Volatile compounds of fresh pineapple (*Ananas comosus* cv. Josapine) in different harvest periods

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Abstract: 'Josapine' is a famous pineapple cultivar as a kind of table fruit with a strong special aromatic fragrance like rose or jasmine, In this paper, ripen fruits were harvested monthly from January to June, and volatile compounds were measured by HS-SPME-GC-MS method to reveal the mysterious aroma compounds. Results showed that 112 volatile compounds totally were found in 5 harvest time. Esters and terpenes are the dominant volatiles among Josapine. February fruits contained the most abundant esters with 80.85% of total volatiles and May fruits contained the richest terpenes with 57.17%. In April and May harvested period, (Z)-β-ocimene was extremely significantly higher than that of other three periods. In March fruit. fruits smelt much more floral than in February and March probably attribute to the abundant (Z)-β-ocimene.

1. Introduction

Pineapple (*Ananas comosus*) is a popular typical tropical and subtropical fruit because of the excellent sweet flavor and pleasant aroma[1], which originated in native Americans and Caribben in the lowland tropics [2]. Pineapple was introduced to China in 1926. Up till now, China is one of the ten top pineapple-producing countries all over the world[3], now occupies almost 70,000 hectare area in China and yielded a crop worth ￥2.7 billion in 2023. As a famous fruit, it can be easily regulated for off-season harvest throughout the year by plant growth regulator such as ethephon. Pineapple fruit was very popular in winter and spring because of slack season for other fruits in China besides its nutrition and flavor. For many years, cultivar 'Comte de Paris' has been the main cultivar of China, occupies more than 70% planting area, which caused lots of problems black heart disease[4] and water heart disease decreasing the quality dramatically[5]. Diverse varieties were demanded deeply to improve and enrich the quality of pineapple.

'Josapine' was a famous pineapple cultivar as a kind of table fruit with strong aromatic fragrance like rose or jasmine, which was bred in Malaysia[6] and introduced to China in 2008. It is early ripen, and easily to handle forcing flowering anytime with high resistance to internal browning. The fruit was small but delicious reminding people of old memory for pineapple decades before. Above all, the special and pleasing aroma, something like rose or jasmine attract the attention and adore of consumers is quite different from the main cultivar ''Comte de Paris'' and new cultivar 'MD-2' [7]. Up till now there was little known about the volatile compounds of 'Josapine'. Hence, it is meaningful to measure and analyze the constituent of volatiles for further breeding program to improve the aroma quality.

It is well known that volatiles is an important actor in pineapple quality evaluation which affects choices of consumers, and nearly 480 VOCs have been identified to date using different analytical techniques, only 40 compounds are reported to contribute to the unique flavor of pineapple, which were predominantly comprised of esters, followed by terpenes, alcohols, aldehydes, and ketones[8]. With the development of instrument and advanced analytic method. SPME-GC-MS (solid phase micro-extraction for enrichment and gas chromatography -mass spectrometry for isolation and qualification) was proved an effective and simple method[9-10] . Pineapple aroma was quite different during different cultivars and harvest periods[11]. In this article, to reveal the special aroma flavor, the volatile components of 'Josapine' were measured in comparison with 'Comte de paris' and 'MD-2', and the aroma quality of fruits in different harvest periods were also measured. 'Josapine' plants were forced from September to December and harvested from February to June. Volatile compounds of 'Josapine' fruits was measured by HS-SPME-GC-MS method to reveal its mysterious aroma and flavor. Ripen fruits harvested monthly from January to June during the first half of the year were determined by SPME-GC-MS method.

2. Materials and methods

2.1. Materials and reagents

The experiment was conducted on Zhanjiang City, Guangdong Province, China. Uniformed pineapple plants were selected and forced by the 40 mg/L ethephon

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(produced by Pengpu chemical factory of shanghai, China) and 0.05% calcium carbonate and 0.05% borax (analytical grade produced by Sinopharm Chemical Reagent Co., Ltd., Shanghai, China) every month from August to December) to April. About 5 months later, fruits became ripe and were harvested in February, March, April, May and June. The fully ripe fruits were picked and applied to analyze the aroma quality. 15 fruits in five harvest time were divided into 3 groups. Pulps from each group were mixed together as one replication, and then were grounded to juices using a food processor.

2.2. The method of measurement

The sample was cut into small pieces about 2-3 mm. then put into 20 mL bottle about 7 gram and enriched by 65um PDMS fiber and assessed by GCMS method by Wei[9] as well as the instruments except that the oven temperature program was revised as follows: oven temperature rising from 40◦C to 120◦C at the elevating rate of 3◦C per minute, then rising to 150◦C at the rate of 5◦C per minute, and finally to 220◦C at the rate of 8◦C per minute.

2.3. Data analysis

DPS 9.5 (a statistical software developed by Zhejiang University, China) was used to analyze the data, Duncan's new multiple range test (MRT) was used to assess differences of the aromatic compounds of ripen pineapple fruit harvested in different month.

3. Results and discussion

3.1. Aromatic compounds in 'Josapine' fruit pulp

The identification was accomplished by comparison of their MS spectra with those present in a reference data library (NIST2005).

The analysis of pulp displayed the presence of 112 volatile compounds totally in 5 harvest time. Figure 1 shows the volatile substance in pulp of 'Josapine' consisted of esters, alkenes, terpenes, ketones, alcohols, alkanes and aldehydes, among of which esters and terpenes are the dominant volatiles. There were few ketones, alcohols, aldehydes and alkenes successively. And only 60 kinds of volatiles existed among fruit pulp in all the five harvest periods.

3.2. The comparison of aromatic compounds in different harvest period

There were 97 volatile compounds in February fruit, 99 in March, 99 in April, 106 in May, and 77 in June. Figure 1 shows the volatile substance in pulp of 'Josapine' consisted of esters, alkenes, terpenes, ketones, alcohols, alkanes and aldehydes, among of which esters and terpenes are the dominant volatiles among 'Josapine' as far as volatile components were concerned, which occupies kinds from 36 to 53, then esters from 29-36. Figure2 showed that the total relative content of volatiles for every group in five different harvest period. Esters and terpenes were still abundant which accounts for more than 94.31% in the total volatiles. The proportion esters varied from 40.72% to 80.85% and terpenes varied from 8.67% to 57.17%, which illustrated that harvest period affected the aroma quality to a great extent.

February fruits contained the most abundant esters with 80.85% of total volatiles and May fruits contained the richest terpenes with 57.17% (Figure2). As for others such as ketones, alcohol and aldehydes, no more than 4% were found in all harvest period. Esters played dominant role in volatiles harvested in February、March and June. However, terpenes and esters divided equally in fruits \harvested in both April and May. Most esters have low boiler and easy to volatilize with strong fruity fragrance but most of terpenes have high boiler which can last much longer than esters with strong floral, woody and spicy fragrance.

Volatiles in pineapple pulp concentrated in no more than 15 substances including methyl 2-ethylbutyrate, methyl hexanoate, methyl octanoate, ethyl octanoate, ethyl octanoate, pineapple methyl ester, (E)-β-ocimene, (Z)-β-ocimene, heptyl acetate, copaene, α-muurolene and so on. Nine kinds of volatiles were compared in table 1 and four massive substance were displayed in Figure 3. Both the relative content of methyl hexanoate and methyl octanoate were highest in February, the relative content of declined month by month since February and to a lowest point on May then increased from May to June. Methyl hexanoate showed an extremely significant increase from 8.61 % to 24.47% ($p < 0.01$). Except April and May, the relative content of methyl hexanoate was highest, which implied that it played an important role in the aroma contribution of pineaple. Table 1 showed that the relative content of (Z)-β-ocimene increased from March with significant difference ($p< 0.05$) but increased extremely rapidly from March to April with extremely significant difference ($p< 0.01$) and reached a climax at 30.42% in April then decreased until June at 12.4%, which was close with that of March at 11.53%. February fruit contains richest methyl hexanoate, Methyl 2-methylbutyrate, α-muurolene, heptyl acetate and methyl octanoate.

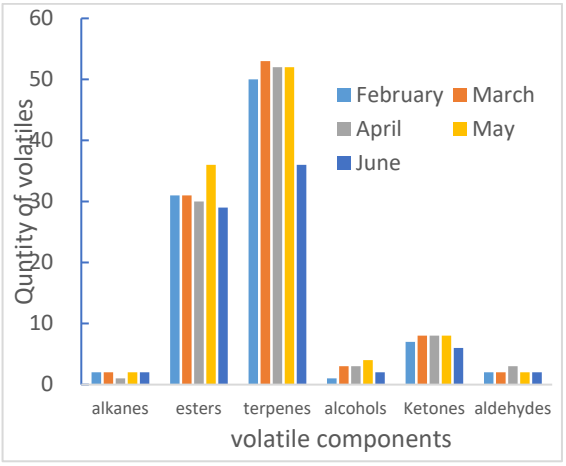

Figure 1. the volatile numbers in different harvest period

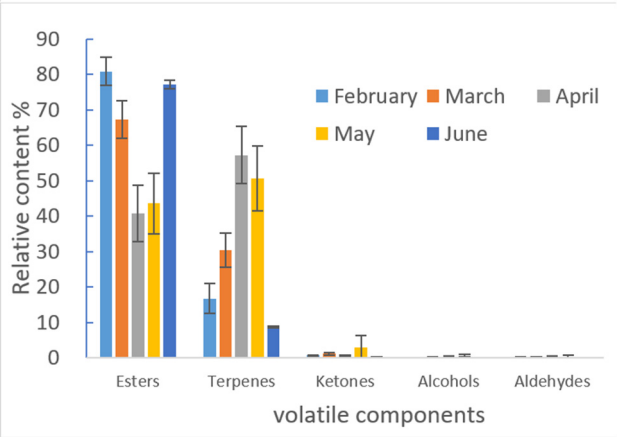

Figure 2. the relative percent of volatile groups in different harvest period

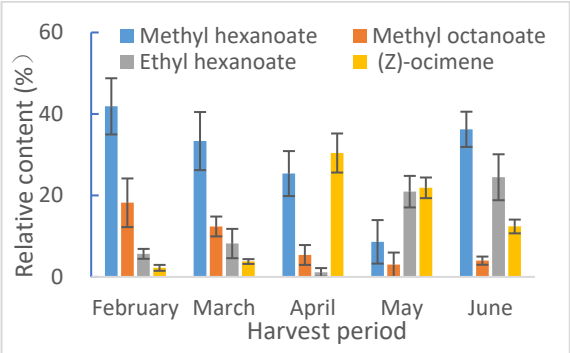

Figure 3. the relative percent of main volatile components in different harvest period

Table 1. The main volatile components in Josapine fruit in different harvest period.

Main components	Harvest period				
	February	March	April	May	June
Methyl hexanoate	41.87aA	33.351abAB	25.38bB	8.61cc	24.47bB
Ethyl hexanoate	5.66cdBC	8.19cB	1.14dC	20.93bA	36.24aA
Methyl octanoate	18.21aA	12.38aA	5.38bB	3.01bB	4.01bB
(Z)-β-ocimene	2.23dD	11.53cC	30.42aA	21.89bB	12.40cC
Copaene	2.17a	2.28a	2.09a	2.09a	0.54b
Heptyl acetate	1.82a	1.24b	1.24b	0.60c	0.66c
α-muurolene	1.56a	1.18ab	0.93b	1.06b	0.43c
Methyl 2-ethyl butyrate	4.91aA	2.72bAB	1.25cB	0.77cB	0.51cB
Pineapple methyl ester	1.66b	2.81a	1.50b	0.49c	0.64c

Different lowercase/capital letters in the same row indicate significance of difference at $p < 0.05$/ $p < 0.01$ levels.

Hexanoic acid ethyl ester was the main ester of pineapple aroma, as highest in February which mean February fruits presents more strong pineapple like aroma with high odor threshold. (Z)-β-Ocimene was richest in April which was significantly higher than other four harvest period. (Z)-β-Ocimene displays a floral, herbal and sweet odor with low odor thresholds. Chen (2015) reported that ocimene release more abundant in 20◦C from 'Purple Ruffles' than in 30◦C and 40◦C[12]. In this article the relative content of (Z)-β-ocimene were highest in April, which explained its variation curve increasing from cold February and decreasing in hot June. According to our smell practice, strong floral odor were also exhibited in April and May.

3.3. The comparison of aromatic compounds in 3 different cultivars

The major volatile compounds in the pulp of three pineapple cultivars was esters, terpenes (table 2). Methyl hexanoate and Methyl 2-methylbotanoate in 'MD-2' were extremely significantly higher than that in Josapine and 'Comte de Paris'. Methyl octanoate, ethyl octanoate and methyl decanoate were richest in 'Comte de Paris' which were extremely significantly higher than other two cultivars. Compared with 'MD-2'and 'Comte de Paris', 'Josapine' contained most abundant (E)-β-ocimene, (Z)-β-ocimene, α-amorphene and allo-ocimene with an extremely significant difference (Table 2). Both heptyl acetate and gepenene was not detected in 'MD-2'and 'Comte de Paris'. It was reported that ocimene presents a floral, rose, spicy and carnation aroma[13]. Allo-ocimene smells like neroli oil with a strong floral aroma. Due to these volatile components the aroma of 'Josapine' fruit was very special and pleasing.

In conclusion, 'Josapine' was a cultivar with strong aroma from February to June representing fruity, floral, woody fragrance. In April and May harvest period, fruits smelt much more floral than in February and March probably attribute to much higher content of (Z)-β-ocimene during the warmer harvest period.

Table 2. The main volatile components in 3 pineapple cultivars harvested in March

Components	Cultivar		
	Josapine	MD-2	Comte de Paris
Methyl 2-methylbotanoate	2.42bB	12.51aA	1.13bB
Methyl hexanoate	33.35aAB	40.94aA	9.91bB
Ethyl hexanoate	8.19a	8.35a	13.90a
(E)-β-ocimene	3.79aA	1.06bB	0.22bB
(Z)-β-ocimene	11.53aA	4.06bB	1.75bB
Heptyl acetate	1.24	/	/
Methyl octanoate	12.38bAB	3.52cB	21.654aA

Allo-ocimene	4.05aA	0.04bB	0.04bB
Ethyl benzoate	0.1	0.68	/
Gepenene	0.11	/	/
Ethyl octanoate	1.77bB	1.38bB	21.1aA
γ-octalactone	0.29a	0.10b	0.10b
Copaene	2.28a	0.82b	1.05b
Methyl decanoate	1.25bB	/	4.03aA
α-amorphene	0.52aA	0.23bB	0.23bB
(+)-aromadendrene	2.43bAB	1.03bB	4.27aA
α-muurolene	1.18aA	0.37bB	0.57bAB

Different lowercase/capital letters in the same row indicate significance of difference at $p < 0.05$/ $p < 0.01$ levels.

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