

Analysis of Phenols and Volatile Metabolites during Lemon Fruit Ripening after Bagging Treatment

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Abstract. Phenols and volatiles in lemon peels at five different stages [130, 144, 158, 172, and 186 days after flowering (DAF)] after bagging treatment were investigated. Lemon fruits harvested at 130 DAF had highest level of total phenolic compounds and total flavonoid. Bagging treatment decreased their content at all five maturity stages. Volatiles identified in lemon peels included 71 components and were divided into seven types according to chemical structure. The changes of volatiles at different stages after bagging varied with the chemical type. Total volatiles and monoterpenes reached the maximum value at 186 DAF in unbagged fruit, and the maximum value stage was advanced after bagging. Except for 172 DAF, the content of total volatiles and monoterpenes was decreased after bagging. Sesquiterpenes accounted for 2.16% to 5.44% of total volatiles. Most sesquiterpenes intermittently decreased with fruit ripening. Sesquiterpene compounds in unbagged fruit at 130 DAF were significantly higher than in bagged fruit, while their variation in two fruit types became small with fruit ripening. The proportion of alcohols and aldehydes reached 3.36% to 7.10% and 1.53% to 3.63%, respectively. Most alcohols and aldehydes were largely formed at the early and middle stages. Bagging treatment decreased their content mainly at 144 DAF. Fruit bagging and maturity stages had important influence on lemon phenols and volatiles, and appropriate harvest period after bagging was crucial for maintaining the nutritional and flavor quality of lemon fruits.

Citrus fruits ripening undergo a series of changes in primary and secondary metabolites, including sugars, organic acid, phenols, carotenoids, limonoids, and volatile terpenoids, which result in flavor and nutritional changes in fruits. These changes make fruit gradually transform toward maturity, and various characteristics combine to form unique

sensory and health properties for each variety at the maturity stage (Lado et al. 2018). Citrus fruits are nonclimacteric and do not have a postripening stage. The quality of harvest is crucial for the commercial value of citrus fruits. Lemon is a popular citrus fruit for high health-promoting ingredients and rich aroma in its peels (González-Molina et al. 2010). As the third most important citrus, lemon can be consumed fresh and also used for industrial extraction of functional ingredients. Fresh consumed lemons require higher sensory and nutritional properties, while high active ingredients are essential for use as industrial raw materials. Understanding the composition changes during the maturation process can help choose the appropriate harvesting time based on different purposes (Magalhães et al. 2023; Yang et al. 2011).

Fruits often suffer from sunburn, fruit cracking, insect attack, and mechanical damage during agricultural production, which lead to decreases in quality and economic loss. To alleviate these problems, growers extensively use agricultural chemicals, which pose great threats to human health and ecosystems (Feng et al. 2014). Bagging is a

physical protection method that isolates fruits from adverse environments, protecting fruits from damage caused by insects, strong light, and mechanical friction and furthermore improving the appearance quality (Yuri et al. 2020). What is more, the microenvironment of fruit development is changed after bagging, which may affect internal quality (Xie et al. 2013). Studies in kiwifruit, apple, grape, and peach have shown that bagging can affect fruit nutrition and flavor quality, and its effects varied among different fruit types and varieties (Liao et al. 2019). Fruit bagging markedly reduced volatile esters and olefins in apple while increasing volatile aldehydes (Feng et al. 2020). Total phenolic compounds in apple were reduced after bagging; among them, anthocyanin and quercetin were the most sensitive compounds (Yuri et al. 2020). Compared with unbagged peach, the content of aldehydes and esters was reduced, while content of alcohols and acids was increased in bagged fruit (Guo et al. 2016). Synthesis of esters in grape was facilitated, while terpenes, ketones, aldehydes, alcohols, and acids were restrained after bagging (Ji et al. 2019).

Bagging is a cultivation measure widely used in lemon production. It can promote the early coloring of lemon fruits and keep the surface clean and tidy; furthermore, it increases the commercial value of the products. In addition, bagging also reduces the post-harvest degreening process and lowers the processing cost (Zhang and Zhou 2019). Bagged lemon fruit is more popular by consumers owing to improved appearance quality and lowered pesticide residue. Jiang et al. (2019) found that the coloring rate of 'Eureka' lemon fruit reached 100% at 116 d after bagging, which is 62 d earlier than control. Chlorophyll in lemon was reduced, and carotenoid was increased by fruit bagging, which contribute to a value increased and color-changed yellow in the peels (Chen et al. 2021). So far, reports on the impact of bagging on the nutritional and flavor composition in lemon during fruit ripening are deficient.

The aim of this study is to determine the influence of bagging on phenols and volatiles in lemon peels during fruit ripening. This study will be conducive to selecting a suitable harvest period with good nutritional and flavor quality or high active ingredients and furthermore to providing a theoretical basis for the better application of bagging in production and meanwhile promoting the development and utilization of lemon peel resources.

Materials and Methods

Materials. The test variety adopted was Meyer lemon, which was planted in the experimental citrus orchard of Chongqing Academy of Agricultural Sciences. Levels of cultivation and management in the orchard were consistent. Bagging treatment was carried out at 90 d after blooming on fruits of the same size on the outer edge of the canopy. The fruit bag was a double-layered bag with an outer yellow layer and an inner black layer, which is opaque and not transparent. Lemon trees with consistent

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growth vigor were selected. Healthy fruits with uniform size and normal fruit shape were treated with shading and flat opening fruit bags, and the unbagged fruits near them were used as controls. The fruits of treatment and control were harvest at 130, 144, 158, 172, and 186 days after flowering (DAF), respectively. Fifteen sample fruits were randomly selected from four directions of lemon trees, and fruits on the outside of the canopy were picked as samples. Then the fruits were cleaned with water and air-dried (Fig. 1). The fruit samples were cut into four equal parts with a knife, and the peels were separated from the flesh. The flesh was used for determination of internal quality index of the lemon. Determination of soluble solids (SSC) using a handheld sugar refractometer and titratable acid (TA) was measured by acid-base titration. A 2,6-dichloroindophenol titration method was used to measure vitamin C (Table 1). The peels were ground into powder in liquid nitrogen. The prepared powder samples were stored in a refrigerator at -80°C for later use.

Phenol determination. Phenols were extracted according to Chen et al. (2015). A total of 0.2 g of peel powder was added to 80% methanol and then examined by ultrasound at 40°C for 60 min. After centrifugation at 4000 rpm for 15 min at 4°C , the supernatant was diluted to 6 ml. The total phenols were measured using the folin phenol method following Xie et al. (2013). The total flavonoid was measured according to the method of Dong et al. (2019).

Volatile determination. The extraction method was that used by Zhang et al. (2020); lemon peel powder (1 g) was weighed accurately and mixed well with 5 mL of saturated sodium chloride solution in a 20-mL head-space flask. Cyclohexanone (266 μg) dissolved in 20 μL of *n*-hexane was added to the flask, before sealing the bottle cap with a polytetrafluoroethylene spacer. 50/30- μm DVB/CAR/PDMS fibers (Supelco Co., Bellefonte, PA, USA) were adopted to collect volatiles. The needle fiber was aged at 250°C for 1 h before the extraction experiment. The solid-phase microextraction program consisted of an

equilibrium time of 6.21 min, an extraction temperature of 42°C , an extraction time of 45 min, and a desorption time of 3.4 min.

A GC-MS-QP2010 device (Shimadzu, Japan) was used to analyze the extracted volatiles from lemon peels. The chromatographic column that was used to dissociate volatiles was a Rxi-5MS capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μm ; J&W Scientific, Folsom, CA, USA). The temperature program of the column oven was as follows: maintained at 34°C for 2 min, increased at $5^{\circ}\text{C}/\text{min}$ to 100°C , increased at $3^{\circ}\text{C}/\text{min}$ to 110°C , increased at $10^{\circ}\text{C}/\text{min}$ to 230°C , and maintained for 2 min. Injection port temperature was 230°C . Helium was used as the carrier gas, the rate of which was maintained at 1.3 mL/min. The inlet was set in a split mode with split ratio of 50:1. The temperatures of the ion source and transmission line were set as 200 and 250°C , respectively. Electron impact mode at 70 eV was used, and the mass spectrometry scanning ranged from 55 to 500 amu.

Volatile components were identified by comparing the mass spectra of the tested compounds with the standard mass spectra of the NIST 2008 library. The compound content was calculated using the internal standard method, and the results are represented as $\mu\text{g}/\text{g}$ fresh weight (He et al. 2013).

Statistical analysis. All samples were set with three replicates. The data were collected by using Microsoft Excel software. SPSS 17.0 (SPSS Inc., Chicago, IL, USA) was used to perform analysis of variance. The significant differences of the means were tested by Tukey's multiple-range test at a level of 0.05.

Results and Discussion

Variations of total phenolic compounds and total flavonoid

Content of total phenolic compounds and total flavonoid changed with fruit ripening and bagging treatment (Table 2). Lemon fruits harvest at 130 DAF had the highest

Table 1. Soluble solids (SSC), titratable acid (TA), and vitamin C (VC) indicators of lemon flesh during ripening after bagging treatment.

Stage	Treatment	SSC, %	TA, %	VC, mg/100 ml
130 DAF	B	8.5	2.45	17.43
	NB	9.0	2.45	18.01
144 DAF	B	9.0	5.00	33.68
	NB	9.2	5.83	31.95
158 DAF	B	8.5	4.50	28.20
	NB	8.5	4.95	31.29
172 DAF	B	8.2	4.54	30.06
	NB	8.4	4.90	31.06
186 DAF	B	7.7	4.13	15.85
	NB	7.8	4.52	17.83

B = bagging, DAF = days after flowering, NB = no bagging (control).

level of total phenolic compounds, the content of which decreased continuously with fruit ripening, other than an increase at 172 DAF. Bagging treatment decreased the content of total phenolic compounds at five stages. Total flavonoid showed similar change trend with total phenolic compounds, and it showed a trend of decreasing before increasing and then decreasing with fruit ripening. Total flavonoid in unbagged fruit ranged from 0.66 to 2.39 mg/g which was higher than 0.44 to 1.86 mg/g in bagged fruit. Phenolic compounds and flavonoid were the important bioactive ingredients of lemon fruit and can prevent many diseases in humans (González-Molina et al. 2010). Lemon peels at early development stages were rich in phenolic compounds and flavonoid, which were good raw materials for the food and pharmaceutical industries. The adverse effects of bagging on phenolic compounds were also found in peach, apple, and sweet orange (Chen et al. 2012; Su et al. 2024; Xie et al. 2013). The reason for the decrease of phenolic compounds may be related to the changes in lighting factors after bagging (Yuri et al. 2020).

Variations in volatiles

Types and total contents of volatiles. Seventy-one volatiles were identified from Meyer lemon peels at five stages. According to the chemical structure, they were divided into seven kinds: monoterpenes, sesquiterpenes, alcohols, aldehydes, ketones, esters, and others. The influence of fruit ripening and bagging treatment on the content of volatiles varied with chemical type. The stage with the highest total volatiles was advanced after bagging, which appeared at 172 DAF in bagged fruit and at 186 DAF in unbagged fruit. The total content of volatiles ranged from 33,713.17 to 39,393.36 $\mu\text{g}/\text{g}$ in unbagged fruit and from 25,773.98 to 39,422.97 $\mu\text{g}/\text{g}$ in bagged fruit. Except for 172 DAF, bagging treatment reduced total content of volatiles at the other four stages (Table 3).

Effects of volatiles by fruit maturation and bagging. Fruit maturation is an important process for forming special flavor quality accompanied by changes in a large number of volatile components. Fruit bagging changed the light and temperature conditions of maturation process and further resulted in metabolism alteration of volatiles (He et al. 2022; Wang et al. 2010).

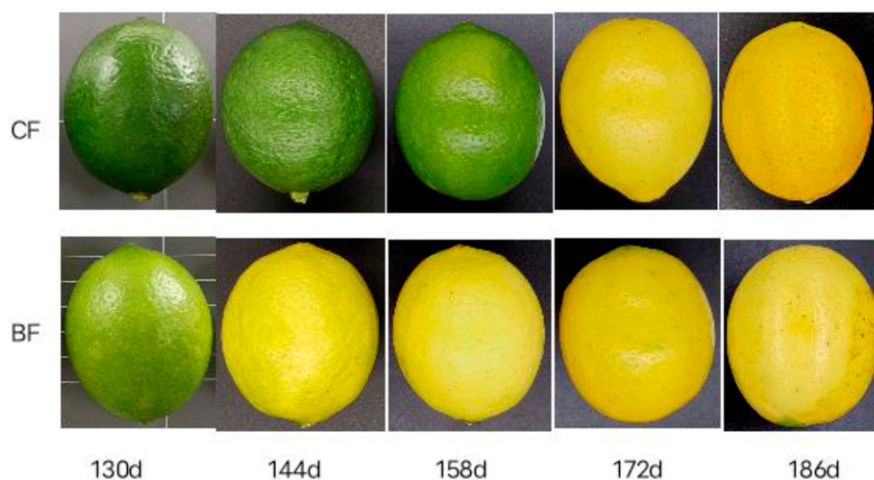


Fig. 1. Fruits of Meyer lemon at different growth stages. 130d, 144d, 158d, 172d, and 186d indicate fruit harvested at different stages of 130, 144, 158, 172, and 186 days after flowering, respectively. CF = unbagged fruit, BF = bagged fruit.

Table 2. Total phenolic compounds and total flavonoid of lemon during fruit ripening after bagging (fresh weight).

Stages	Treatments	Total phenolic compounds, mg/g	Total flavonoid, mg/g	Hesperidin, $\mu\text{g/g}$	Narirutin, $\mu\text{g/g}$	Sinensetin, $\mu\text{g/g}$	Nobiletin, $\mu\text{g/g}$	Tangeretin, $\mu\text{g/g}$
130 DAF	B	4.44 $\pm$ 0.11 a	1.86 $\pm$ 0.13 a	3738.40	14.17	36.37	18.07	26.48
	NB	4.55 $\pm$ 0.09 a	2.39 $\pm$ 0.34 a	4163.87	45.75	37.78	22.42	29.62
144 DAF	B	3.81 $\pm$ 0.02 a	1.64 $\pm$ 0.18 a	3306.77	8.51	35.36	15.14	24.18
	NB	4.03 $\pm$ 0.15 a	1.70 $\pm$ 0.08 a	3651.17	19.63	36.49	17.47	25.89
158 DAF	B	3.27 $\pm$ 0.09 b	0.38 $\pm$ 0.02 b	2982.12	22.60	35.71	15.69	25.36
	NB	3.85 $\pm$ 0.05 a	0.88 $\pm$ 0.06 a	3248.89	21.18	37.20	20.67	29.70
172 DAF	B	3.72 $\pm$ 0.13 b	0.56 $\pm$ 0.11 b	2758.40	20.49	35.21	13.57	24.40
	NB	4.36 $\pm$ 0.09 a	1.04 $\pm$ 0.13 a	2985.17	13.92	36.47	17.89	26.86
186 DAF	B	3.08 $\pm$ 0.13 b	0.44 $\pm$ 0.08 a	2690.14	15.65	34.83	13.18	23.92
	NB	3.67 $\pm$ 0.10 a	0.66 $\pm$ 0.21 a	2880.72	19.09	35.89	15.85	25.55

Different letters indicate significant differences between treatments for the same stage ($P < 0.05$).

B = bagging, DAF = days after flowering, NB = no bagging (control lemon fruit).

The effects of volatiles in lemon by fruit maturation and bagging are shown in Table 3.

Monoterpenes accounted for 78.66% to 88.70% of total volatiles. Fifteen monoterpenes including three chained monoterpenes [β -myrcene, (E)- β -ocimene, (Z)- β -ocimene], seven monocyclic monoterpenes (α -phellandrene, α -terpinene, *p*-cymene, *n*-limonene, γ -terpinene, terpinolene, and 1,3,8-*p*-menthatriene), and five bicyclic monoterpenes (α -thujene, α -pinene, camphene, sabinene, and β -pinene) were identified at five stages. Lemon peels were rich in monocyclic monoterpenes; for example, *n*-limonene was the most abundant monoterpene followed by γ -terpinene and *p*-cymene. Fruit ripening stage and bagging treatment had significant influence on the content of monoterpenes. The change trend of all monoterpene compounds during fruit ripening was similar, intermittently increasing in unbagged fruit and reaching a maximum at 186 DAF. The trend of monoterpenes with fruit ripening was changed after bagging, which presented a trend of increasing first, then decreasing in bagged fruit, and reaching a maximum value at 172. The content of monoterpenes was reduced after bagging treatment at the other four stages, but not at 172 DAF.

Twenty-five sesquiterpenes were detected from lemon peels of different stages and treatments. Content of sesquiterpenes accounted for 2.16% to 5.44% of total volatiles. (E)- α -Bergamotene, β -caryophyllene, α -selinene, and β -bisabolene were the main sesquiterpenes. Sesquiterpene compounds generally decreased in unbagged fruit during ripening, while there was a trend of increasing first and then decreasing in bagged fruit. The inhibitory effect of bagging on sesquiterpenes mainly appeared at early development stages. Sesquiterpenes compounds in unbagged fruit at 130 DAF were significantly higher than in bagged fruit, while their variation in two fruit types became small with fruit ripening. Terpenes are important secondary metabolites produced by fruits, the decrease or increase of which is strictly associated with their biological functions and linked to protection mechanisms against photo-oxidative stress and ultraviolet radiation (Di Rauso Simeone et al. 2020). Liu et al. (2017) found that regulation on peach terpenes by fruit bagging were consistent with effect of ultraviolet-B irradiation treatment. Bagging changes the

lighting conditions of fruits, which may be one of the reasons for the decrease in the synthesis of terpenes with photoprotection effect in fruits.

Alcohols accounted for 3.36% to 7.10% of total volatiles, which included a total of 13 compounds. Linalool and α -terpineol were the most abundant alcohols. Both of them were largely synthesized at early maturation stages and kept stable after notably decreasing at 158 DAF. Bagging treatment had an inhibitory effect on linalool before 158 DAF, while it was slightly increased after that stage. After bagging, α -terpineol was decreased at 144 DAF but increased at other stages. (S)-Perilla alcohol, 1-*p*-menthen-9-ol, nerol, and viridiflorol first decreased and then increased before finally decreasing with fruit ripening, while L-carveol, borneol, and 4-terpineol presented a contrary change trend. The effect of bagging on them varied at different stages. Except (S)-perilla alcohol, the other six compounds were decreased at 144 DAF after bagging, while only three of them were decreased at 186 DAF.

The relative content of aldehydes ranged from 1.53% to 3.63%; altogether, eight compounds belonged to this chemical type. Neral, geranial, and undecanal were largely accumulated at early stage and then decreased with fruit ripening reaching a maximum value at 130 DAF and a minimum value at 186 DAF. Four aldehydes were largely increased and reached their highest level at the middle stage, which included nonanal and decanal at 144 DAF, citronellal at 158 DAF, and perilla aldehyde at 172 DAF. 1-*p*-Menthen-9-al first decreased then increased with fruit ripening. Undecanal was decreased, while perilla aldehyde was increased after bagging regardless of stages. Nonanal and citronellal decreased at the other four stages (excepting 172 DAF) by bagging treatment. Bagging mainly showed inhibitory effect on decanal, 1-*p*-menthen-9-al, neral, and geranial before 158 DAF but had a promotion effect after that time. Aldehydes are important flavor components of some fruits like cucumber, lemon, and peach. The promotion effect of bagging on aldehydes also appear in cucumber and peach, in which there are also consistent changes in key metabolic enzyme activity (Shan et al. 2020; Shen et al. 2014). Aldehyde synthesis pathways in fruits mainly

through oxidization of fatty acids and dehydrogenation of corresponding alcohol, and the key enzyme included lipooxygenases, hydroperoxydelyase, and dehydrogenases (El Hadi et al. 2013). The changes in aldehyde content in fruits may be associated with the regulation of bagging on key enzyme activity in the aldehyde synthesis pathway.

Three ketones, including 2-bornanone, umbellulone, and piperitone, were identified at five stages of two treatments. Content of 2-bornanone was higher than other two compounds and was the dominant ketones. 2-Bornanone increased first and then decreased with fruit ripening, reaching the highest level at 144 DAF. Similar change trends appeared in umbellulone and piperitone, which reached their highest levels at 144 and 158 DAF, respectively. Bagging treatment had decreased effect on ketones mostly at the middle stages, like 2-bornanone and umbellulone at 144 DAF. The ketones in citrus were low; this kind of compound in citrus had been identified, including nootkatone in grapefruit, 1-octen-3-one in sweet orange, and short-chain aliphatic ketones like acetone and 2-butanone in mandarin, which present woody, mushroom, and fruit flavors, respectively. This was different from 2-bornanone in lemon, which presented cool and sweet flavors. Citrus species have different kinds of ketones flavor compounds, which promote formation of various flavor of different genotypes (Huang et al. 2022; Türkmenoğlu and Özmen 2023; Wang et al. 2023).

Five esters were identified in lemon peels, which proportion ranged from 0.13% to 0.21%. Chrysanthenyl acetate and nerol acetate were the predominant esters produced at five stages of two treatments in fruit. Chrysanthenyl acetate had the highest level at 130 DAF and decreased with fruit ripening with the exception of 172 DAF. Nerol acetate first decreased, then increased, and reached the highest level at 186 DAF. Geranyl acetate, perilla acetate, and carvyl acetate had lower content and only appeared at specific stages. Carvyl acetate and perilla acetate were mainly produced at 130 DAF, while carvyl acetate only appeared at 186 DAF. Bagging treatment decreased total esters content before 158 DAF, while it varied little between bagged and unbagged fruit after that stage. Nerol acetate, geranyl acetate, and perilla acetate were the

Table 3. Volatile compositions and contents of Meyer lemon peels at different harvest stages after bagging (µg/g fresh weight).

Compounds	Unbagging					Bagging					F value (significance)		
	130 DAF	144 DAF	158 DAF	172 DAF	186 DAF	202/273.59	144 DAF	158 DAF	172 DAF	186 DAF	B	S	B*S
Monoterpenes	26,695.38	33,113.57	28,627.16	30,591.70	34,941.13	20,273.59	21,337.78	25,983.84	33,766.02	26,142.62			
α-Thujene	71.8 ± 22.86	105.13 ± 26.49	94.25 ± 14.51	101.59 ± 6.55	169.44 ± 60.18	46.15 ± 3.9	51.9 ± 6.21	68.55 ± 8.77	121.08 ± 23.14	86.17 ± 17	14.043***	7.532***	3.591*
α-Pinene	214.62 ± 71.64	313.73 ± 83.61	284.03 ± 47.77	313.24 ± 18.91	512.53 ± 181.47	140.97 ± 16.66	157.17 ± 19.17	211.65 ± 25.94	374.18 ± 71.3	262.11 ± 54.49	12.73***	7.63***	3.494*
Camphene	3.63 ± 1.87	3.69 ± 1.14	3.55 ± 1.07	5.51 ± 0.32	7.52 ± 1.68	2.73 ± 0.55	2.28 ± 0.43	3.23 ± 0.86	6.75 ± 0.75	5.58 ± 0.8	2.952	16.079***	1.979
Sabinene	124.41 ± 34.67	123.49 ± 26.71	93.44 ± 11.02	99.8 ± 7.02	121.31 ± 38.68	45.47 ± 3.78	53.95 ± 5.77	63.3 ± 8.41	117.93 ± 22.64	66.8 ± 11.29	31.949***	1.831	5.211**
β-Pinene	211.25 ± 59.44	305.43 ± 69.76	259.75 ± 32.04	311.11 ± 13.51	447.14 ± 140.35	156.5 ± 17.05	163.78 ± 12.87	210.52 ± 26.07	427.56 ± 65.31	267.76 ± 51.38	7.65*	10.935***	5.242**
β-Myrcene	433.78 ± 120	640.33 ± 158.02	558.24 ± 59.32	597.54 ± 19.02	949.4 ± 373.49	285.45 ± 28.83	345.75 ± 30.87	456.4 ± 51.81	716.41 ± 106.3	492.53 ± 109.45	11.106**	5.84**	3.316*
α-Phellandrene	56.08 ± 8.08	47.5 ± 11.74	44.46 ± 14.04	43.89 ± 5.37	50.82 ± 8.01	33.24 ± 4.26	49.77 ± 10.34	40.7 ± 10.89	49.94 ± 5.39	37.78 ± 8.6	3.383	0.368	2.43
α-Terpinene	73.34 ± 6.97	106.38 ± 18.67	88.76 ± 4.64	88.59 ± 3.22	122.96 ± 35.56	58.83 ± 0.98	60.17 ± 4.17	73.85 ± 7.09	124.81 ± 19.28	100.99 ± 21.44	3.539*	8.276***	6.887***
p-Cymene	553.22 ± 121.13	643.06 ± 64.35	588.42 ± 52.45	685.69 ± 22.43	736.61 ± 43.31	453.58 ± 43.31	474.08 ± 16.91	571.34 ± 52.55	610.79 ± 199.28	676.43 ± 120.18	5.682*	3.83*	0.525
o-Limonene	21,526.09 ± 4633.85	26,364.22 ± 4760.11	22,954.18 ± 1948.55	24,519.93 ± 82.85	26,519.6 ± 2471.77	16,682.63 ± 1328.74	17,389.75 ± 479.87	21,172.98 ± 2015.76	26,760.01 ± 1820.46	20,668 ± 3872.46	14.119***	4.429**	3.473*
(E)-β-OCimene	21.98 ± 5.94	25.56 ± 6.74	20.47 ± 1.24	20.98 ± 0.97	62.18 ± 46.28	14.23 ± 1.81	12.41 ± 0.66	17.11 ± 2.22	26.18 ± 3.8	19.28 ± 4.42	8.704**	3.678*	2.33
(Z)-β-OCimene	61.82 ± 13.42	75.2 ± 16.25	63.12 ± 3.76	59.24 ± 1.34	127.33 ± 76.44	40.93 ± 2.42	37.49 ± 4.12	48.6 ± 4.89	70.27 ± 7.34	52.14 ± 12.51	8.618**	2.164	2.33
γ-Terpinene	2931.5 ± 672.53	3805.31 ± 734.46	3119.05 ± 209.79	3284.1 ± 44.04	4483.07 ± 1082.66	1956.71 ± 169.65	2145.33 ± 159.57	2588.22 ± 270.9	3733.66 ± 331.41	2876.8 ± 624.1	19.757***	5.339**	4.01*
Terpinolene	399.25 ± 52.92	539.58 ± 79.76	442.96 ± 22.33	456.96 ± 6.49	614.49 ± 126.52	344.7 ± 16.14	384.46 ± 12.93	447.2 ± 43.1	612.93 ± 41.75	518.74 ± 100.69	1.587	8.899***	5.297**
1,3,8-p-Menthatriene	12.61 ± 1.12	14.96 ± 1.22	12.49 ± 1.24	11.55 ± 0.64	16.75 ± 8.06	11.45 ± 0.92	9.49 ± 1.11	10.19 ± 0.58	13.51 ± 0.29	11.5 ± 1.74	5.951*	0.849	1.904
Sesquiterpenes	1836.80	1490.52	1492.74	1234.31	1075.82	557.45	734.22	1125.70	1515.95	1204.05			
δ-Elemane	6.71 ± 1.64	6.66 ± 1.27	6.29 ± 0.62	4.5 ± 0.15	4.14 ± 0.49	2.14 ± 0.43	4.91 ± 0.97	5.1 ± 0.86	6.82 ± 0.53	5.33 ± 0.77	6.288*	3.11*	14.146***
α-Cubebene	12.65 ± 3.89	11.93 ± 2.61	11.09 ± 0.81	10.84 ± 1.21	11.47 ± 2.21	4.68 ± 0.39	6.71 ± 0.83	9.1 ± 0.81	11.76 ± 0.39	9.39 ± 2.55	21.573***	1.658	4.699**
Copaene	23 ± 6.32	21.43 ± 4.2	20.11 ± 2	16.06 ± 1.36	16.14 ± 2.74	8.26 ± 1	10.38 ± 0.63	14.63 ± 1.58	20.29 ± 0.73	14.22 ± 2.84	30.121***	1.153	10.045***
β-Elemene	86.78 ± 25.83	98.13 ± 16.84	93.56 ± 8.13	75.81 ± 3.95	68.28 ± 10.59	29.84 ± 3.08	51.25 ± 7.15	73.79 ± 9.44	90.24 ± 6.73	62.53 ± 10.42	27.367***	5.085**	8.885***
(Z)-α-Bergamotene	15.71 ± 8.25	12.86 ± 3.48	14.37 ± 2.39	13.94 ± 1.06	11 ± 0.97	4.54 ± 0.5	6.36 ± 0.85	9.53 ± 1.84	16.76 ± 3.21	9.155**	2.701	4.38	
β-Caryophyllene	141.63 ± 42.7	125.23 ± 26.15	121.05 ± 11.59	102.57 ± 5.42	95.3 ± 15.25	50.68 ± 4.87	61.3 ± 2.49	93.98 ± 10.3	130.23 ± 10.12	91.9 ± 17.25	21.687***	1.819	9.698***
γ-Murolene	8.11 ± 5.33	6.2 ± 1.55	7.19 ± 1.57	7.43 ± 0.9	5.15 ± 0.59	2.14 ± 0.24	3.56 ± 0.3	2.77 ± 0.87	7.96 ± 2.69	5.33 ± 2	9.764**	1.819	2.581
(E)-α-Bergamotene	192.21 ± 53.76	174.84 ± 31.84	167.13 ± 14.82	155.4 ± 3.32	134.43 ± 16.84	62.62 ± 4.3	82.24 ± 6.81	131.89 ± 15.67	202.89 ± 24.39	157.25 ± 24.14	17.788***	4.473**	14.213***
Aromadendrene	8.16 ± 3.32	7.37 ± 1.9	7.88 ± 1.19	3.47 ± 3.55	3.62 ± 2.29	nd	4.17 ± 0.59	5.02 ± 1.05	5.17 ± 3.13	5.52 ± 1.5	6.473*	1.228	5.184**
β-Cubebene	8.84 ± 4.77	6.78 ± 1.66	7.06 ± 1.17	3.74 ± 3.8	3.95 ± 2.5	nd	2.3 ± 2.02	4.92 ± 4.3	5.16 ± 3.1	5.37 ± 1.1	5.902*	0.328	3.473*
(E)-β-Farnesene	61.93 ± 19.93	46.98 ± 9.59	50.73 ± 3.51	33.9 ± 1.49	29.81 ± 5.2	12.19 ± 0.83	17.08 ± 3.51	26.4 ± 3.53	45.15 ± 5.81	35.02 ± 4.67	37.526***	1.195	15.814***
α-Caryophyllene	85.73 ± 23.77	69.88 ± 10.18	66.55 ± 5.92	56.27 ± 4.55	50.38 ± 8.88	28.57 ± 1.12	34.67 ± 4.11	51.06 ± 6.14	67.95 ± 4.08	50.92 ± 9.39	28.48***	1.384	11.893***
β-Santalene	27.29 ± 5.81	20.14 ± 3.24	21.62 ± 1.46	16.87 ± 2.07	14.68 ± 0.85	8.32 ± 1.19	9.35 ± 2.05	13.45 ± 1.78	23.05 ± 4.43	16.63 ± 4.65	23.908***	2.217	13.720***
Bicyclosesquiphellandrene	24.78 ± 9.08	15.73 ± 1.09	14.97 ± 2.24	13.12 ± 1.39	10.28 ± 3.22	7.6 ± 2.36	8.94 ± 2.01	10.83 ± 0.97	18.26 ± 1.43	11.51 ± 1.5	12.22**	2.654	9.448***
α-Murolene	30.91 ± 9.14	19.74 ± 2.05	26.76 ± 4.54	21.06 ± 4.38	17.66 ± 4.02	10.82 ± 0.14	12.36 ± 3.46	15.17 ± 3.99	25.79 ± 4.88	17 ± 2.95	18.017***	2.631	6.812***
Patchoulene	83.08 ± 21.87	60.26 ± 9.02	61.61 ± 2.77	50.4 ± 4.51	43.48 ± 7.18	25.44 ± 1.34	31.27 ± 6.61	45.56 ± 5.35	61.3 ± 6.12	50.76 ± 8.66	25.952***	1.504	14.368***
Germaerene D	143.98 ± 43.59	131.89 ± 23.95	127.43 ± 9.19	101.19 ± 4.76	95.34 ± 19.01	38.08 ± 1.61	55.48 ± 5.83	84.26 ± 10.12	127.74 ± 8.24	92.41 ± 10.16	37.583***	1.849	13.237***
β-Eudesmene	157.69 ± 28.48	117.05 ± 26.67	105.88 ± 7.45	93.46 ± 11.31	80.9 ± 8.92	47.14 ± 2.15	58.82 ± 15.02	92.31 ± 12.44	100.24 ± 9.4	91.29 ± 16.07	32.522***	1.21	15.634***
β-Bisabolene	269.86 ± 63.83	193.74 ± 29.04	194.76 ± 13.06	159.81 ± 15.34	140.97 ± 25.05	85.45 ± 4.34	99.73 ± 22.71	153.47 ± 17.31	187.78 ± 15.38	164.13 ± 27.54	27.81***	1.56	15.153***
γ-Cadinene	324.77 ± 83.07	256.07 ± 34.29	269.11 ± 12.11	213.54 ± 20.7	177.69 ± 33.4	93.8 ± 3.38	127.26 ± 23.17	212.91 ± 25.48	273.74 ± 19.95	231.45 ± 28.45	22.482***	2.633	18.908***
δ-Cadinene	24.83 ± 7.96	17.95 ± 3.86	20.58 ± 1.62	17.42 ± 1.25	12.4 ± 1.9	8 ± 1.11	8.23 ± 4.8	15.22 ± 2.05	18.31 ± 2.98	16.25 ± 3.09	16.631***	2.088	7.733***
1,4-Cadinadiene	74.03 ± 20.33	54.78 ± 7.38	57.41 ± 3.36	47.17 ± 4.64	37.46 ± 4.24	22.03 ± 0.77	29.97 ± 6.26	42.33 ± 3.72	51.03 ± 5.62	43.3 ± 7.45	30.728***	1.672	12.767***
α-Amorphene	6.36 ± 3.76	2.97 ± 2.59	5.41 ± 0.73	4.91 ± 0.77	3.17 ± 0.43	1.25 ± 0	1.95 ± 0	2.99 ± 0	4.7 ± 1.27	3.38 ± 0.88	3.899	0.965	1.214
α-Amorphene B	12.05 ± 6.29	6.94 ± 1.09	9.41 ± 0.9	8.09 ± 1.07	5.37 ± 0.67	2.38 ± 0.42	3.39 ± 1.21	5.42 ± 0.81	8.47 ± 2.03	7.19 ± 1.33	13.176**	1.654	5.87**
Alcohols	5.7 ± 1.64	4.97 ± 0.93	4.76 ± 1.33	3.34 ± 0.57	2.75 ± 0.62	1.48 ± 0.18	2.55 ± 0.88	3.6 ± 0.43	5.13 ± 0.73	3.82 ± 0.53	9.369***	1.234	11.686***
Linalol	1807.73	1640.42	1373.16	1376.18	1321.80	1831.08	1496.34	1465.59	1458.09	1297.89			
(Z)-p-Menth-2-en-1-ol	630.35 ± 87.87	574.82 ± 47.24	484.29 ± 30.35	449.28 ± 25.42	446.06 ± 48.55	587.9 ± 8.53	507.94 ± 50.53	504.31 ± 46.58	461.58 ± 26.25	451.37 ± 44.73	0.722	12.492***	1.023
l-Carveol	6.03 ± 3.3	6.72 ± 0.52	5.41 ± 0.24	5.91 ± 0.35	5.83 ± 1.56	5.96 ± 1.11	4.72 ± 0.28	4.87 ± 0.75	4.88 ± 1.26	3.94 ± 1.36	4.773*	0.617	0.55
Isopulegol	71.02 ± 12.87	73.82 ± 7.33	64.48 ± 3.55	65.17 ± 3.61	76.9 ± 12.53	63.71 ± 2.8	52.1 ± 2.05	60.1 ± 5.42	74.19 ± 5.06	61.13 ± 12.39	7.774*	1.136	3.324*
Borneol	4.95 ± 4.29	nd	3.94 ± 0	8.41 ± 0.99	11.87 ± 2.22	6.86 ± 1.64	nd	5.45 ± 0.05	6.91 ± 1.28	8.02 ± 2.82	0.03	19.534***	2.821
Terpinol	6.78 ± 0.77	10.37 ± 0.53	8.69 ± 2.98	7.26 ± 0.61	7.56 ± 0.64	7.94 ± 1.05	4.68 ± 0.46	9 ± 2.2	10.16 ± 1.03	7.74 ± 1.45	0.195	1.481	7.922***
4-Terpinol	191.64 ± 32.37	211.93 ± 8.98	123.23 ± 18.05	188.38 ± 11.92	189.09 ± 32.83	210.66 ± 14.42	175.15 ± 22.74	155.76 ± 15.67	163.6 ± 11.09	165.86 ± 21.53	0.784	8.021***	3.284*
p-Cymen-8-ol	28.84 ± 4.72	20.23 ± 6.51	14.51 ± 1.49	16.35 ± 6.82	21.06 ± 3.8	32.71 ± 1.8	18.66 ± 6.85	19.28 ± 5.26	30.39 ± 1.44	26.68 ± 4.43	9.435**	7.329***	2.075

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Table 3. (Continued)

Compounds	Unbagging					Bagging					F value (significance)		
	130 DAF	144 DAF	158 DAF	172 DAF	186 DAF	130 DAF	144 DAF	158 DAF	172 DAF	186 DAF	B	S	B*S
α -Terpineol	554.21 $\pm$ 78.17	500.11 $\pm$ 10.89	402.23 $\pm$ 43.41	452.3 $\pm$ 32.18	418.06 $\pm$ 34.36	618.09 $\pm$ 7.13	485.32 $\pm$ 65.41	440.36 $\pm$ 27.56	458.36 $\pm$ 40.75	433.82 $\pm$ 48.92	1.831	14.126***	0.705
Perilla alcohol	26.76 $\pm$ 11.7	28.25 $\pm$ 1.5	30.44 $\pm$ 1.78	5.54 $\pm$ 0.3	9.26 $\pm$ 9.19	19.19 $\pm$ 13.8	31.38 $\pm$ 1.34	33.61 $\pm$ 2.33	22.73 $\pm$ 12.1	3.57 $\pm$ 0.55	0.551	12.16***	2.521
(S)-Perilla alcohol	133.76 $\pm$ 10.83	94.96 $\pm$ 6.42	144.62 $\pm$ 6.1	66.18 $\pm$ 7.48	34.55 $\pm$ 9.68	129.89 $\pm$ 9.53	115.84 $\pm$ 7.55	133.56 $\pm$ 15.78	110.06 $\pm$ 15.83	30.43 $\pm$ 10.72	5.661*	98.406***	7.103***
1-p-Menthen-9-ol	92.17 $\pm$ 12.59	79.53 $\pm$ 3.05	59.98 $\pm$ 5.65	70.95 $\pm$ 5.55	64.75 $\pm$ 7.06	90.76 $\pm$ 3.31	65.6 $\pm$ 8.81	66.88 $\pm$ 1.86	71.09 $\pm$ 4.24	64.14 $\pm$ 7.61	0.532	17.033***	1.907
Nerol	52.82 $\pm$ 12.47	36.74 $\pm$ 3	28.21 $\pm$ 3.98	37.77 $\pm$ 3.8	34.27 $\pm$ 2.4	57.41 $\pm$ 3.21	34.96 $\pm$ 6.26	30.14 $\pm$ 1.84	41.07 $\pm$ 3.1	38.36 $\pm$ 0.26	1.673	21.262***	0.373
Viridiflorol	8.42 $\pm$ 3.72	2.95 $\pm$ 0.71	3.11 $\pm$ 0.81	2.71 $\pm$ 0.36	2.55 $\pm$ 0.21	nd	nd	2.28 $\pm$ 0.33	3.07 $\pm$ 1.11	2.83 $\pm$ 0.53	11.173**	1.192	4.925*
Aldehydes	897.82	795.65	678.78	693.11	601.39	934.35	718.32	686.16	835.72	616.19			
Nonanal	10.13 $\pm$ 2.12	28.97 $\pm$ 23.75	6.65 $\pm$ 0.76	5.43 $\pm$ 0.62	21.51 $\pm$ 25.55	5.4 $\pm$ 0.69	7.58 $\pm$ 0.29	5.36 $\pm$ 0.8	10.3 $\pm$ 1.64	4.61 $\pm$ 0.31	3.804	1.242	1.468
Decanal	35.72 $\pm$ 5.36	35.84 $\pm$ 2.33	25.33 $\pm$ 0.87	29.66 $\pm$ 0	27.73 $\pm$ 6.76	33.27 $\pm$ 0.94	27.74 $\pm$ 4.68	29.48 $\pm$ 1.83	36.61 $\pm$ 5.01	23.04 $\pm$ 2.41	0.257	4.815*	2.858
1-p-Menthen-9-al	74.32 $\pm$ 8.29	65.01 $\pm$ 2.06	43.03 $\pm$ 3.69	54.23 $\pm$ 3.21	55.59 $\pm$ 6.16	71.86 $\pm$ 0.94	56.98 $\pm$ 10.29	52.92 $\pm$ 3.51	61.41 $\pm$ 3.6	57.93 $\pm$ 5.91	1.186	16.277***	2.156
Neral	244.38 $\pm$ 49.66	221.91 $\pm$ 9.28	169.83 $\pm$ 22.91	193.64 $\pm$ 21.06	151.6 $\pm$ 6.28	295.47 $\pm$ 8.83	207.91 $\pm$ 32.99	191.83 $\pm$ 16.52	242.46 $\pm$ 13.41	170.88 $\pm$ 7.56	9.226**	19.775***	2.013
Geraniol	394.49 $\pm$ 60.86	234.77 $\pm$ 20.24	212.2 $\pm$ 65.85	208.93 $\pm$ 20.12	172.47 $\pm$ 16.79	411.47 $\pm$ 31.74	214.67 $\pm$ 34.23	173.65 $\pm$ 8.47	270.98 $\pm$ 24.25	197.21 $\pm$ 5.79	0.509	39.521***	1.946
Citronellal	105.65 $\pm$ 12.38	88.06 $\pm$ 3.46	109.15 $\pm$ 10.18	79.37 $\pm$ 3.39	66.71 $\pm$ 5.51	94.76 $\pm$ 4.06	83.33 $\pm$ 4.78	102.15 $\pm$ 7.92	93.24 $\pm$ 16.02	57.82 $\pm$ 2.66	1.259	24.088***	1.785
Perilla aldehyde	nd	96.73 $\pm$ 13.95	90.84 $\pm$ 12.84	100.3 $\pm$ 3.54	84.71 $\pm$ 7.96	nd	101.19 $\pm$ 16.06	112.68 $\pm$ 10.17	102.05 $\pm$ 2.87	93.11 $\pm$ 11.29	4.244	127.891***	1.093
Undecanal	33.13 $\pm$ 10.45	26.35 $\pm$ 1.82	21.76 $\pm$ 2.72	21.55 $\pm$ 1.24	21.07 $\pm$ 3.04	22.12 $\pm$ 2.35	18.91 $\pm$ 2.97	18.09 $\pm$ 3.29	18.67 $\pm$ 2.3	11.59 $\pm$ 1.53	21.79***	6.395**	1.156
Ketones	17.00	18.58	16.96	16.61	14.42	19.13	14.56	16.90	17.61	14.34			
2-Bornanone	8.87 $\pm$ 2.1	11.81 $\pm$ 3.5	9.31 $\pm$ 2.98	8.17 $\pm$ 0.84	6.91 $\pm$ 1.05	10.75 $\pm$ 1.75	8.22 $\pm$ 0.83	9.11 $\pm$ 0.6	10.23 $\pm$ 1.19	7.4 $\pm$ 1.65	0.36	2.168	2.199
Umbellulone	3.88 $\pm$ 0.89	4.09 $\pm$ 0.56	2.74 $\pm$ 0.26	4.07 $\pm$ 0.2	3.9 $\pm$ 0.49	4.31 $\pm$ 0.33	2.86 $\pm$ 0.67	3.49 $\pm$ 0.37	3.88 $\pm$ 0.02	4.18 $\pm$ 0.53	0.001	4.568**	3.705*
Piperitone	4.25 $\pm$ 0.96	2.68 $\pm$ 0.48	4.91 $\pm$ 4.07	4.37 $\pm$ 0.99	3.61 $\pm$ 0.19	4.07 $\pm$ 1.26	3.47 $\pm$ 0.12	4.3 $\pm$ 2.73	3.5 $\pm$ 0.5	2.76 $\pm$ 0.11	0.337	0.915	0.28
Esters	71.83	50.05	43.81	62.49	61.46	48.19	40.02	43.69	66.71	59.33			
Chrysanthenyl acetate	29.26 $\pm$ 6.45	28.18 $\pm$ 1.83	22.71 $\pm$ 4.59	25.31 $\pm$ 3.5	22.98 $\pm$ 2.59	30.44 $\pm$ 0.62	23.63 $\pm$ 3.3	21.98 $\pm$ 1.09	27.03 $\pm$ 1.53	21.49 $\pm$ 1.89	0.435	5.796**	0.901
Carvyl acetate	nd	nd	nd	nd	2.08 $\pm$ 0.53	nd	nd	nd	nd	nd	46.383***	46.383***	46.383***
Nerol acetate	27.82 $\pm$ 6.57	21.87 $\pm$ 0.58	20.31 $\pm$ 2.09	28.34 $\pm$ 2.97	29.52 $\pm$ 6.49	17.75 $\pm$ 2.16	16.39 $\pm$ 2.34	21.71 $\pm$ 3.36	31.01 $\pm$ 1.42	31.31 $\pm$ 3.93	2.052	11.508***	3.403*
Geranyl acetate	1.65 $\pm$ 0	nd	0.78 $\pm$ 0.13	1.08 $\pm$ 0.31	1.18 $\pm$ 0.17	nd	nd	nd	1.89 $\pm$ 0.07	1.01 $\pm$ 0.13	1.217	20.199***	4.741**
Perilla acetate	13.09 $\pm$ 0	nd	nd	7.75 $\pm$ 0.03	5.7 $\pm$ 0.9	nd	nd	nd	6.78 $\pm$ 2.87	5.52 $\pm$ 1.82	33.586***	46.477***	18.974***
Others	2408.09	1838.15	1480.57	1674.33	1377.33	2110.19	1760.89	1529.28	1762.88	1632.47			
Methyl thymol ether	76.29 $\pm$ 15.27	85.25 $\pm$ 8.24	60.29 $\pm$ 6.81	72.04 $\pm$ 4.85	65.41 $\pm$ 10.31	65.9 $\pm$ 3.64	64.33 $\pm$ 4.76	65.07 $\pm$ 4.73	87.49 $\pm$ 6.71	66.78 $\pm$ 11.81	0.393	3.843*	4.115*
Thymol	2331.8 $\pm$ 309.73	1752.9 $\pm$ 35.78	1420.29 $\pm$ 131.73	1602.29 $\pm$ 157.59	1311.91 $\pm$ 111.17	2044.29 $\pm$ 70.37	1696.57 $\pm$ 224.98	1464.21 $\pm$ 106.34	1675.39 $\pm$ 188.56	1565.69 $\pm$ 141.35	0.008	20.587***	2.152
Total	33,734.65	38,946.93	33,713.17	35,648.74	39,393.36	25,773.98	26,102.14	30,851.16	39,422.97	30,966.9			

*, **, **** Significance at $P \leq 0.05$, 0.01, and 0.001, respectively.
 B = bagging, DAF = days after flowering, NB = no bagging (control lemon fruit), nd = not determined.

major decreased esters at 130 DAF after bagging, while chrysanthenyl acetate and nerol acetate were majorly decreased compounds at 144 DAF. The inhibition of bagging treatment on esters has also been found in peach and apple (Wang et al. 2010, 2024).

Thymol and methyl thymol ether belonged to phenolic compounds and ether, respectively, and their content accounted for 3.5% to 8.19% of total volatiles. Thymol was much higher than methyl thymol ether. Both of the two compounds were mainly formed at early maturation stages, and their maximum value appeared at 130 and 144 DAF, respectively. Thymol increased only at 172 DAF but showed a decrease at other stages. Thymol had strong antibacterial activity, and it had been integrated into edible coatings to prolong the shelf life of fresh fruit like strawberry and blueberry (Ding et al. 2024; Zhang et al. 2022). The high accumulation of thymol in lemon at different stages may be promoted to improve the postharvest storage performance of this fruit according to its harvest stages. Bagging treatment decreased the content of methyl thymol ether and thymol before 158 DAF, while their content slightly increased after that stage.

Conclusions

Phenols and volatiles in lemon peels were influenced by fruit ripening stages and bagging treatment. Total phenolic compounds and total flavonoid generally decreased with fruit ripening and were inhibited after bagging. Volatiles were divided into seven types. Monoterpenes displayed a trend of intermittent increasing with fruit ripening, which was opposite to the trend of most sesquiterpenes. The major alcohols and aldehydes were largely formed at early harvest stages and then decreased with fruit ripening. Bagging inhibits most volatile compounds at early stages ranging from 130 to 158 DAF, and the inhibition effect decreased with fruit ripening. To obtain the highest total volatiles and monoterpenes, 172 DAF was the best suitable harvest period for bagged fruit and 186 DAF was best for control fruit. While to obtain highest oxygenated volatile compounds and phenols, the harvesting period should be advanced to 130 DAF.

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