Comprehensive evaluation of Korla fragrant pears and optimization of plucking time during the harvest period

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Abstract: To optimize the harvest of Korla fragrant pears and to provide a theoretical basis for post-harvest processing, a total of 26 basic indices of Korla fragrant pears, including their morphology, quality, and softening age, were investigated. The harvest period ranged from August 22nd to October 6th, samples were collected every 5 d (totally 10 plucking days, indicated as H1-H10). The results indicated that changes in pectin content were the main causes of softening in fragrant pears. The scavenging of free radicals mainly occurred because of the collaborative effects of superoxide dismutase (SOD) and catalase (CAT). In the harvest periods H1-H3, the hardness, titratable acid (TA), chlorophyll content, density, and diameter of the stone cells, as well as the cellulose and hemicellulose content of the Korla fragrant pears were at their highest. During the periods H7-H10, the single-fruit weight, fruit horizontal and vertical diameter, color coordinates *L*, *a**, *b**, soluble-solids content (SSC), SOD activity, CAT activity, and water-soluble pectin were higher than in the other plucking periods. The highest vitamin C (VC) content and moderate values for a variety of indicators were observed during H3-H7. Variations in the Korla fragrant pears during H1-H3 mainly manifested through changes in softening-related parameters. During H3-H10, changes in the softening-related, aging-related, color-related, and quality indices had a dominant role. On this basis, some suggestions for the post-harvest processing of fragrant pears have been proposed. Fruit, during H1-H3, are suitable for transportation and storage; during H7-H10 are suitable for fresh-eating and further processing; and during H3-H7, exhibited moderate values for a variety of indicators and had the highest commercial value. This research provides a systematic evaluation of the characteristics of mature Korla fragrant pears during the harvest period and can form the basis for fruit quality control and processing of Korla fragrant pears.

Keywords: Korla fragrant pear, harvest period, plucking time, quality, fruit maturity, evaluation, optimization
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1 Introduction

The Korla fragrant pear is a type of fruit unique to China and is known as 'the queen of pears'[1]. This pear has been the subject of a considerable amount of scientific research and has high commercial value. Characterized by its thin skin, fine-textured fruit flesh, plentiful juice, and sweet crisp taste, Korla fragrant pears have been widely consumed and used in jam processing, distilling fruit wine, and the extraction of functional substances[2]. They are nutritious for humans and significantly improve rural economies. Nevertheless, it is currently impossible to obtain perfect fragrant pears through post-harvest processing owing to limitations regarding farmer experience and harvesting modes, which focus only on the single-fruit weight. This greatly decreases consumer satisfaction and the add-on value of these commodities[3]. Therefore, controlling fruit quality during production is a crucial problem in the sustainable development of the Korla fragrant pear industry.

Maturity at harvest is an important factor that determines fruit quality during the process of transportation and commercialization[4,5]. Park et al.[6] studied the influence of harvest periods on the physicochemical indices of 'Wonhwang' pears, finding that early harvests increased the hardness, titratable acid (TA), and starch content, however, they reduced the weight and soluble-solid content (SSC) of the fruit. Late-harvested fruit had a higher sugar content and increased commercial value. Yilmaz et al.[7] studied the enzymatic activity of twelve varieties of tomato when they were green, pink, and red, finding that enzymatic activity varied across the different stages, which could affect their flavor. Razzaq et al.[8] studied mangoes and found that, with maturation, the fruit-softening enzyme activity and antioxidant enzyme activities, including that of peroxidase (POX) and catalase (CAT), significantly intensified, influencing the nutritional quality of the mangoes. Based on the above research, it can be seen that differences in maturity during the harvest period essentially translate into differences in commercial quality. Therefore, it is imperative to explore the variations in the basic indices of Korla fragrant pears to complete a systematic evaluation of their characteristics at maturity. This can help control fruit quality and ascertain which fruit is most suitable for consumers and enterprises.

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However, existing studies on Korla fragrant pears have mainly focussed on comparisons of yield and quality with other pear varieties[9], as well as variations in fruit quality, their chemical constituents, and antioxidant activity under different storage conditions. It was learned that a comprehensive evaluation of Korla fragrant pears during the harvest period and studies on their commercialization have not been reported to date.

In this study, the characteristics of mature Korla fragrant pears were assessed during the harvest period, including twenty-six basic indices concerning the morphology, quality, softening and aging. In order to provide a reliable reference and technological support for harvest and processing standards in the Korla fragrant pear industry, and also acts as a scientific basis to improve fruit quality in production.

2 Materials and methods

2.1 Test samples

The Korla fragrant pear samples were collected from two conventionally managed pear gardens: the high-quality Korla fragrant pears production base in the 10th Production and Construction Corps, Alear City, Xinjiang, China (80°30′E-81°58′E, 40°22′N-40°57′N, elevation: 900-950 m) and the 29th Production and Construction Corps, Korla City, Xinjiang, China (85°30′E-86°30′E, 41°30′N-41°68′N, elevation: 902-924 m). The rootstock was birch-leaf pear and the trees were aged between 17-18 years. The harvest period ranged from August 22nd to October 6th (131-176 d after flowering, effective accumulated temperature: 2996.5°C-3861.5°C). Samples were collected every 5 d (totally 10 plucking days, indicated as H1-H10), with the aim of obtaining fragrant pears at different stages of maturity. During the harvest, 300 fragrant pears (150 from each sampling site) that had carpopodium but no deformations, damage or infection were randomly selected. Subsequently, the samples were mixed to analyze their fruit morphology, quality, softening, and aging-related parameters. Physical indices were tested first, followed by chemical indices. Finally, some samples were stored in −80°C liquid nitrogen (DW-HL668, Zhongke Meiling Cryogenic Technology Co., Ltd., China) to test their enzymatic activity.

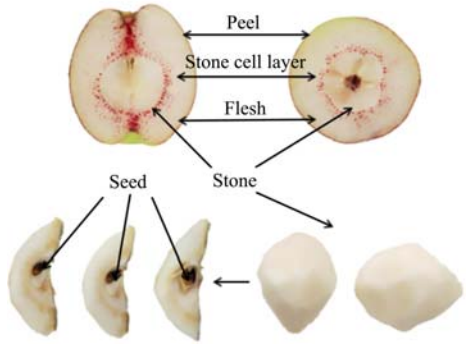

Figure 1 Position of the sampling points on the Korla fragrant pears

2.2 Fruit morphological parameters and color coordinates

2.2.1 Horizontal and vertical diameters of the fruit and the single-fruit weight

The average weights of the Korla fragrant pears at different maturities were determined using a ±0.01 g electronic precision balance (CX2000, Changxie Electronic Technology Co., Ltd., China). The horizontal and vertical diameters (in mm) of the fruit were measured with vernier calipers (NSCING 300, NscingEs Measuring Instrument Co., Ltd., China).

2.2.2 Colour coordinates (L, a*, b*)

A Minolta CR-400 colorimeter (Minolta, Osaka, Japan) was used to measure the skin color at three points (opposite sides of the equator and bottom) of the Korla fragrant pears. The measurement points were chosen outside of blush positions on the fragrant pears. The colorimeter was calibrated using standard white tiles. Further, L denotes the brightness index; a higher value indicates a brighter surface. a* refers to the red-green aberration. If a* is positive, the fruit is red, with a higher value indicating a more reddish appearance. If a* is negative, the fruit is green, with a smaller value indicating a darker green color. b* refers to the yellow-blue aberration. If b* is positive, the fruit is yellow; if b* is negative, the fruit is blue. The higher absolute value of b* indicates the darkness of colors.

2.3 Quality parameters

2.3.1 VC content, SSC, hardness, chlorophyll content, and TA

The vitamin C (VC) content was tested by referring to the China National Standard GB 5009.86-2016: Ascorbic Acid Test in Foods (unit: mg/100g)[10].

The SSC was analyzed following the methods proposed by Lan[11], using a portable sugar measuring refractometer. A juice sample with the seeds removed was dripped onto the refractometer (unit: %).

To test the hardness, 15 fragrant pears were selected and a universal fruit hardness meter GY-4 was used to measure four uniformly distributed points along the equator of the fruit. The pericarp at the measuring point was removed with a small knife and the hardness meter was then slowly inserted perpendicular to the measuring point until the marked standard was reached. The mean of the measurements was subsequently calculated (unit: kg/cm^2).

To analyze the pericarp chlorophyll, China's agricultural standards were followed[12], with certain revisions. Three fragrant pears were randomly selected and a thin layer of pericarp was collected using a curved knife. Next, an abraser was added to the prepared sample slurry. Then, 1 g of this slurry was added to a 50 mL mixture of absolute ethyl alcohol and acetone (1:1); the mixture was subsequently placed in the dark at room temperature for 12 h after being sealed with a membrane. Next, the samples were filtered, and the filtrate was poured into a 50 mL volumetric flask and dissolved to a constant volume of 50 mL. Finally, a mixture of absolute ethyl alcohol and acetone was used as a blank solution to calibrate the spectrophotometer and the absorbance values of the samples were measured at 645 nm and 663 nm. The mean absorbance values were calculated and the chlorophyll in the pericarp was calculated according to Equation (1).

$$\omega = \frac{(8.05 \times A_1 + 20.29 \times A_2) \times \upsilon}{1000 \times m} \quad (1)$$

where, ω is the pericarp chlorophyll content, mg/g; A_1 is the absorbance of the solution at 663 nm; A_2 is the absorbance of the solution at 645 nm; υ is the volume of the tested sample, mL; m is the mass of the sample, g.

Measurement of TA: The TA was measured by using NaOH solution titration method[13]. Distilled water was first used to grind 1 g sample. Then, the grinding fluid was heated in a water bath for 30 min at a constant temperature (80°C) and centrifuged for 10 min at 4000 r/min. Thereafter, the supernatant was collected. Distilled water was added to the precipitate, and the above-mentioned steps were repeated. Then, the supernatant was mixed with water to 25 mL. The extract (20 mL) was used for organic acid titration. The average value was obtained after three

repeated tests.

2.3.2 Diameter and density of the stone cells

Referring to the methods of Liu[14] and Cai et al.[15], 10 pears were randomly selected for each test. Slices (1.0 cm×1.0 cm× 0.3 cm) were collected from near the pericarp, the flesh, and the core by hand and then fixed using FAA stationary liquid (90 mL 50% ethyl alcohol, 5 mL glacial acetic acid, and 5 mL formalin). Paraffin slices (13-20 μm thick) were prepared through the process sequence of dehydration, waxing, cladding, slicing, dewaxing, rehydration, dehydration, red staining, and sealing. The density of the stone cells was determined under a 10×10 microscope view. For each sample position, 10 slices were tested and the mean was calculated. The diameter of the stone cells was measured using an ECLIPSE Ni-U microscope (Nikon, Japan). and ten measurements were performed for each position. The mean was calculated to represent the diameter of the stone cells of the Korla fragrant pears.

2.4 Softening-related parameters

2.4.1 Pectin, cellulose, and hemicellulose content of cell walls

The pectin content of the cell walls of the fragrant pears was measured by referring to the methods of Wang et al.[16]. Water-soluble pectin, ionic pectin, and covalently bonded pectin were measured (unit: mg/g).

The cellulose and hemicellulose content of the cell walls of the fragrant pears was also measured by referring to the methods of Wang et al.[16] and Maria et al.[17] A standard curve was created using glucose standards. The cellulose and hemicellulose contents were measured via anthrone colorimetry (unit: mg/g).

2.4.2 Pectinase activity, cellulase activity, and β-glucosidase activity

The pectinase and β-glucosidase activity in the cell walls of the fragrant pears was tested by referring to the Modern Phytophysiology Experimental Guidelines[18]. The cellulase activity (unit: U/g) was tested by referring to the methods of Cao et al.[19]

2.5 Ageing-related parameters

2.5.1 Test of respiration intensity

In light of a study by Xu[20], an alkali adsorption static method was used and the mean of ten replicates tests was calculated (CO_2/kg·h).

2.5.2 Relative conductivity

By referring to the methods of An et al.[21], holes were made on the equator of the fragrant pears using a 10 mm punch. Flesh tissue was collected from the fragrant pears and formed into uniform discs of tissue. The 2.0 g samples, dried via a filtering method, were collected and put into a beaker, into which 50 mL distilled water was added; the solution was then incubated under (25±1)°C for 30 min. Following this, the conductivity (D_0) was measured. The samples were then placed in a boiling water bath for 15 min before being quickly cooled to measure the conductivity again (D_1). The relative conductivity D (in %) was calculated as follows:

$$D=\frac{D_0}{D_1}\times 100\% \tag{2}$$

2.5.3 Superoxide dismutase (SOD), peroxidase (POD), and catalase (CAT) activity

The SOD activity of the fragrant pears was tested according to the Modern Phytophysiology Experimental Guidelines[18] (unit: U/g).

The POD and CAT activity was tested according to the methods proposed by Li[22] (unit: U/g).

2.6 Static pressure damage test

A static pressure damage test was performed to measure the pressure resistance of fragrant pears during transportation and storage. Before the test, the WD-D3 electronic universal testing machine (Zhuoji Instrument Company, China) was preheated for 30 min. Fragrant pears with weights of (120±5) g were chosen. The equators of the fragrant pears were compressed between rigid plates for 15 s. According to an early pre-test, the bio-yield point of the Korla fragrant pears was at a deformation of 5 mm. Therefore, 5 mm, 7 mm, 9 mm, 11 mm, and 13 mm deformations were chosen in the static loading range of the fragrant pears. At each level, fifteen fragrant pear samples were tested three times.

To complete browning, the fragrant pears were left at room temperature (mean temperature is 20°C) for 24 h after the static-pressure treatment. The fragrant pears were approximately oval in shape. Since the damaged regions were also approximately oval, the damaged areas were calculated using the bruise-measurement method for fruit and vegetables, proposed by Mohenin[23].

$$A=\pi ab \tag{3}$$

where, A is the brown stained area of the damage to the fragrant pears, mm^2; a and b are the long and short axes of the oval damaged areas of the fragrant pears, respectively, mm. The static pressure damage of the fragrant pears was based on the mean results across five levels.

2.7 Statistical analysis

In this study, variance analysis of the data was performed and the results were viewed as statistically significant if the significance was higher than 0.05. Additionally, the number of variables in the data matrix was decreased using principal component analysis (PCA) to facilitate the selection of distinguishing parameters. On this basis, there are different degrees of correlation and overlapping information among different basic indices. However, mass information may hinder the analysis. Also, it is difficult to characterize harvest period correlation with quality. Therefore, it is necessary to construct characteristic variables of the basic indices of Korla fragrant pears to explore implicit variables that are hidden in the original variables and are difficult to measure. The scores of characteristic variables (common factors) calculated using factor analysis were used for a comprehensive evaluation of the harvest period. All data were based on the means of three replicates tests, unless stated otherwise, and were expressed as a mean±standard error.

3 Results and analysis

3.1 Changes in the morphology and color of Korla fragrant pears with maturity

3.1.1 Fruit horizontal, vertical diameters, single-fruit weight, L, a*, and b*

It can be seen from Table 1 that both the horizontal and vertical diameters of the fragrant pears gradually increased throughout the harvest period. The vertical diameter of the fruit experienced rapid growth from H1 to H6. The growth rate declined after H7 and the vertical diameter of the fruit exhibited no significant changes after H9. The horizontal diameter of the fruit increased significantly, by about 12.49 mm, from H1 to H10, but it only increased by about 5.09 mm from H5 to H10, indicating a decreased growth rate in these stages. Similarly, the single-fruit weight gradually increased with the increase of horizontal and vertical diameter, with an average increase of about 20 g from H1 to H10. The increase in the horizontal and vertical diameters was

mainly caused by the fast expansion of the fruit cells along the vertical and horizontal axes during the early harvest period[24]. However, the horizontal and vertical diameters of the fruit were essentially constant in the later stages of maturity.

Table 1 Changes in the morphology and color of Korla fragrant pears during the harvest period

Harvest period	Vertical diameters/mm	Horizontal diameters/mm	Sing-fruit weight/g	*L*	*a**	*b**
H1	50.42^{d}±7.73	40.22^{b}±3.69	109.86^{b}±15.26	59.26^{d}±2.68	-9.71^{c}±1.11	43.25^{c}±1.44
H2	51.42^{d}±4.47	40.75^{b}±5.74	110.92^{b}±25.97	59.83^{cd}±1.43	-9.45^{c}±2.04	43.54^{c}±2.21
H3	53.93^{c}±6.24	42.42^{b}±4.34	116.57^{b}±12.49	60.52^{cd}±2.45	-8.96^{c}±2.03	43.98^{c}±1.54
H4	56.85^{c}±3.66	45.72^{b}±3.82	119.54^{b}±19.08	61.18^{c}±1.73	-8.14^{bc}±1.12	44.55^{bc}±2.05
H5	60.27^{bc}±3.07	47.62^{a}±3.90	121.59^{b}±12.23	62.16^{bc}±1.43	-7.72^{bc}±1.31	44.91^{bc}±1.92
H6	64.44^{b}±6.84	49.17^{a}±4.05	122.45^{ab}±17.58	63.34^{b}±2.64	-7.08^{b}±2.26	45.79^{b}±2.64
H7	67.18^{ab}±6.32	50.81^{a}±3.29	125.55^{ab}±14.45	64.57^{ab}±2.79	-6.15^{ab}±1.27	46.42^{ab}±2.54
H8	67.32^{ab}±5.71	51.58^{a}±3.54	126.27^{ab}±17.73	65.29^{a}±1.23	-5.99^{ab}±2.18	46.88^{ab}±2.95
H9	69.57^{a}±8.74	51.98^{a}±3.84	129.04^{a}±21.89	65.82^{a}±1.90	-5.31^{a}±1.29	47.25^{ab}±2.68
H10	70.27^{a}±4.40	52.71^{a}±4.14	129.48^{a}±18.72	66.05^{a}±2.05	-4.99^{a}±1.24	47.53^{a}±2.83

Note: Means followed by the same letter within the column are not significantly different, $p<0.05$.

The *L*, *a**, and *b** indices are very important because they change significantly in some fruits during different stages of maturity. For example, apricots change from green to yellow at maturity. The *L* of the Korla fragrant pears exhibited an upward trend throughout the plucking period, indicating that fragrant pears that are harvested later are brighter in color. The red-green index *a** was negative, indicating that the Korla fragrant pears were green. In many fruit species, immature fruits are green; however, Korla fragrant pears remain green even at full maturity. The *a** values started at −9.71 at H1 and increased to −4.99 at H10, showing significant changes relative to the early-harvested fruit; the Korla fragrant pears turned from deep green to light green. *b** represents the yellow-blue aberration, which continuously increased throughout the harvest period. This reveals that the late-harvested Korla fragrant pears were more yellow than the early-harvested fruit. This is in line with the conclusions of Pan[25].

3.2 Changes in quality with maturity

3.2.1 Vitamin C (VC), hardness, chlorophyll content, TA, SSC, diameter, and density of stone cells

VC is one of the most important indices related to the nutritive value of fresh fruits, as it offers resistance to oxidation, scavenges free radicals, and delays the aging process after harvest[26]. It can be seen from Table 2 that the VC content in the Korla fragrant pears gradually increased from H1 to H6, reaching a peak at H6, before declining. The VC content at H6 was 82.5% higher than that at H1. The VC content at different stages of maturity could be influenced by effective precursor concentrations (sugars) and VC decomposition or cycling rates[27].

Hardness is one of the most important indices to measure the commercial viability of fruits and is an essential index for recognizing variations in fruit characteristics and quality. The hardness of the fruit is vital for its supply, transportation, and economics[28]. The hardness of the Korla fragrant pears gradually declined throughout the harvest period. It decreased from an initial 7.78 kg/cm^2 to 5.75 kg/cm^2. Brummell et al.[29] demonstrated that the internal factors determining the hardness of fruit include the binding forces among cells, the mechanical strength of cell components, and cell turgidity. External changes are manifested by intercellular processes, such as the decomposition of glue-like substances, lignin, cellulose, etc. This process is accompanied by changes in the structural constituents of cell walls and relevant enzymes; therefore, hardness declines as a collaborative consequence of multiple factors.

The appearance and color of the pericarp is an important metric for consumers to judge fruit quality. Color changes in Korla fragrant pears were significantly influenced by the chlorophyll content. Table 2 shows that the chlorophyll content continuously decreased throughout the harvest period. The reduction in chlorophyll content was not significant during H1-H3; however, from H1 to H10, it decreased by 53.60%, from 0.3401 mg/g to 0.1578 mg/g. Unlike common green vegetables and fruits, which exhibit yellowing and other changes in color as signs of maturity and aging, the pericarp color of Korla fragrant pears only changes from deep green to light green, never turning completely to yellow (based on the measured *L*, *a**, and *b** indices and practical observations), although the chlorophyll quickly degraded during the harvest period.

Malic acid is the primary acid in Korla fragrant pears, and it is the major contributor to its flavor. It can be seen from Table 2 that the TA remained largely stable from H6-H10, showing only minor differences. In general, the TA content continuously decreased across the harvest period. This was mainly caused by respiratory effects because the acid is consumed as a respiratory substrate. Some organic acids are transformed into sugars and accompanied by fruit enlargement. The increase in moisture content triggers the dilution of acids, decreasing the TA content of fragrant pears in the harvest period. This conforms to conclusions drawn from research on Dangshan pears[30].

The SSC is an index for evaluating the quality of fragrant pears. It has a complex composition that directly influences the flavor, taste, and nutrition level of fruits[31]. As nutritious substances are formed through the synthesis of carbon atoms, fruits with high sugar content have the benefits of promoted growth, delayed aging, and protection of the integrity of mitochondria and cytomembranes. The SSC was relatively low in young Korla fragrant pears. With the growth and development of the fragrant pears, substances like starch are hydrolyzed into saccharose, glucose, and fructose; The SSC reached a maximum at H10. This agrees with conclusions drawn by Wang[32] on Fengshui pears during the harvest period.

Stone cells are a type of common thick-walled tissue cell in pears, which form clusters of multiple cells. During the formation of stone cell clusters, cell division occurs layer-by-layer around the first-formed stone cells, and these newly divided cells later also become stone cells. As shown in Table 2, the size of the stone cells did not change significantly from H1-H10 but it did gradually decrease. The density of the stone cells changed significantly from H1-H10. Liu[14] pointed out that the number of stone cells is largely determined in the early-developmental stages of pears and will not increase during later growth. Instead, the number of stone cells per unit volume of flesh gradually decreases with

increases in fruit volume, and this essentially stabilizes two months before maturity. Reductions in the size and density of stone cells in Korla fragrant pears during the harvest period had two causes. Firstly, lignin decreases as the fruit matures, which decomposes some stone cells. Secondly, polygalacturonase activity is strengthened and pectin hydrolyzation starts, destroying the binding forces among stone cells. As a result, individual stone cells are removed from stone cell clusters, which decreases the diameter and density of the stone cell clusters[15]. It is important to note that the diameter of the stone cells in Korla fragrant pears was larger than those of Zaosu pears (144.84 μm) and Jinfeng pears (183.00 μm)[14]; however, Korla fragrant pears have a finer texture and fewer impurities, which is related to the low density of stone cells. The same characteristics were also observed in Dangshan pears[33].

Table 2 Changes in the quality parameters of Korla fragrant pears during the harvest period

Harvest period	VC content /mg·100 g^{-1}	Hardness /kg·cm^{-2}	Chlorophyll content /mg·g^{-1}	TA/%	SSC/%	Stone cell's diameters /μm	Stone cell's density (individual/visual field)
H1	4.65^{b}±0.97	7.78^{a}±1.62	0.3401^{a}±0.0892	0.123^{a}±0.014	10.64^{b}±1.65	308.11^{ns}±98.62	9.66^{a}±2.69
H2	4.97^{b}±1.15	7.6^{a}±1.57	0.3372^{a}±0.0569	0.121^{a}±0.025	10.75^{b}±1.61	303.91^{ns}±85.93	8.74^{ab}±3.49
H3	5.67^{b}±0.68	7.47^{a}±1.22	0.3184^{a}±0.0609	0.115^{ab}±0.011	10.93^{b}±0.71	299.17^{ns}±76.92	7.42^{b}±2.21
H4	7.01^{ab}±1.82	6.95^{ab}±1.31	0.2937^{ab}±0.0432	0.102^{ab}±0.009	11.33^{b}±0.96	294.65^{ns}±66.52	6.31^{bc}±3.15
H5	8.23^{ab}±1.99	6.72^{ab}±0.96	0.2631^{ab}±0.0312	0.103^{ab}±0.013	11.83^{ab}±2.16	291.48^{ns}±77.75	5.88^{bc}±2.83
H6	8.49^{a}±1.94	6.25^{b}±1.79	0.2245^{b}±0.0598	0.095^{b}±0.008	12.07^{ab}±1.71	289.84^{ns}±87.46	5.14^{c}±2.56
H7	8.32^{ab}±1.86	5.93^{b}±1.87	0.1994^{b}±0.0423	0.084^{b}±0.007	12.6^{ab}±1.66	284.19^{ns}±81.97	4.57^{cd}±2.57
H8	7.98^{ab}±2.17	5.85^{b}±1.06	0.1846^{b}±0.0209	0.083^{b}±0.007	12.87^{ab}±1.93	283.18^{ns}±72.09	3.58^{cd}±1.36
H9	7.17^{ab}±1.63	5.79^{b}±1.20	0.1785^{b}±0.0122	0.075^{b}±0.015	13.01^{ab}±1.38	281.67^{ns}±83.56	2.93^{d}±1.79
H10	6.64^{ab}±1.61	5.75^{b}±1.67	0.1578^{b}±0.0171	0.071^{b}±0.017	13.13^{a}±2.05	280.15^{ns}±79.98	2.57^{d}±2.15

Note: Means followed by the same letter within the column are not significantly different, p<0.05. NS: No significance. The same as below.

3.3 Changes in softening-related parameters with maturity

3.3.1 Water-soluble pectin, ionic pectin, covalently bonded pectin, cellulose, hemicellulose, pectinase activity, cellulase activity, and β-glucosidase activity

Pectin substances are the main ingredients of the primary wall and the middle lamellae of cell walls[34]. Pectins can be divided into water-soluble pectin, ionic pectin, and covalently bonded pectin[20]. It can be seen from Table 3a that the water-soluble pectin content in the Korla fragrant pears changed significantly from H1-H4, with a rapid increase from the initial 5.67 mg/g to 18.26 mg/g. Further, it presented a stable upward trend after H4. Ionic pectin exhibited similar changes in early harvest stages but remained largely stable from H7-H10. In contrast, the insoluble covalently bonded pectin content increased only slightly during H1-H3 but continuously declined by 34%, from its peak at 16.44 mg/g to 10.85 mg/g. Redgwell et al.[35] and Ahmed et al.[36] pointed out that the most significant changes in pectin substances in fruit during maturation manifest owing to an increase in soluble pectin content and a reduction in insoluble pectin content, which is consistent with the variation in pectin observed in the Korla fragrant pears. However, the covalently bonded pectin content of the Korla fragrant pears was relatively high compared to that of peaches' duration maturation[37], which may be beneficial for maintaining the fruit texture of fragrant pears at maturity.

Cellulose and hemicellulose serve as the backbone of the cell wall. Table 3 shows that the cellulose and hemicellulose in Korla fragrant pears generally decreased from H1 to H10, but the variation was not significant (p<0.05). The cellulose content of the Korla fragrant pears reached about 10.55 mg/g at H10. The hemicellulose content was higher than the cellulose content, and decreased to about 14.36 mg/g at maturity. Xu[20] pointed out that cellulose and hemicellulose content increase during the young fruit stages. With the fast growth of fruits, the cellulose and hemicellulose contents decrease to meet the needs of cell expansion. As fruits mature, the cellulose and hemicellulose contents tend to decrease insignificantly at stable rates. This agrees with the findings in this study.

Table 3 Changes of softening-related parameters during the harvest period

Harvest period	Water-soluble pectin/mg·g^{-1}	Ionic bonding pectin/mg·g^{-1}	Covalently bonded pectin/mg·g^{-1}	Cellulose content/mg·g^{-1}	Hemicellulose content/mg·g^{-1}	Pectinase activity/U·g^{-1}	Cellulase activity/U·g^{-1}	β-glucosidase activity/U·g^{-1}
H1	5.67^{e}±0.86	4.77^{c}±1.68	13.46^{ab}±2.39	12.04^{ns}±2.11	15.57^{ns}±0.95	0.48^{b}±0.04	1.24^{ns}±0.08	0.35^{b}±0.07
H2	9.28^{d}±1.24	8.94^{bc}±2.19	14.68^{ab}±0.51	11.53^{ns}±1.32	14.83^{ns}±2.20	0.66^{b}±0.17	1.18^{ns}±0.25	0.36^{b}±0.09
H3	14.56^{c}±1.72	14.33^{b}±5.56	16.44^{a}±3.19	11.08^{ns}±2.03	14.34^{ns}±1.94	0.78^{ab}±0.05	1.15^{ns}±0.14	0.37^{b}±0.04
H4	18.26^{b}±1.41	18.12^{ab}±2.85	15.59^{ab}±2.39	10.87^{ns}±1.31	14.55^{ns}±0.78	0.96^{ab}±0.29	1.18^{ns}±0.06	0.38^{b}±0.03
H5	19.91^{b}±0.49	19.32^{ab}±3.12	14.81^{ab}±1.49	10.75^{ns}±3.32	14.52^{ns}±0.51	0.98^{ab}±0.14	1.21^{ns}±0.13	0.41^{ab}±0.06
H6	20.73^{b}±2.65	19.68^{ab}±4.10	13.71^{ab}±0.88	10.52^{ns}±0.85	14.31^{ns}±2.17	1.01^{ab}±0.20	1.26^{ns}±0.10	0.44^{ab}±0.07
H7	21.47^{ab}±1.27	20.78^{a}±1.75	12.95^{b}±1.22	10.38^{ns}±1.97	14.27^{ns}±0.14	1.03^{ab}±0.32	1.27^{ns}±0.09	0.45^{ab}±0.03
H8	22.21^{ab}±2.08	20.67^{a}±3.88	12.84^{b}±1.12	10.45^{ns}±3.11	14.35^{ns}±0.89	1.06^{a}±0.21	1.29^{ns}±0.23	0.44^{ab}±0.06
H9	23.77^{ab}±3.38	20.09^{a}±2.86	12.09^{b}±2.10	10.25^{ns}±1.85	14.31^{ns}±0.45	1.12^{a}±0.30	1.28^{ns}±0.16	0.46^{ab}±0.07
H10	24.06^{a}±1.59	20.44^{a}±4.10	10.85^{b}±2.72	10.55^{ns}±1.24	14.36^{ns}±2.46	1.14^{a}±0.23	1.29^{ns}±0.25	0.48^{a}±0.04

The pectinase activity increased continuously throughout the harvest period by 137.5%, from an initial 0.48 U/g at H1 to a peak of 1.14 U/g at H10, which corresponds to the increases in water-soluble pectin and ionic pectin content seen in this study. Cellulase (CX) in fruit can promote the decomposition of cellulose, which implies that the disassembly of fruit cells is occurring, leading to softening[38]. It can be seen from Table 3 that the cellulase activity in the Korla fragrant pears changed only slightly during the harvest period, which conformed with the changes in cellulose content. Therefore, it is speculated that the cellulose and hemicellulose decomposition of the Korla fragrant pears were not the main causes of fruit softening during the harvest period. β-glucosidase is another enzyme related to the degradation of cell walls and can make some components of cell walls unstable.

Moreover, it can facilitate the degradation or dissolution of pectin by degrading branched polyaldehyde acids[39]. β-glucosidase was largely stable during H1-H4 and reached a peak (about 0.48 U/g) at H10, which was 37% higher than the initial value.

3.4 Changes in aging-related parameters with maturity

3.4.1 Respiration intensity, relative conductivity, SOD, POD, CAT

As shown in Table 4, Korla fragrant pears exhibited no climacteric rise throughout the harvest period, and the respiration intensity decreased to the lowest level in the final harvest stage, which is conducive to sugar accumulation in fruits[40]. Cui et al.[41] found that Qiu Xiang pears exhibited a climacteric rise in the harvest period and their tissues quickly disintegrated within the two weeks after harvest, making it extremely difficult for them to be stored. However, Wan Xiang pears showed respiration intensity peaks during the storage period after the harvest in mid-September, and the fruits softened slowly, which are good characteristics for long-term storage. The variation in the respiration intensity of the Korla fragrant pears was similar to that of the Wan Xiang pears. Therefore, such characteristics at maturity might be one of the reasons for the long-term storage ability of fragrant pears. Further, relative conductivity can characterize the degree of damage to the cytomembrane. The relative conductivity of the Korla fragrant pears continuously decreased during H1-H8, but increased to 23.69% at H10, though the differences were not significant. This suggests there were no significant changes in the functioning of the cytomembranes of the Korla fragrant pears. However, the relative conductivity slightly increased in the final stage of the harvest period, which indicates that some damage occurred to the cytomembranes of the Korla fragrant pears, and there was increased membrane permeability with time. This, eventually, would lead to a degradation in the quality of the Korla fragrant pears at the cellular level.

SOD, POD, and CAT together form the biological antioxidant enzyme system. SOD is the first antioxidase that plays a role in the reactive oxygen species (ROS) scavenging system. Plants may generate ROS and free radicals, which are harmful to cells under adverse conditions. SOD can catalyze a disproportionate reaction to eliminate ROS in biological cells and transform it into H_2O_2 and O_2, which are beneficial for maintaining a relatively low superoxide anion level to protect the cytomembrane structure. SOD activity exhibited no significant changes during H1-H4, but this value quickly increased after H4 by 261.18%, to 19.54 U/g at H10. Similarly, the CAT activity showed no significant changes during H1-H3, but began to gradually increase after H4. The CAT activity at H10 was 124.24% higher than that at H1. This could be attributed to the significant increase in H_2O_2 in the late stages of the harvest period. The POD activity was the highest at H1, at 57.56 U/g. Subsequently, the POD activity quickly dropped and stabilized at about 29.50 U/g after H6. POD can facilitate the transformation of carbohydrates in the tissues into lignin and can, thereby, intensify the degree of lignification[42]. Lee et al.[43] pointed out that POD activity in pears is relatively low and decreases with the diameter and density of stone cells in fruit, leading to textural changes. Based on the above discussion, it is speculated that the decreasing POD activity during the harvest period might be influenced by the decelerating lignification and degradation of the stone cells.

Table 4 Changes of ageing-related parameters during the harvest period

Harvest period	Respiration intensity/mg·(kg·h)$^{-1}$	Relative conductivity/%	SOD activity/U·g^{-1}	POD activity/U·g^{-1}	CAT activity/U·g^{-1}
H1	37.47^{a}±3.31	27.18^{ns}±5.01	5.41^{e}±0.83	57.56^{a}±6.64	0.33^{c}±0.13
H2	36.53^{a}±3.04	26.67^{ns}±1.89	6.92^{e}±1.24	55.74a^{b}±7.93	0.35^{c}±0.03
H3	35.22^{ab}±2.63	25.74^{ns}±3.79	6.65^{e}±0.84	46.92^{b}±5.03	0.36^{c}±0.05
H4	32.84^{ab}±5.37	24.16^{ns}±1.84	7.64^{de}±1.25	43.41^{b}±5.29	0.42^{bc}±0.05
H5	28.86^{b}±4.43	23.83^{ns}±0.62	9.16^{d}±0.98	38.82^{bc}±5.69	0.46^{bc}±0.11
H6	26.78^{bc}±2.61	23.23^{ns}±3.20	11.61^{c}±0.37	38.16^{bc}±7.15	0.53^{b}±0.12
H7	21.96^{c}±4.58	22.16^{ns}±3.21	14.93^{b}±0.46	31.87^{c}±5.44	0.59^{ab}±0.14
H8	20.47^{c}±3.12	21.87^{ns}±5.70	15.78^{b}±1.61	29.5^{c}±3.53	0.68^{ab}±0.07
H9	19.63^{c}±5.58	22.77^{ns}±3.91	18.46^{a}±2.14	27.71^{c}±5.33	0.72^{a}±0.07
H10	18.69^{c}±3.21	23.69^{ns}±3.86	19.54^{a}±0.52	26.96^{c}±3.18	0.74^{a}±0.04

3.5 Comprehensive evaluation of Korla fragrant pear during the harvest period

In the picking period of Korla fragrant pears, with the ripening of fruit, pears with different picking maturity will have significant changes in fruit shape, color, flavor, texture, and other aspects, accompanied by softening and aging, which will ultimately affect the commercial use and consumer acceptance of fruit. Hence, the six indices (fruit horizontal, vertical diameters, single-fruit weight, *L*, *a**, and *b**) were classified as the basic morphology parameters of the Korla fragrant pear. The seven indices representing fruit texture, taste, and nutrition (VC, hardness, chlorophyll content, TA, SSC, diameter, and density of stone cells) were classified as the basic quality parameters of the Korla fragrant pear. The cell wall components (pectin, cellulose, hemicellulose) and cell wall degrading enzymes (pectinase, cellulase, β-glucosidase) are classified as the basic softening parameters of the Korla fragrant pear. Respiration intensity, antioxidant enzyme systems (POD, SOD, CAT), and relative conductivity are classified as the basic parameters of the senescence of the Korla fragrant pear.

Correlation analyses of the morphological, quality, softening and aging parameters during the harvest period were carried out. A visual analysis of these basic indices was undertaken using PCA. The first two principal components explained 96.373% of the total variance of the original 26 variables (PC1 88.851% and PC2 8.030%), and the eigenvalue of these two principal components was higher than 1. The number of components chosen is either based on the eigenvalue being greater than 1 or on the number of components that contribute to explaining more than 80% of the variance. Hence, the first two principal two components were chosen in this study. Based on the generated biplot of the principal components (Figure 2) and the variation in the indices during the harvest period, it can be seen that the hardness, TA, chlorophyll content, density, and diameter of the stone cells, cellulose content and hemicellulose content were highest during H1-H3. The single-fruit weight, fruit horizontal and vertical diameter, *L*, a^*, b^*, SSC, SOD activity, CAT activity, and water-soluble pectin during H7-H10 were higher than those in the other periods. The highest VC content and moderate values for a

variety of indicators were achieved during H3-H7.

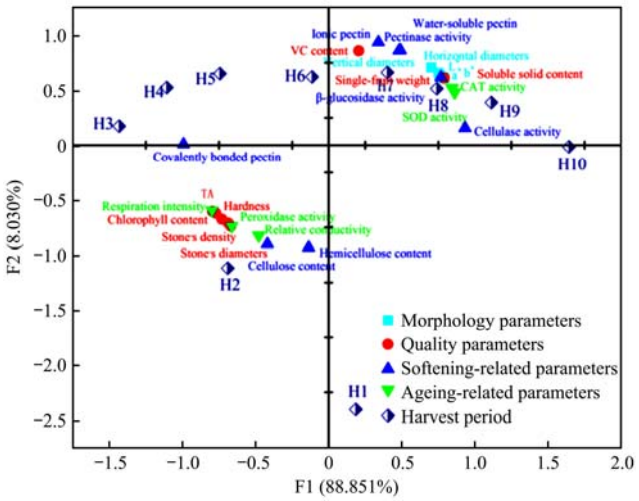

Figure 2 Biplot of the principal components

To facilitate a comprehensive evaluation of the Korla fragrant pears during the harvest period, a coefficient matrix of the different components was estimated using a regression method, through which the scores for the two principal components were calculated. The scores of the two principal components reflected changes in the characteristics of Korla fragrant pears at maturity during H1-H10 from two perspectives. It can be seen from Figure 3 that the scores for the first factor (F1) continuously decreased during H1-H3, but continuously increased from −1.143 to 1.643 during H3-H10. Changes in the characteristics during the middle and late period fruits mainly manifested as changes in the softening-related, aging-related, color-related, and quality-related parameters. The scores for F2 jumped quickly from −2.404 to 0.174 during H1-H3. Subsequently, these slowly increased before decreasing again during H4-H10, fluctuating within the range of −0.1 to 0.7. This indicates that changes in the softening-related parameters were the key factors influencing variations in the characteristics of the Korla fragrant pears in the early-harvest stage. Hence, it can be inferred that variations in the characteristics of Korla fragrant pears at maturity were mainly governed by changes in softening-related parameters during H1-H3, and subsequent changes in softening-related, ageing-related, color-related, and quality-related parameters during H3-H10.

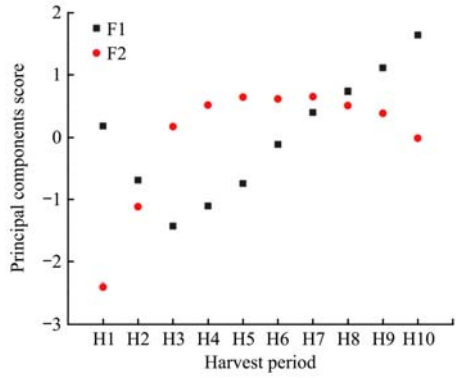

Figure 3 Changes in newly constructed variables

4 Discussion

The harvest period (harvest maturity) is closely related to fruit quality. In this study, the hardness, TA, density, and diameter of stone cells, SSC, single-fruit weight, and the horizontal and vertical diameters of Korla fragrant pears gradually increased with delays in plucking. Guang[44] and Xu[20] also demonstrated that the hardness, TA, density, and diameter of stone cells of fruits gradually decrease, while indices like SSC gradually increase with delays in plucking. In the present study, although the hardness of the fruit was at a relatively high level during H1–H3, the pericarp exhibited less red coloration than many other fruits in the late plucking period. Moreover, the single-fruit weight, fruit horizontal and vertical diameter, VC content, and SSC were lower and were accompanied by poorer flavor and taste. Park et al.[6] also found, in a study on the plucking period of 'Wonhwang' pears, that the hardness and the TA content of the fruit were relatively high in the early periods, but had low single-fruit weight, low SSC, and poor taste. Nevertheless, early harvested fruits exhibited better storage performance than late-harvested fruit. In Figure 4, it can be seen that fragrant pears that were harvested during H1-H3 exhibited better resistance to static pressure. From the perspective of storage and transportation, this suggests that Korla fragrant pears should be harvested during H1-H3. However, Korla fragrant pears during H1–H3 had lower single-fruit weight, lower nutrient accumulation, and poorer taste, which are disadvantageous for increasing their commercial value. In contrast, Korla fragrant pears, during H7–H10, have a good appearance and shape, high SSC and VC content, low TA content, and low stone cell diameter and density, indicating that they will have taste pleasant. However, Korla fragrant pears during H7–H10 softened quickly at room temperature, resulting in a short shelf-life and poor storage and transportation performance[45]. These fruits are suitable for fresh eating and juice processing. During H3–H7, the fruit had relatively high single-fruit weight, hardness, and SSC, while the VC content was maintained at a higher level. Moreover, the diameter and density of the stone cells were acceptable and the static pressure resistance was stronger. Jia et al.[46] indicated that the harvest of 'Yulu Xiang' pears at the appropriate time could preserve relatively high VC levels in the fruit. Korla fragrant pears, during H3-H7, had special pericarp colors, flavors, and tastes. These features are advantageous for marketing and further processing after storage, thus supplying the highest commercial value.

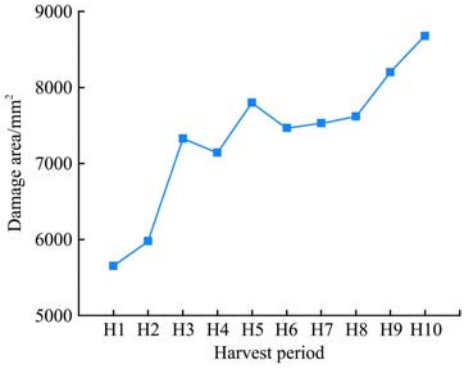

Figure 4 Relationship between harvest period and static pressure damage area

Aging is a physiological phenomenon that occurs with the softening of fruit, which is an important indicator of fruit maturity[29]. In this study, the pectin in early harvested pears was mainly insoluble covalently bonded pectin. With delays in plucking, the water-soluble pectin and pectinase content in the fruit continuously increased, as did the β-glucosidase activity. Variations in these indices can directly influence the softening of Korla fragrant pears. It is important to note that the cellulose and hemicellulose content exhibited no significant changes. This

means that changes in the structure and composition of the cell walls, especially changes in pectin composition, were the main causes of softening in the Korla fragrant pears during the harvest period. Additionally, the ROS generation capacity was enhanced during the maturation and aging of the fruit, thus increasing the free radical levels in cells, and causing damage. Further, there are enzymatic and non-enzymatic free radical scavenging systems in cells. The enzymatic free radical scavenging system includes SOD, POD, CAT, and so on[47]. SOD is an extremely important enzyme for the oxidative metabolism of plants, and it is used to eliminate O_2, through disproportionate reactions that generate nontoxic O_2 and low-toxicity H_2O_2. The H_2O_2 is further decomposed into O_2 and H_2O by CAT or POD, thus restricting the reaction between O_2^- and H_2O_2, which generates •OH[48]. During the harvest period, POD activity continuously decreased after H1 and was stable until H6. POD is generally classified as a free radical scavenging enzyme and thought to be able to delay aging. It has also been reported that POD can catalyze tomatine or a-ketone-γ-methyl thiobutyric acid to generate ethylene[49] or transform carbohydrates in tissues into lignin, intensifying lignification[42]. Based on these studies, POD activity fluctuation might be related to the lignification and degradation of stone cells. The SOD and CAT activity continuously increased during H4, indicating that the scavenging of free radicals in the Korla fragrant pears could mainly be attributed to the collaborative effects of SOD and CAT.

The PCA and factor analysis were important steps in the comprehensive evaluation of the Korla fragrant pears during the harvest period. The correlations identified between the physiological and physical characteristics of the fruit are valuable for post-harvest applications and quality assessment in different stages of the harvest period. In this study, the characteristics of the Korla fragrant pears in different stages of the harvest period were successfully characterized, which aided the analysis and classification of Korla fragrant pears during the harvest period from the perspectives of morphology, and quality, softening, and aging of the fruit. This method favors the harvesting of fruit for different commercial uses and quality control based on the characteristics of Korla fragrant pears at maturity; however, more research is required to promote the development of the fruit industry. This study emphasized the physical and chemical indices of fragrant pears but did not consider natural environmental factors (e.g. light and nutrition). Therefore, quantifying the relationship between natural environmental factors, characteristics at maturity, and the post-harvest quality of fragrant pears at different stages of plucking should be discussed in the future.

5 Conclusions

To the best of our knowledge, this is the first study to evaluate Korla fragrant pears during the harvest period that involves the basic indices of morphology, color, quality, softening, and aging. This can facilitate the development of harvest and processing standards in the fragrant pear industry. Some major conclusions can be drawn from this study, as follows:

1) The plucking period can greatly influence the characteristics of Korla fragrant pears at maturity. Variation in the basic indices measured followed certain patterns, with some of these indices following very similar trends. The softening of the fruit was mainly caused by changes in pectin, and the scavenging of free radicals was the collaborative result of SOD and CAT.

2) The hardness, TA, chlorophyll content, density and diameter of the stone cells, cellulose and hemicellulose content reached peaks during H1-H3. The single-fruit weight, fruit horizontal and vertical diameter, *L*, *a**, *b**, SSC, SOD activity, CAT activity, and water-soluble pectin during H7-H10 were higher than those in the other plucking periods. The VC content was the highest and a variety of indicators had moderate values during H3-H7.

3) Changes in the quality of the Korla fragrant pears mainly manifested as variations in the softening-related parameters during H1-H3 and variations in the softening-related, aging-related, color-related, and quality indices during H3-H10.

4) Fruit during H1-H3 were suitable for storage and transportation. Fruit during H7-H10 had the highest quality and were suitable for fresh eating and further processing. Further, fruit during H3-H7 exhibited moderate values for a variety of indicators and had the highest overall commercial value.

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