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RESEARCH ARTICLE

Changes in Sugar Metabolism and Fruit Quality of Different Pear Cultivars During Cold Storage

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Abstract

This study evaluated the changes in sugar metabolism and fruit quality of different pear cultivars during cold storage using seven major commercial pear cultivars belonging to different *Pyrus* species, such as *P. bretschneideri* Rehd. ("Huangguan," "Yali"), *P. pyrifolia* Nakai. ("Wonhwang," "Hosui"), *P. ussuriensis* Maxim. ("Jingbai," "Nanguo"), and *P. communis* L. ("Bartlett"). The firmness, respiration rate, titratable acidity, total soluble solids, sugar content, and enzyme activity of the seven pear cultivars were investigated. SPSS was used for analyzing the significance of different indexes. Results showed that fructose was the dominant sugar, accounting for > 60% of total sugars, followed by glucose and sucrose. The respiration peak of almost all cultivars appeared within 60 days. The levels of fructose, glucose, sucrose, and total soluble solids increased within 90 days and then generally decreased. Acid invertase showed the highest activity among all pear cultivars, followed by neutral invertase, sucrose synthetase, and sucrose phosphate synthetase during storage.

Keywords Pear cultivar · Sugar metabolism · Enzyme activity · Quality change

Introduction

The pear belongs to the family Rosaceae, subfamily Pomoideae, and genus *Pyrus* L. [1]. It is an important commercial fruit crop widely planted in 76 countries and regions throughout the world (http://faostat.fao.org). Pear cultivars from around the world might have been derived from four major *Pyrus* species, i.e., *P. communis* L., *P. bretschneideri* Rehd., *P. ussuriensis* Maxim., and *P. pyrifolia* Nakai. Pear flesh is juicy, sweet, and delicious; it has high nutritional values and contains rich contents of sugar, fruit acid, protein, fat, minerals (such as calcium, phosphorus, and iron), phenolic compounds, and antioxidants [2–4]. Sugars are the primary biochemical component of pears. The species and the quantity of sugars have a direct influence on the characteristics of fruit flavor, such as the intensity or quality of sweetness. Moreover, they are important factors for fruit quality [5]. Fruit sugar metabolism is associated with the metabolism of proteins, nucleic acids, lipids, and secondary metabolites (pigments, vitamins, aromatic volatiles) [6, 7], and it is considered as the center of the whole organism metabolism [8]. Several studies have reported that sugar-metabolizing enzymes are associated with sugar content in the process of fruit development [9, 10]. Yao et al. [11] analyzed the sugar components and the contents of the fruit of 98 pear cultivars and observed that sucrose content exhibited great variability in different cultivars. Although several studies have investigated pear sugar metabolism and the related enzyme activity in the process of fruit development [12, 13], there is a lack of studies focusing on the systemic analysis of the changes in pear sugar contents and the related enzyme activities in different pear cultivars during postharvest storage. Comprehensive compositional data about sugar metabolism mechanisms of different pear cultivars at the postharvest phase are still deficient and fragmentary.

In this study, we selected four pear varieties, *P. bretschneideri* Rehd. ("Huangguan," "Yali"), *P. pyrifolia* Nakai.

Yanan Zhao and Jinhui Geng made the same contribution to the paper.

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("Wonhwang," "Hosui"), *P. ussuriensis* Maxim. ("Jingbai," "Nanguo"), and *P. communis* L. ("Bartlett"), to determine the respiration rate, titratable acidity (TA), soluble solids, and the changes in sugar metabolism and the related enzyme activity during cold storage. The aim of our study was to determine the sugar metabolism patterns and the key regulatory enzymes of different pear cultivars during storage. The results of these analyses would provide a scientific basis for postharvest fresh-keeping technology and have a positive effect in maintaining the postharvest qualities and prolonging the shelf life. In addition, more detailed information regarding the changes in different pear fruit qualities can guide consumers to determine the best purchasing date.

Materials and Methods

Plant Materials and Treatments

The seven different pear cultivars selected in this study are listed in Table 1. *P. bretschneideri* Rehd. ("Huangguan," "Yali") and *P. pyrifolia* Nakai. ("Wonhwang,"

Table 1 Relevant characteristics of the seven pear cultivars selected for sugar metabolism studies

Cultivar name	Genus	Growing areas	Harvest date	Peel color	Fruit type	After ripening	Other characteristics
Huangguan 	*P. bretschneideri* Rehd.	East Asia	Early	Yellow or yellowish green	Oval	No need	Cold resistance; juicy; less stone cells
Yali 	*P. bretschneideri* Rehd.	East Asia	Late	Yellow or yellowish green	Oval	No need	Cold resistance; juicy; less stone cells
Wonhwang 	*P. pyrifolia* Nakai	East Asia	Mid	Brown or yellowish green	Round or oblate	No need	Light aroma; more stone cells; poor cold resistance
Hosui 	*P. pyrifolia* Nakai	East Asia	Early	Brown or yellowish green	Round or oblate	No need	Light aroma; more stone cells; poor cold resistance
Jingbai 	*P. ussuriensis* Maxim	East Asia	Mid	Yellow or yellowish green	Round or oblate	Need	No aroma; small size but high yield; good cold resistance
Nanguo 	*P. ussuriensis* Maxim	East Asia	Late	Yellow or yellowish green	Round or oblate	Need	No aroma; small size but high yield; good cold resistance
Bartlett 	*P. communis* L.	Europe, America, Africa, Australia	Mid	Yellow or red	Calabash shape or obovate	Need	Rich aroma; fleshy; shortest storage life

"Hosui") were collected from the science and technology demonstration garden of Xinji City in Hebei Province. *P. ussuriensis* Maxim. ("Jingbai," "Nanguo") and *P. communis* L. ("Bartlett") were collected from the Plantation of Lijia Town, Suizhong County, Liaoning Province. All cultivars were harvested at recoverable maturity. Samples were selected for uniformity of color, size, and firmness, packaged using a wrapping paper, and placed in a carton lined with polyvinyl chloride (PVC) after drying the surface moisture. After treatment, all fruits were placed in a chamber at 0 ± 0.5 °C and relative humidity of 85–90%. Measurements of the Bartlett variety were taken every 10 days, whereas the other samples were measured once every 30 days. From each species, 10 samples were taken and the peel was removed, after which the fruit flesh was cut into small pieces, immediately frozen in liquid nitrogen, and then stored at − 80 °C for further experiments. All samples were prepared with three replicates.

Firmness and Respiration Rate

Fruit firmness was determined using a GY-4 fruit hardness meter equipped with a probe of 8 mm diameter that can penetrate till 10 mm. Measurements were performed at two opposite points along the equator of the fruit, with the peel removed. The value was automatically calculated and expressed in N. The respiration rate was measured using a GXH-3051 infrared carbon dioxide analyzer at room temperature (randomly selected three fruits, air velocity of 0.5 mL min^{-1}, 30 min).

TA and Total Soluble Solids (TSS)

To assess TA, frozen pulps were quickly ground into powder in liquid nitrogen. For 10 g of the sample, 40 mL distilled water was added to dissolve the sample, centrifuged at 13,400*g* for 5 min, and then the supernatant was collected. Thirty milliliters of distilled water was added to precipitate, and the above-mentioned steps were repeated. Then, supernatants were mixed with water to 100 mL. Twenty milliliters of supernatant was antagonized to pH 8.0 by 0.1 mol L^{-1} NaOH. TSS were measured using a PAL-1 pocket refractometer.

Sugar Content and Enzyme Activity

The sucrose, fructose, and glucose were extracted and measured by Agilent 1200 HPLC, where acetonitrile/MQ mixture (8:2) was at the mobile phase, the flow rate of the sampler was 1 mL min^{-1}, and the sampling volume was 20 µL. Representative flesh samples (2 g) of the pear fruit from different varieties were ground using a ceramic mortar and pestle precooled with liquid nitrogen, treated in a microwave within 30 s, and mixed with 15 mL MQ. After centrifugation at 13,400*g*, the supernatant was transferred to a tube, and the precipitation was resuspended in 3 mL MQ and extracted again. Two times volume of supernatant was mixed vigorously with MQ to 10 mL as the sample, which was filtered through a 0.45-µm filter for three times. We selected the external standard method to quantify the sugar contents.

The activities of acid invertase (AIV) and neutral invertase (NIV) were evaluated according to Ref. [14] with few modifications. Briefly, 0.2 mL of crude enzyme extract and 0.8 mL of AIV enzyme reaction solution [10 g L^{-1} sucrose, 0.1 mol L^{-1} acetate buffer (pH = 5.5)] were incubated for 30 min at 37 °C, and the reaction was stopped by boiling in water for 10 min. After cooling, 0.5 mL of DNS reagent was added in a boiling water bath, incubated for 10 min, and mixed with 20 mL water, and then the A_{510} value was determined. The activities were evaluated by determining the amount of glucose produced from sucrose. The same process was used for determining the NI activity, except that the AIV enzyme reaction solution was substituted with the NI enzyme reaction solution (10 g L^{-1} sucrose, 0.1 mol L^{-1} phosphate buffer (pH = 7.5), 5 mmol L^{-1} $MgCl_2$, 1 mmol L^{-1} EDTA). The activities of sucrose synthetase (SS) and sucrose phosphate synthetase (SPS) were evaluated as follows: 50 µL of crude enzyme extract and 50 µL of SS enzyme reaction solution [4 mmol L^{-1} uridine diphosphate glucose (UDPG), 0.06 mol L^{-1} fructose, 15 mmol L^{-1} $MgCl_2$, 0.1 mol L^{-1} phosphate buffer (pH = 8.0)] were incubated for 30 min at 37 °C, 0.2 mL of 30% KOH solution was added, and then the reaction was stopped by boiling in water for 10 min. After cooling, the A_{291} value was determined. The same process was repeated for evaluating the SPS activity, except that the SS enzyme reaction solution was substituted with the SPS enzyme reaction solution [4 mmol L^{-1} UDPG, 0.06 mol L^{-1} fructose-6-phosphate, 15 mmol L^{-1} $MgCl_2$, 0.1 mol L^{-1} phosphate buffer (pH = 8.0)].

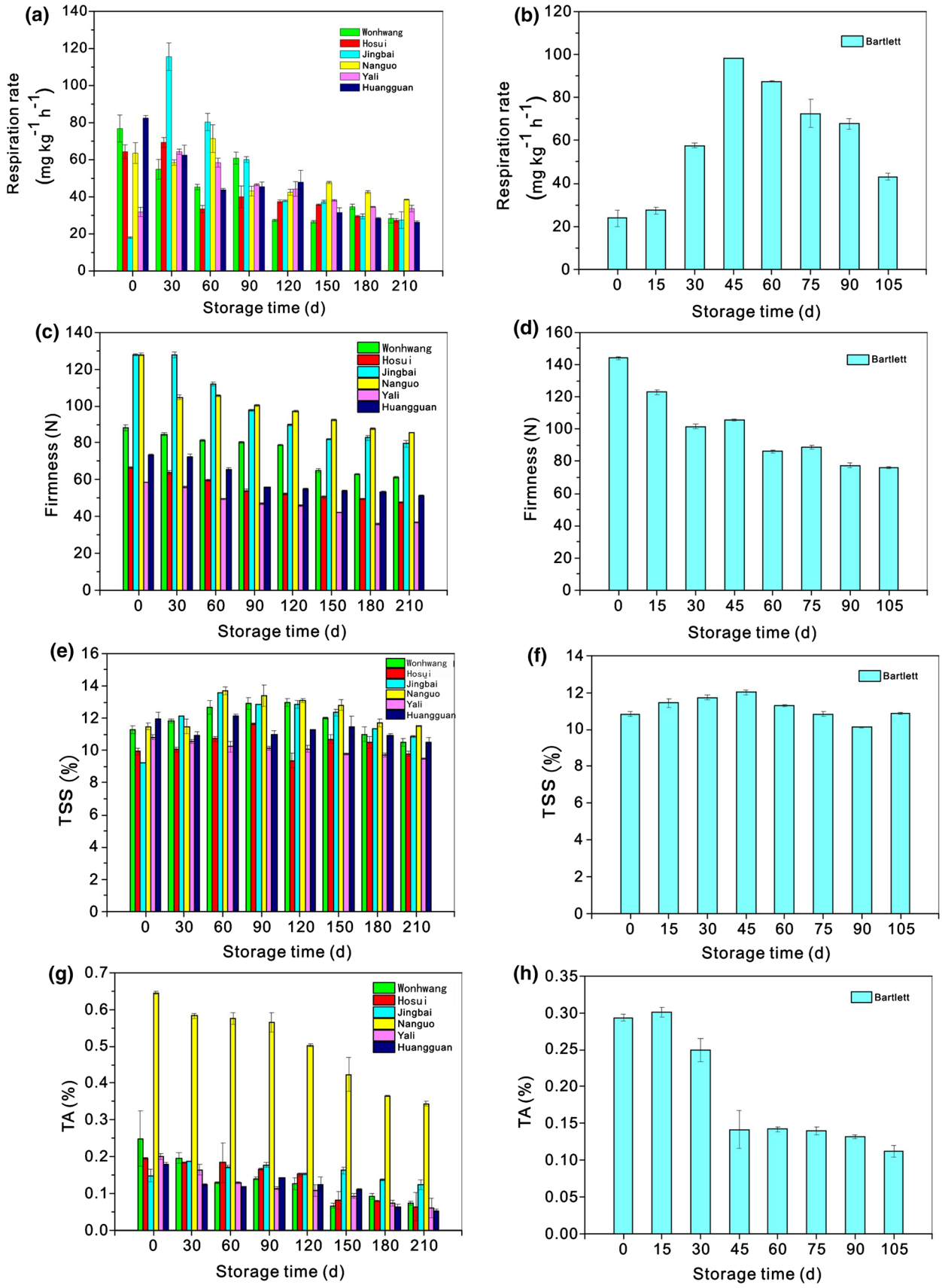
Statistical Analysis

The data were presented as mean ± SD of three independent experiments. Figures were drawn using the OriginPro 9.0 software. Difference analysis and correlation analysis were performed using the software of statistical package for the social sciences (SPSS) version 22.0 for Windows.

Results

Changes in the Respiration Rate of Different Cultivars

Pear is a typical respiratory climacteric fruit, and obvious respiration climacteric is observed in the storage period.


(a)
Respiration rate (mg kg-1 h-1)
Storage time (d)
Wonhwang
Hosui
Jingbai
Nanguo
Yali
Huangguan
(b)
Respiration rate (mg kg-1 h-1)
Storage time (d)
Bartlett
(c)
Firmness (N)
Storage time (d)
Wonhwang
Hosui
Jingbai
Nanguo
Yali
Huangguan
(d)
Firmness (N)
Storage time (d)
Bartlett
(e)
TSS (%)
Storage time (d)
Wonhwang
Hosui
Jingbai
Nanguo
Yali
Huangguan
(f)
TSS (%)
Storage time (d)
Bartlett
(g)
TA (%)
Storage time (d)
Wonhwang
Hosui
Jingbai
Nanguo
Yali
Huangguan
(h)
TA (%)
Storage time (d)
Bartlett

◂**Fig. 1** Changes in **a**, **b** respiration rate, **c**, **d** firmness, **e**, **f** TSS, and **g**, **h** TA of different pear cultivars during cold storage. CO_2 production per hour implies respiration rate. Values represent the mean ± SD of three replicates

With the climacteric peak of respiration, fruit quality reaches the best condition and then decreases. Differences were observed in the respiration rate of different cultivars during the cold storage period (Fig. 1a and b). Most of the pear cultivars reached respiration peaks within 90 days. Meanwhile, obvious respiration climacteric was observed in "Jingbai" (Fig. 1a) and Bartlett (Fig. 1b) varieties, wherein the peak values for "Jingbai" were 6.4 times higher than those of the fruit obtained immediately after harvest, while the peak values for "Bartlett" were 4.1 times higher than those of the fruit obtained immediately after harvest. At the end of the storage period, the respiration rate of all pear cultivars was lower than 40 mg CO_2 kg^{-1} h^{-1}.

Changes in Firmness, TSS, and TA

Because one of the best indicators of the ripening stage is flesh firmness, this parameter was measured for each pear variety. During the storage period, the firmness of the fruit from different cultivars steadily decreased. The hardness of *P. ussuriensis* Maxim. was higher than that of other cultivars, and the lowest was for "Yali" (Fig. 1c). Changes in the firmness of *P. bretschneideri* Rehd. and *P. pyrifolia* Nakai. were slow.

The content of TSS in all the pear fruits increased early and then decreased. As shown in Fig. 1e and f, the content of TSS in *P. ussuriensis* Maxim. was the highest. The TA content in all varieties decreased continuously, and Nanguo had the highest TA content (Fig. 1g). The content of TA ranged from high to low in the order of *P. ussuriensis* Maxim., *P. communis* L., *P. pyrifolia* Nakai., and *P. bretschneideri* Rehd. (Fig. 1g and h).

Changes in Sugar Content During Cold Storage

The major components of soluble sugars in all pear fruits that ranged from high to low were fructose, glucose, and sucrose. Fructose was the predominant soluble sugar in the pear fruit (60% in *P. pyrifolia* Nakai. and *P. bretschneideri* Rehd., and 70% in *P. ussuriensis* Maxim. and Bartlett varieties), as shown in Fig. 2. Interestingly, there was a significant difference in the sucrose content (Fig. 2c) of different cultivars of pears (4–22%). The content of fructose and glucose in all the fruits increased and then generally decreased. Sucrose content was decreased continuously, but "Wonhwang" and "Bartlett" were an exception (Fig. 2c).

During the whole storage period, the sugar content in *P. ussuriensis* Maxim. and *P. communis* L. was higher than that in *P. pyrifolia* Nakai. and *P. bretschneideri*. At the end of the storage period, Bartlett variety had the highest fructose content (Fig. 2e) and *P. ussuriensis* Maxim. had the highest glucose content (Fig. 2b). The content of total sugar (Fig. 2d) ranged from high to low in *P. communis* L., *P. ussuriensis* Maxim., *P. bretschneideri* Rehd., and *P. pyrifolia* Nakai.

Changes in Enzyme Activity Involved in Soluble Sugar Metabolism

The dynamic changes in enzyme activity were monitored between different pear cultivars during cold storage. In all the pear varieties, the enzyme activities that ranged from high to low were AI, NI, SS, and SPS, as shown in Figs. 3 and 2f. This result demonstrated that AI and NI play important roles in sugar metabolism and pear is a hexose-accumulation-type fruit. The time to reach enzyme activity peak was significantly different between different pear cultivars. The level of AI activity was higher during the earlier storage in "Huangguan," "Hosui," "Jingbai," and "Nangguo" varieties. In "Wonhwang" and "Bartlett" varieties, it was further higher during the later storage period (Figs. 2f and 3a). The level of NI activity was also higher at the mid-storage period in "Jingbai" and "Nanguo" varieties, but the NI activity peak appeared on the 150th day in "Huangguan" (Fig. 3b). Compared with AI and NI activities, the activities of SS and SPS were found to be lower in all pear cultivars and increased during the middle and later periods in all cultivars. SS and SPS exhibited a higher activity in *P. ussuriensis* Maxim. (Fig. 3c and d). In *P. pyrifolia* Nakai. ("Wonhwang," "Hosui"), the changes in AIV activity were distinct, but no clear changes were observed in NI, SS, or SPS activities. Moreover, the changing trend of enzyme activity was highly similar in *P. ussuriensis* Maxim. ("Jingbai," "Nanguo").

Discussion

Relationship Between Sugar Contents and Enzyme Activity in Different Pear Cultivars

Sugar accumulation is important for fruit quality. Fruits with a higher sucrose content have better flavor and sweet taste, which is possibly due to the fact that sucrose remains for a longer time than other sugars in the mouth [15]. As such, sucrose metabolism is the primary mode of sugar metabolism. Therefore, several researchers have attempted to explore the mechanism of sugar accumulation by studying the changes in enzyme activity related to sucrose metabolism in different fruits. In general, the major function of AI and NI is to catalyze sucrose hydrolysis [5, 16]. It was reported that the enhanced invertase activity leads to a reduction in sucrose and an increase in reducing sugar, whereas SPS and

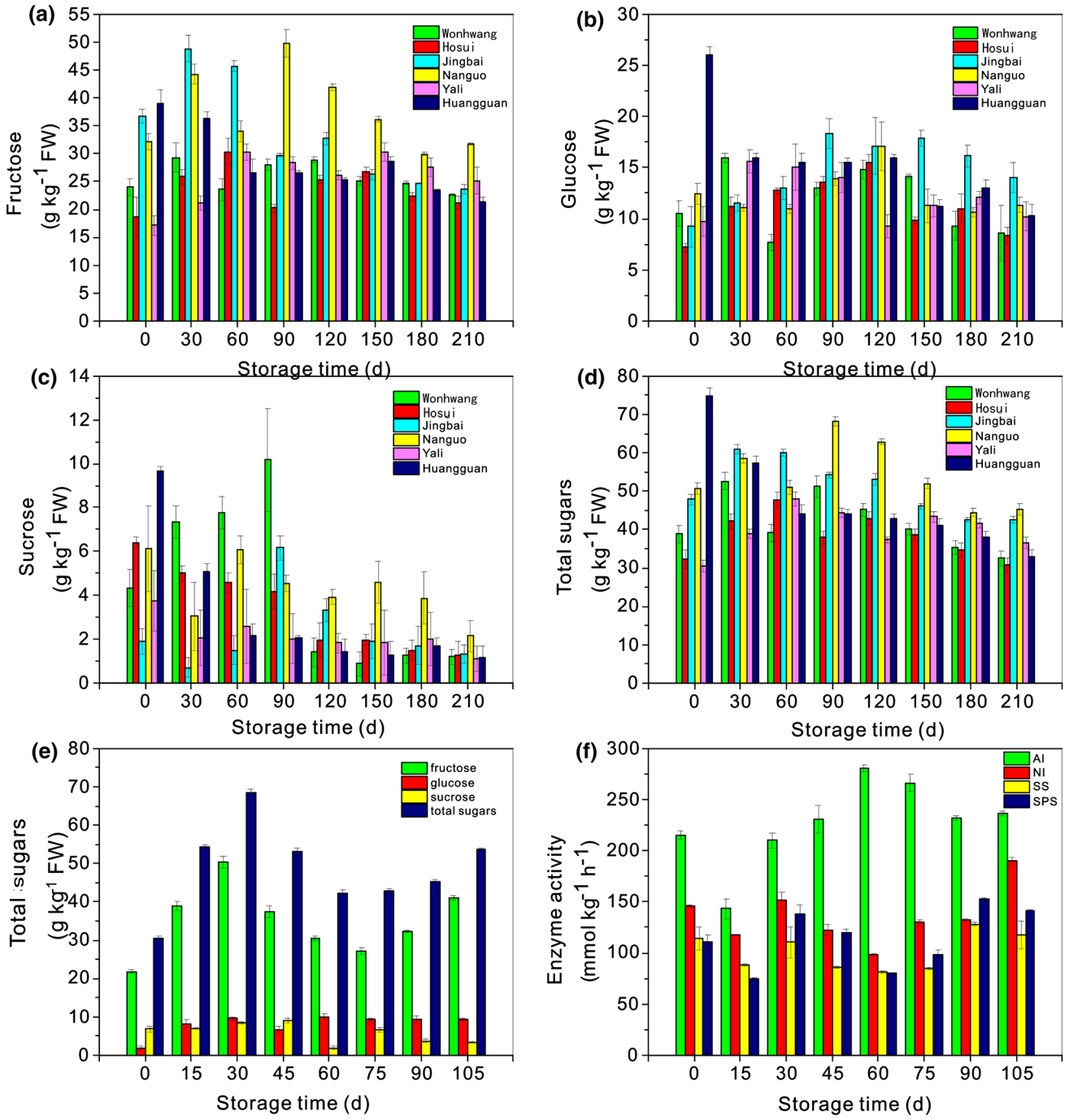


Fig. 2 **a**–**d** Changes in sugar content (fructose, glucose, sucrose, and total sugars) of different pear cultivars during cold storage. **e** Changes in total sugar content of Bartlett variety during cold storage. **f** Changes in enzyme activity of Bartlett variety during cold storage. FW indicates that the results are expressed on a fresh weight basis. Values represent the mean ± SD of three replicates

SS are involved in sucrose synthesis [17, 18]. Higher levels of AI and NI activities were observed in several hexose-accumulation-type fruits such as apple [19], strawberry [20], and pineapple [21], whereas sucrose-accumulation-type fruits such as sugarcane [10, 22], melon [8], and banana [23] showed higher SS and SPS activities [24]. In our study, we found that fructose was the dominant sugar, accounting for > 60% of total sugars, followed by glucose and sucrose, in all mature pear cultivars, as shown in Fig. 2. Furthermore, AI activity was significant in all the varieties during storage (Fig. 2f and a). These results confirmed that pear is a hexose-accumulation-type fruit.

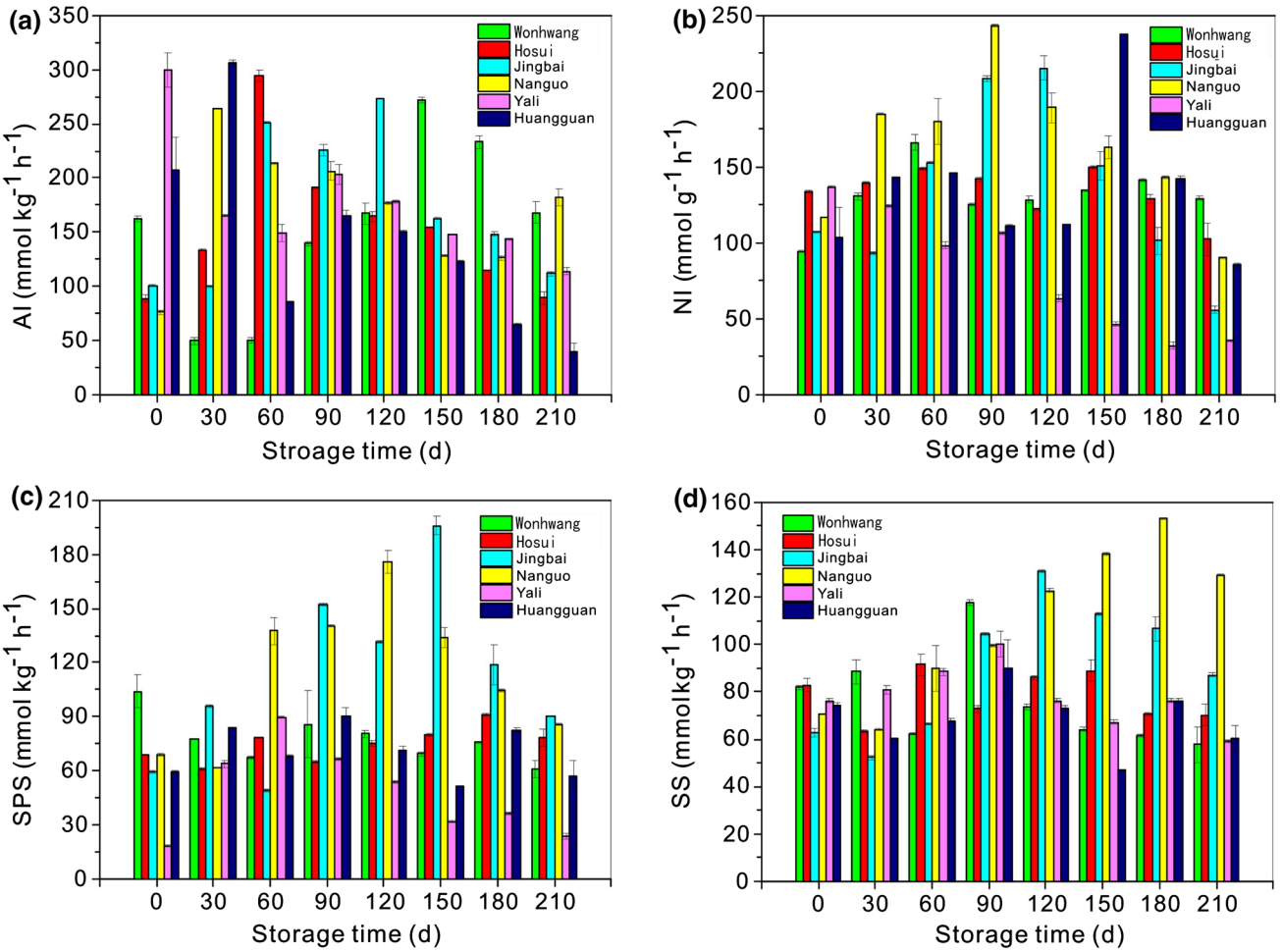


Fig. 3 Changes in enzyme activities of different pear cultivars during cold storage. **a** AI; **b** NI; **c** SPS; **d** SS. Values represent the mean ± SD of three replicates

Correlation analysis of sugar content and enzyme activity revealed that AI activity during storage was positively correlated with fructose and glucose in all the pear cultivars, except "Yali" (Table 2). The accumulation peak of fructose in "Nanguo" appeared on days 25 and 86, which was consistent with NI and AI activity peaks (Figs. 2, 3a and b). The AI activity in "Hosui" at the end stage of storage was very high, which was related to the increase in sucrose content and the decrease in the contents of fructose and glucose (Figs. 2a, b and 3a). A positive correlation between SPS activity and sucrose content was detected, which was consistent with the fact that SPS catalyzes UDPG and 6-phosphoric acid fructose (6-P-F) compound sucrose phosphate. Furthermore, SPS activity was higher in *P. ussuriensis* Maxim. ("Jingbai" and "Nanguo") than in other varieties (Fig. 3c); thus, at the end of the storage, the sucrose content of Nanguo was significantly higher than those of other pear varieties (Fig. 2c).

SS plays a role in the decomposition of sucrose, and some studies have indicated different results for SS activity [25]. In our study, we also observed a positive correlation between reducing sugar (glucose or fructose) content and SS activity in "Yali," "Nanguo," and "Hosui" varieties. This result indicated that SS is a reversible enzyme; it catalyzes not only the synthesis of sucrose but also sucrose decomposition [26]. The reversible catalytic reaction is as follows: fructose + UDPG ↔ sucrose + UDP. The optimal pH for sucrose synthesis is 8.0–9.5, and the optimal pH for sucrose decomposition is 5.5–6.5. In fact, there are two different types of SS, SS I (SS cleavage) and SS II (SS synthesis) [27]. SS I catalyzes the sucrose decomposition in immature fruits, whereas SS II catalyzes the synthesis of sucrose in ripe fruits [28, 29].

To summarize, the relationship between sugar accumulation and enzyme activity was established in this study. SPS and SS activities are involved in catalyzing sucrose biosynthesis; in contrast, AI, NI, and SS cleavage lead to hexose accumulation [16, 30]. AI plays a very important role in the regulation of sugar metabolism; it directly leads to the

Table 2 Correlation analysis among respiration rate, TSS, TA, sugar contents, and enzyme activities in different pear cultivars

Wonhwang	Respiration rate	TSS	TA	Fructose	Glucose	Sucrose	AI	NI	SS	SPS
Respiration rate		0.364	0.894*	0.095	0.001	0.677	−0.444	−0.526	0.648	0.795*
TSS	−0.123		0.402	0.508	0.397	0.516	−0.296	−0.052	0.550	0.480
TA	0.700	0.109		0.257	0.190	0.490	−0.509	−0.580	0.496	0.825*
Fructose	−0.178	−0.065	0.089		0.869**	0.338	−0.253	−0.111	0.632	0.257
Glucose	−0.238	0.090	0.261	0.438		0.074	0.023	−0.291	0.478	0.232
Sucrose	0.798*	0.238	0.895**	−0.100	−0.163		−0.742	0.108	0.745*	0.264
AI	−0.253	0.424	0.392	0.723*	0.621	0.181		−0.246	−0.298	−0.019
NI	0.282	0.629	0.461	0.466	0.203	0.492	0.599		−0.394	−0.769*
SS	−0.261	0.015	0.147	0.454	0.167	0.050	0.532	0.380		0.570
SPS	0.763*	−0.110	−0.703	0.171	−0.075	−0.692	−0.007	−0.209	0.284	Hosui
Jingbai	Respiration rate	TSS	TA	Fructose	Glucose	Sucrose	AI	NI	SS	SPS
Respiration rate		0.559	0.820*	0.792*	−0.178	−0.127	0.076	0.056	−0.504	−0.162
TSS	0.125		0.567	0.220	0.570	0.310	0.779*	0.637	0.303	0.306
TA	0.731*	0.217		0.656	−0.003	0.239	0.260	0.453	−0.303	0.113
Fructose	−0.139	0.411	0.429		−0.646	−0.336	0.002	−0.047	0.755*	−0.607
Glucose	−0.316	0.397	0.234	0.519		0.604	0.567	0.600	0.883**	0.839**
Sucrose	0.701	0.450	0.643	−0.113	0.026		0.559	0.766*	0.513	0.443
AI	0.083	0.253	0.151	0.603	0.009	−0.396		0.863**	0.542	0.215
NI	0.041	0.704	0.430	0.857**	0.415	0.208	0.517		0.561	0.465
SS	−0.727*	0.072	−0.900**	−0.359	−0.002	−0.352	−0.339	−0.213		0.741*
SPS	−0.253	0.866**	−0.097	0.308	0.630	0.205	0.058	0.553	0.423	Nanguo
Yali	Respiration rate	TSS	TA	Fructose	Glucose	Sucrose	AI	NI	SS	SPS
Respiration rate		0.390	0.323	0.116	0.832*	−0.021	−0.191	0.476	0.533	0.869**
TSS	0.474		0.989**	−0.659	0.230	0.829*	0.805*	0.953**	0.466	0.229
TA	0.868**	0.632		−0.702	0.188	0.851**	0.812*	0.936**	0.363	0.125
Fructose	0.902**	0.428	0.772*		0.246	−0.529	−0.670	−0.579	0.167	0.429
Glucose	0.939**	0.573	0.841**	0.778*		−0.001	−0.232	0.395	0.633	0.777*
Sucrose	0.949**	0.489	0.778*	0.927*	0.932**		0.862**	0.731*	0.340	−0.019
AI	0.776*	0.094	0.680	0.842**	0.544	0.626		0.735*	0.324	−0.211
NI	−0.276	0.266	−0.047	0.082	−0.346	−0.248	0.017		0.584	0.333
SS	0.238	−0.010	0.312	−0.079	0.397	0.154	0.033	−0.621		0.738*
SPS	0.069	−0.322	0.063	−0.035	0.021	−0.05	0.332	−0.277	0.686	Huangguan
Bartlett	Respiration rate	TSS	TA	Fructose	Glucose	Sucrose	AI	NI	SS	SPS
Respiration rate		0.403	−0.917**	0.034	0.430	−0.067	0.664	−0.459	−0.429	0.048
TSS			−0.108	0.625	0.267	0.483	−0.166	−0.256	−0.634	−0.287
TA				0.165	−0.423	0.170	−0.758*	0.315	0.309	−0.135
Fructose					0.527	0.270	−0.363	0.324	0.108	0.333
Glucose						−0.388	0.248	−0.072	−0.145	0.075
Sucrose							−0.478	0.007	−0.186	0.008
AI								−0.080	−0.128	0.122
NI									0.669	0.669
SS										0.822*

* and ** indicate significance at 0.05 and 0.01 levels, respectively

change in sugar content and indirectly affects the quality of the pear fruit during the storage period. However, a similar correlation was not observed between sugar contents and enzyme activity in different pear cultivars (Table 2). The changes in pear fruit quality properties are variety dependent. This may be related to the respiratory metabolism, the

Table 3 Variance analysis of pear fruit quality between the first and last day of storage

Cultivar	Firmness (N)		Respiration rate (mg kg^{-1} h^{-1})		TA (%)		TSS (%)	
	First day	Last day	First day	Last day	First day	Last day	First day	Last day
Huangguan	73.35bB	51.3eE	82.4aA	26.35hH	0.18dDE	0.05hH	11.93aA	10.5cC
Yali	58.5eE	36.9gG	31.9dD	33.69cC	0.2dD	0.06fF	10.83aA	9.5eE
Wonhwang	88.2cC	61.2dD	76.9bB	28.35eE	0.25cC	0.07eE	11.3aA	10.5cC
Hosui	66.51dD	47.7fF	64.3cC	27.34gG	0.15eF	0.06gG	9.93aA	9.8dD
Jingbai	128aA	79.451bB	18fF	27.46fF	0.15eF	0.11cC	9.23aA	10.86bB
Nanguo	128aA	85.508aA	63.6cC	38.46bB	0.64aA	0.36aA	11.48aA	11.5aA
Bartlett	144aA	75.9cC	23.8eE	43.12aA	0.29bB	0.23bB	10.83aA	10.87bB
Cultivar	Fructose (g kg^{-1} FW)		Glucose (g kg^{-1} FW)		Sucrose (g kg^{-1} FW)		Total sugars (g kg^{-1} FW)	
	First day	Last day	First day	Last day	First day	Last day	First day	Last day
Huangguan	38.98aA	21.34fF	26aA	10.34cC	9.67aA	1.13fF	74.68aA	32.81eE
Yali	17.1eE	25.2cC	9.7deCD	10.23dC	3.74cdBc	1.1gF	30.54eD	36.53dD
Wonhwang	23.96dD	22.65eE	10.5cdBC	8.57gF	4.34bcdBC	1.19eE	38.84dc	32.41eE
Hosui	18.64eE	21.24fF	7.18eD	8.36hG	6.41bB	1.25dD	32.23efD	30.85gG
Jingbai	36.72bB	23.6dD	9.26dCD	14.01aA	1.89dD	1.3hG	47.87cB	38.91cC
Nanguo	32.18cC	31.69bB	12.5bcAB	11.35bB	6.13bcB	2.15bB	50.79bB	45.19bB
Bartlett	21.81dD	40.93aA	1.78fE	9.33eD	6.86bAB	3.39aA	30.45fD	53.65aA

Lower case letters indicate levels of significance at $P < 0.05$; capital letters indicate levels of significance at $P < 0.01$

changes in the internal environment of different varieties of the pear fruit, and the external environmental and other factors during the process of cold storage.

Effect of Changes in Respiration Rate on Metabolites

The pear is a typical respiratory climacteric fruit, and in this study, the pear fruit flavor significantly increased as it reached its respiration peak on the 45th day and then decreased (Fig. 1a and b). A significant correlation was observed between the respiration rate and levels of TA, fructose, glucose, and sucrose and AI activity among different pear cultivars (Table 2), which suggested that the respiration rate has an important influence on the changes in fruit quality during the storage period. Studies on climacteric fruits such as *Annona* [31] and mango [32] have demonstrated that as the respiratory peak reaches along with the degradation of polysaccharides and the accumulation of monosaccharides, the fruit quality becomes better. However, the fruit firmness declined rapidly, and the fruit quality increased obviously and then decreased after the climacteric peak. Among the seven pear cultivars, *P. ussuriensis* Maxim. ("Jingbai," "Nanguo") and *P. communis* L. ("Bartlett") experienced ripening and softening obviously (Fig. 1c and d). Bartlett had a short storage life when it became completely mature [33].

Moreover, a significant correlation was observed between fruit respiration rate and TA content. We had previously investigated the organic acid metabolism of "Huangguan" during postharvest storage and found that the titratable acid content in the peel and flesh was significantly decreased [34, 35]. In this study, we also obtained a similar result, i.e., the TA content in all the varieties decreased continuously (Fig. 1g and h). This may be explained by the fact that organic acid was consumed as the substrate in the Krebs cycle (TCA cycle), glycolysis, and glyconeogenesis. In sugarcane fruit, the AI and NI activities initially increased in cane juice and hydrolyzed sucrose into glucose and fructose irreversibly during storage [36]. Later, with an increase in the storage period, the stem of sugarcane lost water, and the acidity of sugarcane juice increased, which produced other harmful substances (microbes), leading to changes that caused a decrease in the invertase activities and recoverable sugars [37]. In our study, we found a similar change, i.e., in all the pear varieties, the AI and NI activities initially increased and then decreased during cold storage (Fig. 3a and b). However, the NI activity was lower than AI activity, and the change was nonsignificant. This may be due to the decline of TA (Fig. 1g and h), which led to the deviation from the optimum pH of AI and NI and the decrease in sucrose invertase activities. In addition, with the accumulation of harmful substances, the contents of fructose, glucose, and sucrose decreased. These results show that the sugar loss during postharvest storage is the result of the fruit's own breathing consumption and changes in invertase activity.

Analysis of Optimum Storage Period Based on Quality Changes of Different Pear Cultivars

The pear flavor significantly increased as the pear reached the respiration peak and then decreased. Most of the pear cultivars reached respiration peaks on the 60th day (Fig. 1a and b), the sugar contents reached the maximum value, and the fruit flavor and quality were the best. Changes in the respiration rate were significant in *P. ussuriensis* Maxim. ("Jingbai," "Nanguo") and *P. communis* L. ("Bartlett") (Fig. 1); these fruit varieties experienced ripening and softening obviously and had a short storage life when they became fully mature. Combining with the results of the physiological and biochemical index with the organoleptic assessment (Table 3), we concluded that at the beginning of storage period, the fruit quality ranged from high to low in "Huangguan," "Yali," "Wonhwang," "Hosui," "Jingbai," "Nanguo," and "Bartlett" varieties. At the end of the storage period, the ranking of the quality of different pear cultivars was "Bartlett," "Nanguo," "Jingbai," "Huangguan," "Yali," "Wonhwang," and "Hosui." Consumers can select the optimum storage period for determining an edible fruit according to the quality changes of different pear cultivars.

Conclusion

We selected four pear varieties, *P. bretschneideri* Rehd. ("Huangguan," "Yali"), *P. pyrifolia* Nakai. ("Wonhwang," "Hosui"), *P. ussuriensis* Maxim. ("Jingbai," "Nanguo"), and *P. communis* L. ("Bartlett") to analyze and summarize the changes in respiration rate, TA, soluble solids, sugar content, and the related enzyme activities during cold storage period. A significant correlation was detected between sugar content and enzyme activity. Therefore, we can regulate the quality of the pear fruit by adjusting the metabolic pathway of the enzymes.

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