The maximum value of the difference between calcium–magnesium foliar fertilizer and the control group was 0.0127 U/mL and the minimum value was 0.0036 U/mL. Comprehensive analysis of the two kinds of foliar fertilizer indicated that calcium–magnesium foliar fertilizer treatment had a greater impact on pectinase activity for Korla fragrant pear fruit. The difference of pectinase activity between the two foliar fertilizers was small until the fruit ripening stage.

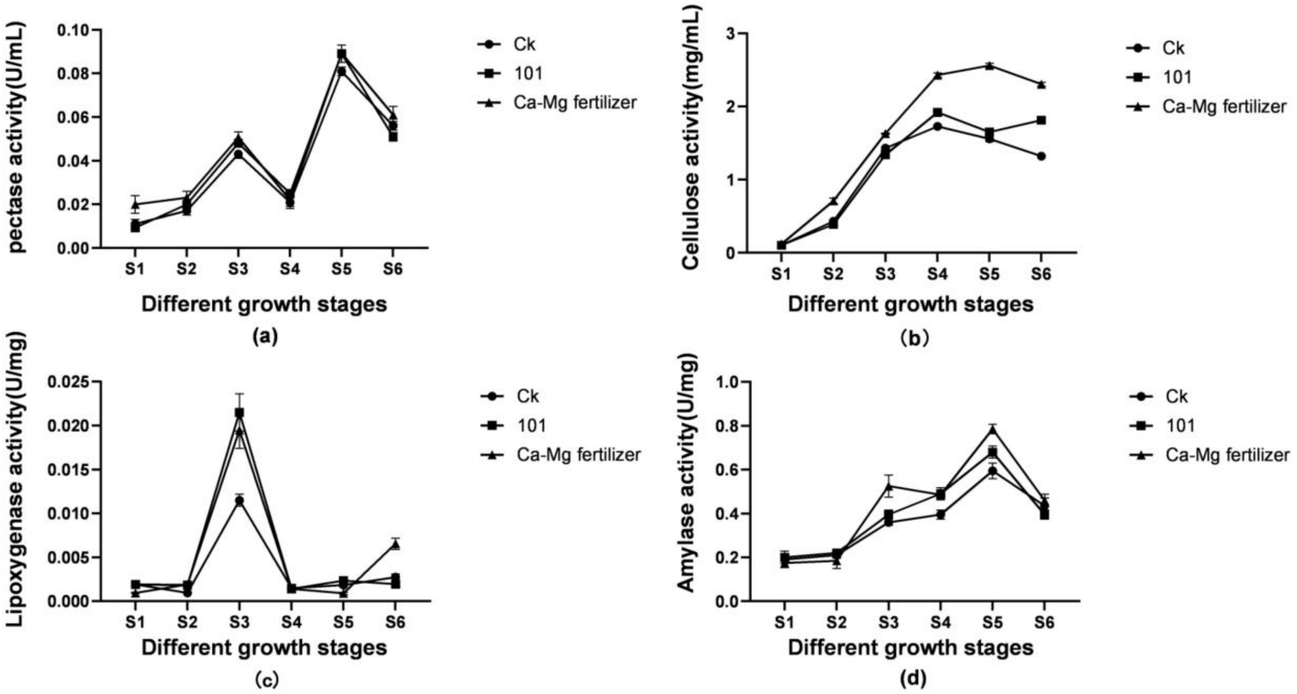

Figure 6. Effects of different foliar fertilizers on pectinase activity, cellulase activity, lipoxygenase activity, and amylase activity of the Korla fragrant pear. (**a**) Effects of different foliar fertilizers on pectinase activity during the development of Korla fragrant pear persistent calyx fruit. (**b**) Effects of different foliar fertilizers on cellulase activity during the development of Korla fragrant pear persistent calyx fruit. (**c**) Effects of different foliar fertilizers on lipoxygenase activity during the development of Korla fragrant pear persistent calyx fruit. (**d**) Effects of different foliar fertilizers on amylase activity during the development of Korla fragrant pear persistent calyx fruit.

3.3.2. Effects of Different Foliar Fertilizers on Cellulase Activity during the Development of Korla Fragrant Pear Persistent Calyx Fruit

As shown in Figure 6b, during the development of the Korla fragrant pear, the cellulase activity experienced an overall upward trend. Fruit treated with different foliar fertilizers were different: the cellulase activity increased slowly after 101 fertilizer treatment, then decreased, and increased again. After the treatment of calcium–magnesium foliar fertilizer, the cellulase activity first increased and then decreased slowly but tended to be stable as a whole. Calcium–magnesium foliar fertilizer showed higher cellulase activity than 101 and the control group and showed different enzyme activities. The cellulase activity of 101 foliar fertilizer was higher than that of the control group in the first three periods, and the cellulase activity of 101 foliar fertilizer was lower than that of the control group in the last three periods. The differences in enzyme activity between foliar calcium–magnesium fertilizer and foliar 101 fertilizer were 0.6 mg/mL and 0.05 mg/mL, respectively. The maximum difference in enzyme activity between 101 leaf fertilizer and the control group was 0.2 mg/mL and the minimum value was 0.04 mg/mL.

3.3.3. Effects of Different Foliar Fertilizers on Lipoxygenase Activity during the Development of Korla Fragrant Pear Persistent Calyx Fruit

As shown in Figure 6c, lipoxygenase activity first increased and then decreased during the development of the Korla fragrant pear. The activity of lipoxygenase showed a low state in the young fruit period, reached a peak in the S3 period, then decreased and maintained a low level. The lipoxygenase activity of fruit treated with 101 foliar fertilizer was higher than that treated with calcium–magnesium foliar fertilizer. The lipoxygenase activity of 101 foliar fertilizer was higher than that of calcium–magnesium foliar fertilizer and the control group, and the difference was significant at the S3 stage. The maximum difference of fruit enzyme activity between 101 foliar fertilizer and calcium–magnesium foliar fertilizer was 0.002 U/mg. The maximum value of the difference between the enzyme activity of the fruit after treatment with the control group was 0.011 U/mg and the minimum value was 0.0001 U/mg. The maximum difference of fruit enzyme activity between calcium–magnesium foliar fertilizer and the control group was 0.0009 U/mg and the minimum value was 0.0001 U/mg.

3.3.4. Effect of Different Foliar Fertilizers on Amylase Activity during the Development of Korla Fragrant Pear Persistent Calyx Fruit

As shown in Figure 6d, during the development of the Korla fragrant pear, the activity of amylase first increased, then decreased, then increased, and then decreased, reaching the highest activity in S5. The amylase activity of calcium–magnesium foliar fertilizer was higher than that of 101 foliar fertilizer and the control group, and the difference was significant at the S5 stage. The difference of enzyme activity between calcium–magnesium foliar fertilizer and 101 foliar fertilizer was 0.108 U/mg and 0.0367 U/mg. The maximum difference of enzyme activity between calcium–magnesium foliar fertilizer and the control group was 0.167 U/mg and the minimum was 0.073 U/mg. The maximum value of the enzyme activity difference between 101 foliar fertilizer and the control group was 0.059 U/mg and the minimum value was 0.025 U/mg.

3.4. Correlation Analysis between Texture Parameters and Cell Wall Enzyme Activity

Correlation analysis showed that hardness and gumminess, hardness and chewiness, chewiness and gumminess, cellulase and adhesiveness, amylase and adhesiveness, amylase and cellulase, pectinase and amylase, and pectinase and cellulase were significantly correlated. It showed that there was a significant impact, with hardness and adhesion, and springiness and pectinase significantly correlated, with correlation coefficients of 0.47 and 0.50, respectively. It showed that there was a very significant impact. There was a significant negative correlation between adhesiveness and gumminess, with a correlation coefficient of 0.39. There was little correlation between hardness and springiness, amylase, pectinase, and lipoxygenase, with correlation coefficients of 0.45, 0.34, 0.01, and 0.20, respectively. The correlation between springiness and adhesiveness, gumminess, chewiness, cellulase, pectinase, and lipoxygenase was small, with correlation coefficients of 0.35, 0.03, 0.08, 0.46, 0.47, and 0.31, respectively. The correlation between gumminess and cellulase, amylase, and lipoxygenase was low, with correlation coefficients of 0.13, 0.01, and 0.31, respectively. There was little correlation between chewy and cellulase, amylase, and lipoxygenase, with correlation coefficients of 0.33, 0.22, and 0.18, respectively. A lesser correlation coefficient of 0.06 was found between cellulase and lipoxygenase (Figure 7).

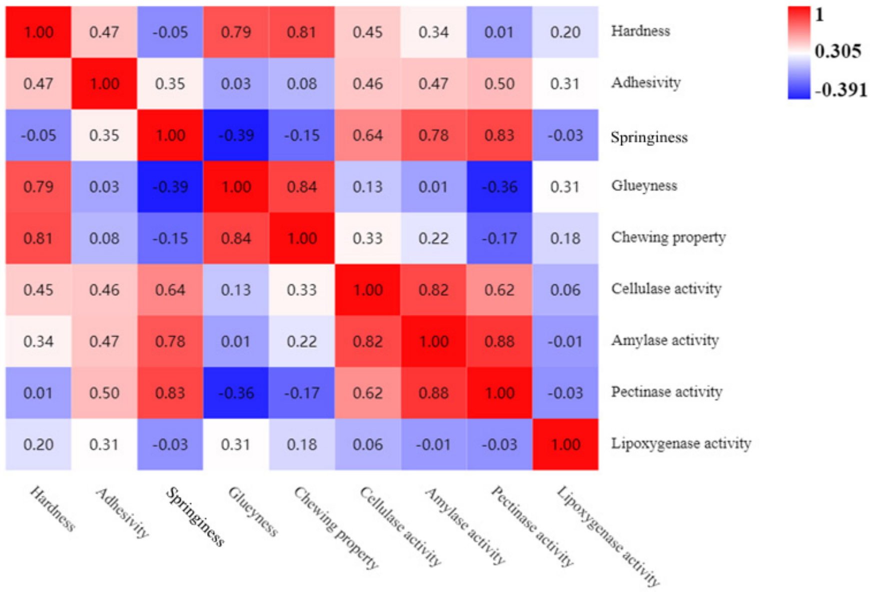

Figure 7. Correlation analysis between texture parameters and cell enzyme activity of the Korla fragrant pear.

4. Discussion

4.1. Texture Change Characteristics of Korla Fragrant Pear Fruit

As hardness can reflect the internal quality of pears, it is often used as a first-class indicator to distinguish external and commercial fruit [24]. Fruit texture is an important index of fruit quality. Wang et al. found that there is also a correlation between texture parameters, and hardness and elasticity are important parameters reflecting the texture of pulp [25]. When the fruit hardness is high, although it is suitable for long-distance transportation, the fruit juice is small and the taste is poor; when the fruit hardness is small, although the flesh is soft, the refreshing degree is reduced and it is vulnerable to external mechanical damage [26]. Texture analyzer can simulate human chewing movement to compress the fruit twice, and TPA can obtain various texture parameters such as hardness, elasticity, cohesiveness, gumminess, and chewiness. The results of Zhang et al. showed that the application of fruit and vegetable calcium fertilizer in the field could increase the fruit hardness of the Korla fragrant pear, and it only reached a significant difference level under extreme calcium treatment [27]. The above test was consistent with the results of this test; however, the fruit hardness of Korla fragrant pears treated with calcium and magnesium foliar fertilizer at the S6 stage was lower than that of the other treatments. The difference in the results may be due to differences in tree age, tree growth, growth environment, and the type and concentration of foliar fertilizers applied. Magnesium is one of the essential mineral elements for the growth of fruit trees, which is the constituent element of chlorophyll. It is also an enzyme activator, which accelerates the enzymatic reaction and promotes metabolism. Many scholars' studies have shown that magnesium has a significant role in promoting fruit tree growth and improving fruit quality [28]. The results of this experiment showed that calcium and magnesium foliar fertilizer treatment reduced the hardness of Korla fragrant pear fruit and improved fruit quality in the late stages of fruit growth and development; however, if the calcium concentration is too high, it may lead to increased fruit hardening. Other fruit trees use a recommended reasonable control of calcium and magnesium concentration, as well as the proportion of calcium and magnesium fertilizer.

4.2. Effects of Different Foliar Fertilizer Treatments on Fruit Hardness and Related Degradation Enzyme Activities of the Korla Fragrant Pear

Pectin is the main component of the primary cell wall and middle pectin layer, which affects fruit quality. Zhao et al. studied the role of protopectin in cell wall degrading enzymes with strawberries as experimental material [29]. With the maturation of strawberries, the amount of protopectin decreased and soluble pectin content increased. The results of this experiment showed that 101 foliar fertilizer promoted the increase of pectin content, followed by calcium and magnesium foliar fertilizer, but both were higher than the control group. This experiment further demonstrated the previous conclusions and proved that the two foliar fertilizers could reduce fruit hardening. The inclusions of starch serve a supportive function in the cell and maintain cellular turgor. This inclusion is rapidly converted to soluble glucose after starch decomposition and then metabolism, leading to weakened cell swelling and softening of the fruit. The experimental results showed that. There was a significant increase in amylase activity in fruit treated with calcium–magnesium foliar fertilizer, which effectively reduced the hardening of fruit. Zhou et al. studied the effects of different concentrations of salt boron solution on callose in cabbage. The results showed that the callose content in the surface papilla cells of cabbage decreased significantly [30]. This conclusion is consistent with the effect of calcium and magnesium foliar fertilizer on fruit callose in this experiment, and there is a significant decreasing trend. It was proved that the hardness of the fruit was reduced and fruit quality was improved after the treatment of calcium and magnesium foliar fertilizer.

Fruit softening is closely related to cell wall degrading enzyme activity. Starch inclusions play a supporting role in cells and maintain cell turgor. These inclusions are rapidly converted into soluble glucose after starch decomposition, which is then metabolized, resulting in reduced cell expansion and fruit softening. The experimental results showed that the amylase activity of fruit treated with calcium and magnesium foliar fertilizer was significantly increased, which effectively reduced the hardening of fruit. Previous studies have found that starch can be hydrolyzed into soluble sugar under the catalysis of amylase, which reduces the swelling power of cells and ultimately leads to fruit softening [31,32]. This experiment further demonstrated the previous conclusions. At the same time, in red raspberry fruit, there was a significant positive correlation between the change of starch content and fruit hardness [33]. This experiment showed that the amylase activity of calcium and magnesium foliar fertilizer treatment was higher than that of other groups. It was proved that calcium and magnesium foliar fertilizer treatment could reduce fruit hardness and improve fruit quality, which further demonstrated the previous conclusions [34]. Cellulase depolymerizes cellulose into low molecular sugars, while pectinase can remove the ester group of the galactose carboxyl group on the pectin molecular chain and make the uronic acid residue generate polygalacturonic acid, which provides the substrate for the action of polygalacturonase. The synergistic effect of cell wall degrading enzymes leads to reduced intercellular connections and unstable cell wall components, resulting in fruit softening and decay [35]. Some studies have found that cellulase activity increased by six times with strawberry ripening. At the same time, researchers observed the cellulase activity of two strawberry varieties dynamically and found that cellulase activity increased with fruit ripening [36]. In this experiment, by comparing the cellulase activity of three different treatments of pear fruit in each critical period, it was found that the cellulase activity of fruit treated with 101 foliar fertilizer and calcium–magnesium foliar fertilizer mainly showed an upward trend, which further demonstrated the previous conclusions. Other studies have found that in the process of fruit ripening and softening of the Qiuzi pear, cellulase mainly plays a role in the middle stage of fruit ripening and softening of Nanguo and Anli pears, but has no obvious effect in the later stage [37]. The results of this experiment showed that calcium and magnesium foliar fertilizer could increase the activity of cellulase, thereby reducing the hardening phenomenon of fruit, and this effect was most obvious in the middle stage of fruit development. Different to the results of this experiment in the later stage, it may be caused by different tree age, tree growth, growth environment, and the type and concentration of foliar fertilizer applied. Lipoxygenase directly acts on fruit softening by catalyzing lipid peroxidation of biofilm, resulting in increased or even degraded plasma membrane permeability and cell dysfunction, and promoting fruit ripening and softening and quality decline [38]. Zhang et al. studied lipoxygenase activity during the ripening of melon, and their results showed that most of the linear lipids were synthesized by lipoxygenase and were highly expressed during fruit ripening [39]. The results of this experiment showed that the lipoxygenase activity of Korla fragrant pears treated with 101 foliar fertilizer was higher than those treated with calcium–magnesium foliar fertilizer, and the two foliar fertilizer treatments were higher than the control group, which proved that the two foliar fertilizers could reduce the fruit hardening phenomenon. This further demonstrates the conclusions of predecessors. Xu et al. used the Korla fragrant pear as the experimental material to regularly detect the activities of pectinase, cellulase, and β-glucosidase during fruit development. These results indicated that the activity of hydrolase increased [40], which was consistent with the changes of related cell wall components and the results of this study.

4.3. Practical Application of Foliar Fertilizer Treatment in the Korla Fragrant Pear

In the production of fruit trees, in order to pursue quick acting, people apply a large quantity of chemical fertilizers for a long time; this not only leads to soil hardening but also the trace elements in the fertilizer are quickly fixed by the soil, resulting in a very low content of available trace elements in the soil, which will cause serious nutrient deficiency and cannot meet the normal growth and fruit development of fruit trees [41]. Spraying foliar fertilizer reasonably makes up for the shortcomings and defects of crop root topdressing and is an efficient and practical trace element fertilizer [42]. Studies have shown that foliar fertilizer also has the effect of improving fruit quality and it has been widely used in fruit tree production [43–45]. However, foliar fertilizer has the disadvantages of low absorption and failure when mixed with some pesticides, so it cannot be used as the main fertilization method. It is necessary to give full play to the effect of increasing yield and quality on the basis of root fertilization. In the application of calcium and magnesium foliar fertilizer, the Korla fragrant pear should pay attention to the best application effect in the young fruit growth period and fruit expansion period, and also pay attention to the concentration of calcium and magnesium elements to achieve the effect of precise fertilization.

5. Conclusions

The Korla fragrant pear has created huge economic benefits in the pear fruit market because of its refreshing taste, but the fruit hardening of calyx fruit has seriously affected the fruit quality of the Korla fragrant pear. It limits the development and promotion of the Korla pear. The results of this experiment showed that the application of calcium and magnesium foliar fertilizer treatment in the young fruit growth period and fruit enlargement period could increase the activity of degrading enzymes and reduce the fruit hardness of the calyx fruit. Therefore, spraying calcium and magnesium foliar fertilizer in time during the development of the Korla fragrant pear can improve fruit quality, which has a certain application value and a promotional prospect in the production practice of the Korla fragrant pear.

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