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Changes in the volatile compounds and chemical and physical properties of Yali pear (*Pyrus bertschneideri* Reld) during storage

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Abstract

Volatiles from stored Yali pear (*Pyrus bertschneideri* Reld) were studied using high-resolution gas chromatography and the solid-phase microextraction (SPME) method of gas chromatography/mass spectrometry (GC/MS). The dominant components were ethyl butanoate, ethyl hexanoate, α-farnesene, hexanal, ethyl acetate, hexyl acetate, ethanol and so on. By using GC-olfactometry, it was demonstrated that the volatile compounds from SPME were responsible for the aroma of Yali pear. The levels of sugars, organic acids, and phenolic acids in Yali pear were investigated using high-performance liquid chromatography. Fructose was the dominant sugar, followed by glucose and sucrose. With increasing storage time, sucrose levels decreased, however fructose and glucose levels did not changed remarkably. There was a slight decrease in flesh firmness during storage. The general soluble solids concentration, slightly decreased after 5 months storage. Some aroma volatile components increased during storage, while others decreased, especially the esters. The organic acids and phenolic acids also changed. Yali pear flavor was affected by changes in the levels of volatile compounds, and chemical and physical properties.

Keywords: *Pyrus bertschneideri* Reld; Yali pear; Volatile compounds; GC-olfactometry; Chemical and physical properties; Storage; Solid phase microextraction (SPME)

1. Introduction

China is an important producer of the Yali pear. Yali pear (*Pyrus bertschneideri* Reld) belongs to super white pear and has been grown in China for more than 2000 years. It is popular with consumers for its unique fragrance, subtle aroma, sweetness, and crispness. It has good eating quality with few stone cells. It matures in middle and late September, and is typically harvested at minimum soluble solids concentration of 10°Brix–12°Brix.

Sugar and organic acid content has a marked influence on the sensory quality of fruit. Phenolic acids and their derivatives are widely distributed in plants and perform a range of essential metabolic functions. The levels of phenolic acids in plants can be affected by the processes of germination, drying, ripening, storage, processing (Maga, 1978), fruit development (Ayaz, Kadioglu, & Reunanen, 1997) and maturation (Hanna, Naim, Rousel, & Zehavi, 1991). They may contribute to the dark color, bitter taste, and objectionable flavor of some fruits, leaves and seeds (Lee & Nagy, 1990; Maga, 1978; Makkar, Dawra, & Singh, 1991). There is currently little information available on changes in the levels of sugars, acids, and phenolic acids of Yali pear fruits during storage.

Aroma is one of the most important sensory attributes of fruit. Fruit flavor is particularly sensitive to compositional alterations. The volatile compounds that

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contribute to fruit flavor are produced through metabolic pathways during ripening, harvest, post-harvest and storage, and are influenced by many factors related to species, variety, and technological treatments (Rizzolo, Lombardi, Vanoli, & Polesello, 1995). There is a considerable amount of literature that examines the volatile components of fruits such as apple, orange, mango, and berries. There have been many studies on pear, however most of these are limited to examination of physiology and non-volatile chemical constituents. There also have 20 papers on pear aroma since 1997, for example, changes in the composition of French pear during maturationing (Haruyasu, 1990); and the volatile constituents of Asian pear (*Pyrus serotina*) (Takeoka, Buttery, & Flath, 1992). However, there have been few studies on the volatile compounds and the chemical and physical properties of Yali pear during storage.

2. Materials and methods

2.1. Chemicals

All chemicals used in the experiment were of analytical or HPLC grade, purchased from Sigma. They were fructose, sucrose, glucose, gallic acid, chlorogenic acid, caffeic acid, epicatechin, *p*-coumaric acid, quercetin, quercitrin, morin, phloretin xylogucoside, quinin acid, malic acid, shikimic acid, lactic acid, acetic acid, citric acid, fumaric acid, and succinic acid.

2.2. Plant materials

Yali pear fruits (*P. bertschneideri* Reld) were harvested from Daxing horticultural field, Beijing, China, on September 26, 2003. Mean SSC was 12°Brix. Yali pear fruits were loosely packed inside a conventional modular bulk container with a polyliner, and stored at 0 °C, 80–90% relative humidity. The humidity inside the polyliner was approximately 95%. The air was exchanged with fans four times daily to remove ethylene.

2.3. Design of experiments

The volatile compounds of Yali pear fruits 1, 2, 3, 4 and 5 months after storage were analyzed. Flesh firmness, composition of sugars, organic acids, and phenolic acids during storage were also determined.

2.4. Measurement of flesh firmness

Flesh firmness was measured using a handheld electrometer (model GY-1, Mudan River, China). Ten fruits were randomly selected from 30 to 40 stored fruit at each storage period of 1, 2, 3, 4 and 5 months. Flesh firmness of each fruit was measured twice, on opposing sides of the fruit equator. The skin was removed using a slicerst to a 1-mm cutting depth, prior to being placed on a hard surface and held firmly during testing (Watkins & Harman, 1981).

2.5. Measurement of soluble solids concentration

Soluble solids concentration (SSC) was measured using a manual refractometer (model ATC-1 E, Brix 0–32%, ATAGO, Japan). Ten fruits were randomly selected from 30 to 40 stored fruit at each storage period of 1, 2, 3, 4 and 5 months. The fruit flesh, excluding core and seeds, were milled into slurry with a juice extractor, centrifuged to remove coarse particles, and the resultant pear juice used for SSC measurement. Each sample was measured in triplicate.

2.6. Standard materials

Standards of sugars, organic acids, phenolic acids were used to obtain the linear curve. Linearity of the response to RI and UV detectors was tested for each compound with five different concentrations prepared in distilled water, K_2HPO_4 solution, and methanol solution. All correlation coefficients were in the required range. Qualitative and quantitative of the sugars, the organic acids and the phenolic acids were according to their respective standard material and the linear curve method.

2.7. Composition of sugars

Composition of sugars was determined using the method of Dolenc and Stampar (1997) with modifications. Samples were prepared from 7 to 10 fresh Yali pear fruits after each storage period of 1, 2, 3, 4 and 5 months. Fruit flesh excluding cores and seeds were squeezed into juice using a commercial turmix blender. The juice was pooled, and then filtered through filter paper. The fruit juice (5 ml) was diluted to 100 ml with bidistilled water, then centrifuged in a refrigerated centrifuge at 3000*g* for 10 min. The extract was filtered through 0.45-μm Millipore filters and a 20-μl sample used for HPLC analysis of sugars. The analysis were done in triplicate The HPLC conditions were as follows: column, PRONTOSIL, 120-10-Amino 10.0 μm, 250 × 4.6–mm i.d. (KNAUER, Germany); solvent CH_3CN/H_2O = 85:15 as mobile phase, at a flow rate of 1.5 ml/min, at 30 °C; equipped with RI detector (model K-2301, KNAUER, Germany).

2.8. Composition of organic acids

Fruits were treated as for organic acids determination. Fruit juice (2 ml) was diluted to 50 ml with $K_2HPO_4 \cdot 3H_2O$ (0.01 M, pH 2.6), and centrifuged in a

refrigerated centrifuge at 3000*g* for 10 min. The extract was filtered through 0.45-μm Millipore Organic filters and a 20-μml sample used for HPLC analysis of organic acids. Each sample was measured in triplicate. The HPLC conditions were as follows: column, PRONTOSIL, 120-10-C_{18}H 10.0 μm, 250 × 4.6-mm i.d. (KNAUER, Germany); solvent 0.01 M $K_2HPO_4 \cdot 3$-H_2O, pH 2.6, as mobile phase, at a flow rate of 0.5 ml/min, at 30 °C, equipped with UV detector with wavelength set to 210 nm (model K-2301, KNAUER, Germany).

2.9. Extraction of phenolic acids

Seven to ten Yali pear fruits were selected after 1, 2, 3, 4 and 5 months of storage, and the fruit flesh, excluding cores and seeds was pressed into juice. Ten milliliter pear juice was extracted three times with ethyl acetate (10 ml the first time, then 5 ml for the remaining times). The combined extracts were dried with pure nitrogen each time the extracts were removed. The residue was dissolved in methanol (1 ml), and then filtered through a 0.45-μm organic membrane prior to HPLC analysis. The separation of phenolic compounds was performed on a Knauer HPLC (Germany), equipped with BF-2000 chemstation software, a K-001 pump, and a PRONTOSIL 120-10-C_{18} (150 × 4.6-mm i.d.) column was operated at the temperature of 30 °C. The mobile phase consisted of 100% (v/v) acetonitrile (eluent A) and 0.1% (v/v) formic acid in water (eluent B). The gradient program was as follows: 2%A + 98%B (0–5 min), 12%A + 88%B (5–25 min), 45%A + 55%B (25 min–50 min), and performed at 280 nm, with a flow rate of 1.0 ml/min. The injection volume for all samples was 20 μl. Each sample was measured in triplicate.

2.10. Isolation of volatile compounds using solid-phase microextraction (SPME)

Seven to ten fruit were washed, and the fruit flesh excluding cores and seeds pressed into pulp. Eight milliliter pulp was quickly transferred into a 15-ml headspace flask containing 2.2 g NaCl, in order to minmize the loss of volatile components and avoid browning. The volatiles were sampled by manual headspace solid phase microextraction at 40 °C while stirring. The fibre (100 μm PDMS, Supelco) was pierced into the injection port of the GC/MS after 30 min of sampling, and then desorbed at 250 °C for 10 min. Each analytical sample was measured in triplicate.

2.11. GC chromatography-olfactometry

Five olfactometry panelists, trained in GC-sniffing and odor recognition, sniffed the humidified efluent of the GC four times each. The intensity of each compound with aroma activity was recorded on a sliding scale from 0 to 15 and the aroma quality noted. The output of the variable potentiometer was connected to a separate channel in the ChromPerfect software and used to gather GC-FID data, simultaneously . The equipment and software used are detailed in (Bazemore, Goodner, & Rouseff, 1999). A compound was deemed aroma active if it was detected in at least half of all sniffs (8–16 runs). The intensity of each run was normalized so the highest intensity had a score of 100. The normalized intensities of all the runs where the compound was detected were then averaged. If the compound was not detected in a run its value was treated as missing, not zero.

2.12. Gas chromatography/mass spectrometry condition

A Hewlett–Packard 6890 GC/MS with a flame ionization detector and GC-olfactometric port (J&W Scientific inc., Germany) was used, with the injector and detector maintained at 250 and 270 °C, respectively, (Fig. 6). The column dimensions were 0.32 mm i.d. × 30 m, 0.5-μm film thickness (Hewlett–Packard). The carrier gas (He) had a flow rate of 40 ml/min. The temperature program was: isothermal at 35 °C for 2 min, increase to 250 °C at 4 °C/min, then hold for 15 min. The analysis was done in triplicate. Compounds were identified by matching mass spectra with the NIST98 library of standard compounds. When available, MS identifications were confirmed by comparing GC retention times with authentic compounds.

2.13. Statistical analysis

The results were statistically evaluated by one way analysis of variance (AVOVA). Statistical differences with *P*-values under 0.05 were considered significant. All experiments were replicated three times.

3. Results and discussion

3.1. Change in flesh firmness

Flesh firmness is a very important physical property of fruit tissue, as it directly affects eating quality and texture. A change in flesh firmness may be due to modification of the chemical structure of the cell wall, for example Sakurai and Nevins (1997) ascribed the massive breakdown of avocado cell walls to hydrolysis of polysaccharides by hydrolytic enzymes. In contrast, Yali pear firmness decreased very slowly during storage (Fig. 1). The response of flesh firmness to storage conditions and harvest date was dependent on the growing location.

As shown in Fig. 1, during Yali pear storage the firmness is maintained to a certain degree, which is not common in other fruits. For example, during Kiwifruit

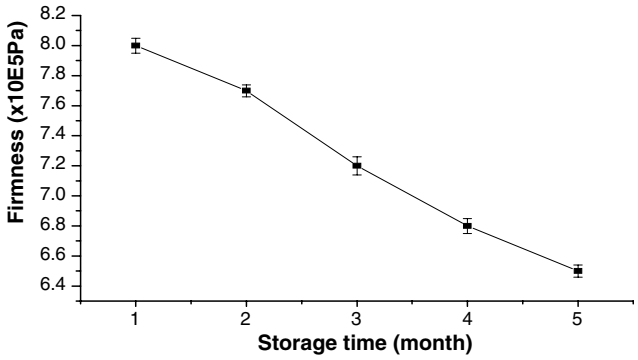


Fig. 1. Changes of Yali pear firmness during storage.

development, the flesh softens remarkably, so the fruit has tendency to be damaged during handling and transportation from the field to the market. However, during storage, Yali pear flesh remained hard enough to avoid damage during transport. Previous work of Ning (1981) also described a similar pattern of Yali pear firmness change.

3.2. Change in SSC

Yali pear SSC developed with fruit ripening, increasing slowly from 1 month after storage (11°Brix) (stage 1) to 3 month after storage (12°Brix) (stage 3), stabilising from stage 3 to stage 4, and then decreasing only slightly (Fig. 2). Total changes in SSC were not remarkable. This suggests that even if Yali pear fruits were stored for half a year, SSC would change only slightly, and the flesh quality could be maintained during the storage period. These findings are similar to the results of Yu (1996) and Cheng (2000).

3.3. Change in composition of sugars

The most important sugars were fructose, glucose and sucrose (Fig. 3). Fructose and glucose content increased from stage 1 to stage 3, while there was a general decline in sucrose content. This may be ascribed to the

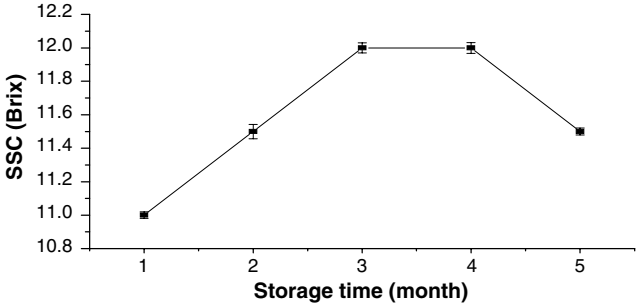


Fig. 2. Changes of Yali pear SSC during storage.

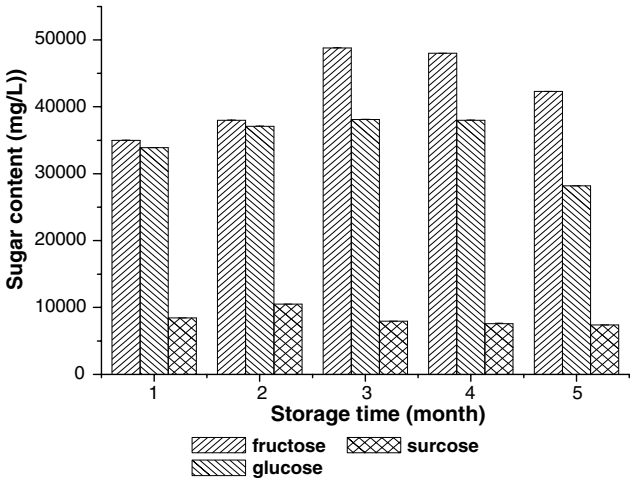
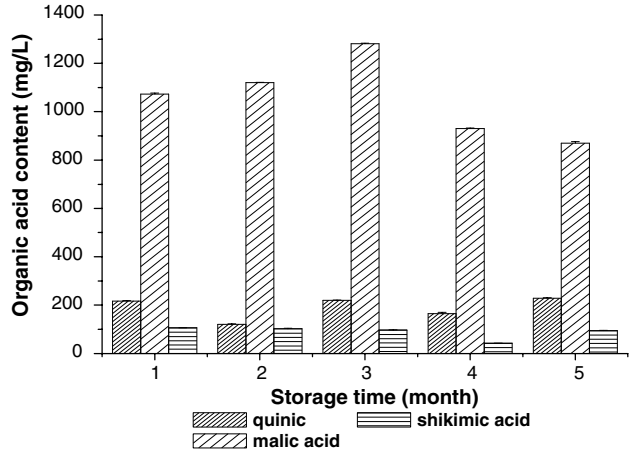


Fig. 3. Changes of Yali pear sugars content during storage.

hydrolysis of sucrose by sucrase to yield glucose and fructose from stage 1 to stage 3. Sucrose content increased slightly after stage 3, perhaps due to fruit respiration



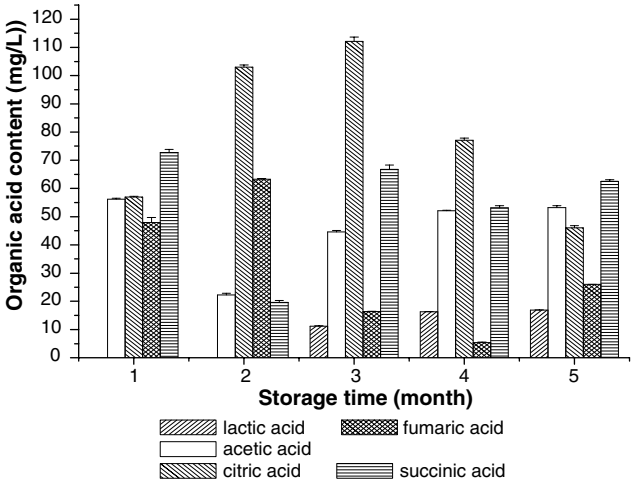


Fig. 4. Changes of Yali pear organic acids content during storage.

and loss of water during Yali pear storage, and decrease in the hydrolysis of starch, as indicated by a concurrent decrease in fructose and glucose content.

There have been numerous studies showing increasing levels of fructose, glucose and sucrose at advanced stages of fruit maturity (Ackerman, Fischer, & Amado, 1992; Gao & Wang, 1983). Gao and Wang (1983) indicated that during kuerle fragrant pear storage period, there was a close relationship between the sugar change and pear respiration change. During the first storage period, pear respiration increased and the sugar was consumed. But with the hydrolysis of starch, the individual sugar content of pear dropped slightly. In the middle of the storage, Kuerle fragrant pear respiration intensity decreased, and consumed little of the individual sugars. With a long storage time, the pear respiration intensity

Table 1
Volatile compounds identified from SPME/GC/MS

Retention time (min)	Compound name	Relative content (%)	Odor description
1.62	Ethanol	3.03	Alcohol
2.38	Ethyl acetate	3.68	Fruity, pineapple
3.03	Acetic acid	0.29	Sour
3.69	Propanoic acid, ethyl ester	0.17	Banana, apple
4.75	Propanoic acid, 2-methyl- ethyl ester	0.32	Sweet, fruity, apple, banana
5.75	Hexanal	6.16	Green, fruity
5.85	Butanoic acid, ethyl ester	16.62	Strawberry, apple, banana
6.25	Acetic acid, butyl ester	0.47	Fruity, pear
7.20	2-Butenoic acid, ethyl ester	0.28	
7.38	Butanoic acid, 2-methyl, ethyl ester	0.67	Green, apple, floral
8.06	1-Hexanol	0.14	Grass, fruity
9.06	Butyl acid, propyl ester	0.01	Pineapple
9.16	Pentyl acid, ethyl ester	0.27	Apple
10.58	Ethyl tiglate	0.14	Fruity
12.87	Hexanoic acid, ethyl ester	26.14	Fruity, green, apple, brandy, wine-like
13.09	3-Hexenoic acid, ethyl ester(Z)	0.24	Fatty, citrus-like
13.24	3-Hexenoic acid, ethyl ester(E)	0.23	
13.37	Acetic acid, hexyl ester	1.10	Apple, pear, floral
13.49	2-Hexen-1-ol, acetate	0.09	
13.95	Eucalyptol	0.04	
14.53	2-Hexenoic acid, ethyl ester	0.81	Green
15.00	2-Dodecenal	0.03	
15.54	1-Octanol	0.09	
16.57	Heptanoic acid, ethyl ester	0.18	Fruity
16.62	3-(Methylthio) propanoic acid, ethyl ester	0.16	
16.76	Nonanal	0.06	Fatty
17.66	Hexanoic acid, 3-hydroxy-ethyl ester	0.05	
19.18	Benzoic acid, ethyl ester	0.06	Fruity
19.90	4-Octenoic acid, ethyl ester	0.03	
20.19	Octanoic acid, ethyl ester	1.23	Floral
20.45	Decanal	0.03	
20.69	Acetic acid, octyl ester	0.32	Pear, fruity
21.84	Benzeneacetic acid, ethyl ester	0.07	Floral
21.91	2-Octenoic acid, ethyl ester	0.43	
21.98	9,12-Octadecadienoic acid (Z,Z)	0.04	
23.50	(E,E)2,4-Decadienal	0.03	Green
24.23	2,4-Decadien-1-ol	0.10	
26.15	Butanoic acid, butyl ester	0.02	Pear, pineapple
26.89	Decanoic acid, ethyl ester	0.02	
26.99	Tetradecane	0.03	
28.67	5,9-Undecadien-2-one-6,10-dimethyl	0.02	
29.17	2,4-*trans*,*cis*-ethyl decadienoate	0.45	Pear
30.10	Pentadecane	0.03	
30.41	α-Farnesene	15.57	
30.52	Butylated hydrotoluene	0.25	
32.92	Diethyl phthalate	0.02	
33.03	Hexadecane	0.10	
38.48	Eicosane	0.01	
40.50	1-Octadecene	0.05	
42.55	Dibutyl phthalate	0.16	
43.95	Isopropyl palmitate	1.06	

was increased, and the total sugar content was reduced. Our results were similar to this.

3.4. Change in the composition of organic acids

Quinic acid, malic acid, shikimic acid, lactic acid, acetic acid, citric acid, fumaric acid, and succinic acid were detected in the Yali pear fruits (Fig. 4). Ackerman et al. (1992) reported citric acid as the most and malic acid as the second most abundant acid determined in an apple variety throughout ripening and storage in a decreasing trend. But in Yali pear, the most important acid was malic acid, followed by citric acid. Malic and citric acid contents increased from stage 1 to stage 3, then decreased. The reason for this may be attributed to a dilution effect caused by mass increase during the cell growth phase. After storage, increased respiration is also responsible for the decline since malic acid is the principle metabolic substrate together with the sugar. Acetic acid content decreased during storage, possibly due to its function as respiration precursor, or its role in the synthesis of esters. Lactic acid was not detected in stage 1 and 2, but was detected after stage 2. Our result was similar to Zhang (1990).

3.5. Isolation of volatile compounds using SPME

See Table 1.

3.6. Change in volatile compounds during storage

Fifty-one compounds were obtained from SPME (Table 1). The most important volatile compounds were hexanoic acid, ethyl ester (26.14%), butanoic acid, ethyl ester (16.62%), α-farnesene (15.57%), hexanal (6.16%), acetic acid, ethyl ester (3.68%), and acetic acid, hexyl ester (1.1%). Isopropyl palmitate was also found in Yali pear, and probably originated from Yali pear waxiness in the pear skin or from the SPME extraction procedure.

Takeoka et al. (1992) reported that the following compounds are important contributors to pear aroma: ethyl 2-methybutanoate, ethyl hexanoate, ethyl butanoate, ethyl 2-methypropanoate, hexyl acetate, ethyl heptanoate, hexanal, ethyl pentanoate, and ethyl propanoate.

Shiota, Minami, and Sawa (1981) reported that the principal volatile components in the La France Pear were ethyl, propyl, butyl and hexyl acetates. In our research, the gas chromatography-olfactometry (sniffing) was used to determine whether the compounds from SPME method have significant contributor to Yali pear. From the odor description (Table 1), we can conclude that the compounds from SPME could account for the aroma of Yali pear, which agrees with the previous work on the aroma components of Asian pear and European pears.

As shown in Fig. 5, ethyl acetate, ethyl butyrate, ethyl hexanoate, hexyl acetate, and ethanol contents increased from stage 1 to stage 4, then decreased. Hexanal and α-farnesene contents decreased, while 2,4-*trans*,*cis*-ethyl decadienoate changed slightly during this period. Zhang (1990) found that the volatiles of climacteric fruit accumulated after the respiratory climacteric, but decreased during storage. Yali pear fruit follows this pattern. Hexanal content was concentrated in the skin of the immature Yali pear fruit after harvest, but decreased during storage as fruit matured. Hexyl acetate and *trans*,*cis*-2,4-ethyl decadienoate are important contributors to the flavor composition of Bartlett pear (Heinz & Jennings, 1966). These compounds were detected in Yali pear fruits, however further research is required to determine whether *trans*,*cis*-2, 4-ethyl decadienoate is also the key flavor compound of Yali pear (*P. serotina*). α-Farnesene was a major compound in Yali pear (*P. serotina*). Shiota et al. (1981) suggested that α-farnesene could be responsible for the "fresh green" odour of the La France pear fruit, since it was the main volatile compound of Japanese pear peel. The role of α-farnesene in Yali pear fruit requires further study.

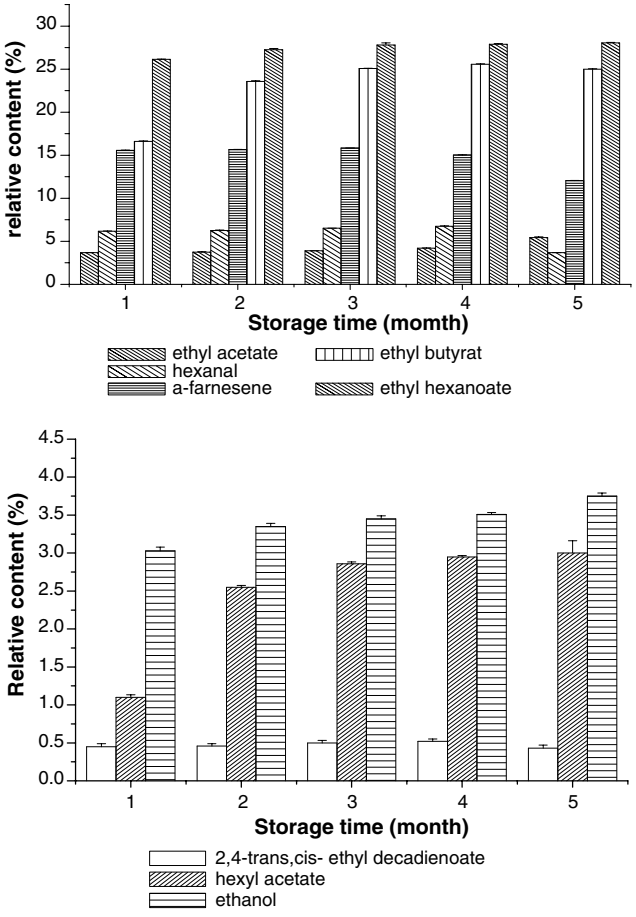


Fig. 5. Changes of Yali pear volatiles content during storage.

Table 2
The changes of the phenolic acid compounds during the storage time (mg/l)

Compounds name	Stage 1 (month)	Stage 2 (month)	Stage 3 (month)	Stage 4 (month)	Stage 5 (month)
Chlorogenic acid	148.6 ± 0.3	158 ± 0.2	145.6 ± 0.7	130.1 ± 0.5	128.5 ± 0.3
Quercitrin	2.07 ± 0.2	1.023 ± 0.2	2.007 ± 0.3	1.449 ± 0.0	1.651 ± 0.2
Phloretin xylogucoside	1.288 ± 0.3	1.794 ± 0.5	1.749 ± 0.2	1.869 ± 0.3	1.789 ± 0.0
Morin	7.547 ± 0.0	4.558 ± 0.3	4.171 ± 0.3	5.037 ± 0.3	5.162 ± 0.5
Quercetin	1.896 ± 0.3	1.924 ± 0.2	1.819 ± 0.0	1.969 ± 0.5	1.406 ± 0.3

In general, European pears are ripened by storage after picking. During storage, the fruit develops a soft flesh and a strong pleasant aroma, and becomes suitable for eating. The changes in the composition of volatile components during maturation were also investigated by Shiota et al. (1981). The results from this showed that the concentration of the esters, especially ethyl, propyl, butyl and hexyl acetates, increased markedly with increasing maturity of the fruit. During our research, Yali pear did not developed a soft flesh like European pears, although the firmness did decease. So perhaps there were different changes of volatile compounds between European pears and Yali pear.

3.7. Change in the composition of phenolic acids

Chlorogenic acid was the dominant phenolic acid, followed by morin, quercetin, phloretin xylogucoside, and quercitrin (Table 2). Zhang (1990) determined that the phenolic acid content of apple was relatively stable during maturation and storage. There is little literature about the functions of individual phenolic acids in Yali pear, although Wu, Zhou, and Wang (1992) reported that chlorogenic acid was the main contributor to core browning of Yali pear fruits.

The composition of phenolic acids in the Yali pear fruits changed during storage, especially the content of chlorogenic acid. From stage 1 (148 mg/l) to stage 5 (128.5 mg/l), total phenolic acid content decreased. This can be explained by the concurrent decrease in polyphenoloxidase (PPO) activity. Change in the composition of phenolic acids has little effect on the flavor of Yali pear fruit, however further research is required to determine the affects on processing for juice and other products. Ju (1991) reported that with the ripening of Yali pear, the phenolic acids dropped. Harel, Mayer, and Shain (1966) described a similar pattern of apple phenolic acid change.

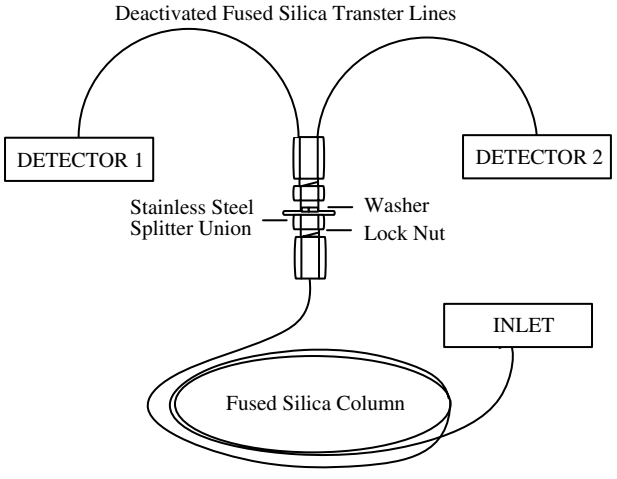


Fig. 6. GC-olfactometry sniffing equipment.

4. Conclusion

The physical and chemical properties of Yali pear fruits were investigated after 1, 2, 3, 4, and 5 months of storage. Flesh firmness and SSC changed slightly. The composition of sugars, organic acids, and phenolic acids was determined by HPLC. Fructose was the dominant sugar in Yali pear fruit, and malic acid was the principal organic acid, and chlorogenic acid the most important phenolic acid. The volatiles of Yali pear (*P. serotina*) during storage were first studied by high-resolution gas chromatography and GC/MS using SPME, and then further by using the GC-olfactometry port. The results demonstrated that the volatile flavor compounds from SPME were responsible for the aroma of Yali pear fruits. The volatiles and physical and chemical properties of Yali pear fruits change during storage, however, the cultivar retains good quality during storage.

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