**Effect of Peeling Method on The Volatile Flavor of Citrus Fruit**

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**Abstract.** Analysis on the volatile flavor of whole peeled citrus fruit with different peeling methods, by solid phase micro extraction-gas chromatography-mass spectrometry (SPME-GC-MS). Totally 50 aromatic components were found, and main components were as follows: limonene (69.32%-78.88%), valencene (8.14%-12.98%), α-pinene (1.01%-1.02%), γ-terpinene (0.27%-0.35%), 3-methyl-butanal (0.75%-1.10%), (E)-2-hexenal (0.47%-0.73%), 1-octanol (0.25%-0.50%), limanol (0.70%-2.16%), ethyl butyrate (0.28%-2.59%), ethyl caproate (5.39%), ethyl acid ethyl ester (0.29%-1.86%), geranylacetone (0.07%-0.25%). The volatile flavor components of the three treatments were 34, 36 and 50, respectively. Enzymatic peeling method was used for detection of limonene, (E)-2-hexenal flavor. Conventional (artificial) peeling method was used for detection of limonene, (E)-2-hexenal flavor, ethyl hexanoate, hexyl butyrate flavor.

**1. Introduction**

Citrus fruit consists of exocarp, mesocarp and endocarp, and the capsule coat is a part of endocarp. The whole peeled citrus fruit refers to the whole naked fruit ball obtained by peeling and decapsulation. Through minimally invasive processing of citrus fruit, to maintain its unique structure and shape, because it is rich in a variety of nutrients and tastes good, it is also known as MP (minimum processed) product [1]. After processing, the naked fruit ball can be peeled and can also be dispersed into orange juice cells, which can be added into the fruit drinks to realize the cross season supply throughout the year. The main methods of peel and encapsulation of citrus fruits are: acid-base two-step method, phosphate alkali method, EDTA complexing agent assistant method [4], acid enzyme combination method, enzyme method, etc [3,4]. At present, enzymatic peel and encapsulation is the research hotspot.

Volatile flavor is one of the most important factors affecting fruit quality and processing quality. SPME was commercialized by Supelco Co., Ltd. in 1993 [5]. As a relatively mature sample pretreatment technology, SPME has been widely used to extract volatile and semi volatile compounds from food matrix [6]. GC/MS as a routine detection method has been widely used in the analysis of volatile components, often using the combination of mass spectrometry retrieval and retention index analysis to ensure the accuracy of component identification. There are a lot of reports on the volatile flavor of orange by solid-phase microextraction (SPME) combined with gas chromatography and mass spectrometry GC/MS, but most of them focus on the aspects of orange juice, orange peel or essential oil [7,8], and there are few reports on the study of the volatile flavor of peeled whole orange. The
The purpose of this study was to analyze the volatile flavor components of peeled citrus fruits by headspace SPME (HS-SPME) combined with GC/MS, and to explore the effect of peeling methods on the volatile flavor of peeled citrus fruits, so as to provide a reference for the further processing and application of peeled citrus fruits.

2. Methods

2.1. Preparation of samples
Enzymatic peeling and decapsulation: one third of the citrus fruits are peeled by enzymatic method, and the fruit balls are rinsed with water for 3 minutes and then dried; artificial peeling and decapsulation (traditional way): one third of the citrus fruits remove the outer and middle skin of the fruit by scalding, and remove the coating with acid and alkali, and the fruit balls are rinsed with water for 3 minutes and then dried; artificial Juicing: one third The whole fruit sample is not peeled, and the fresh fruit is washed, cut and half juiced for comparison.

All the whole fruit samples are crushed by a crusher, filtered by 300 mesh filter gauze, 5ml juice is taken, added into 20 mL screw mouth extraction bottle, balanced at 40°C for 15 min, then taken out, accurately added into 1 μL (cyclohexanone) standard in the extraction bottle, inserted into SPME extraction head in the extraction bottle, and extracted at 40°C for 30 min, retracted the extraction head for 5 min, and each sample is three times parallel.

2.2. Chromatographic condition
Chromatographic column: HP-5MS quartz capillary column (30 m×0.25 mm, 0.25 μm); temperature rise procedure: start temperature 40°C, rise to 100°C at 40°C/min, keep for 1.5 min, then rise to 102°C at 0.5°C/min, keep for 5.5 min, then rise to 140°C at 20°C/min, keep for 7.4 min, finally rise to 250°C at 15°C/min, keep for 2 min; analytical temperature of sample inlet 250°C, no split injection; the flow rate of carrier gas (He) is 1 mL/min.

2.3. Mass spectrum condition
EI ion source; electronic energy 70 eV; transmission line temperature 280°C; ion source temperature 230°C; four pole temperature 150°C; mass scanning range m/z 40-350.

2.4. Qualitative and quantitative analysis
Qualitative analysis: all compounds are matched by the atlas Library (NIST 11.LIB) configured by Agilent system, and C5-C20 n-alkanes are used as the standard. Under the same temperature programmed condition, the corresponding retention index is calculated by its retention time. The similarity, retention index and related literature reports are integrated to qualitative analysis. Linear warming formula of retention index adopted: RI=100n+100(tx–tn)/tn+1–tn, Tn+1 and Tn represent the retention time of components and n-alkanes with carbon number of n+1 and n respectively, tx is the retention time of tested components, and t_{n+1}>t_x>t_n.

Quantitative analysis: internal standard method is used for semi quantitative analysis, and the calculation formula of volatile flavor substance content of various products is as follows: volatile flavor content (μg/g) = (component peak area×quality of internal standard)/(peak area of internal standard×sample weight).

3. Results and analysis

3.1. Total ion chromatogram
In order to understand the effect of different peeling methods on the types and contents of volatile components in the whole fruit of citrus, enzymatic and artificial peeling and decapsulation methods were used, and artificial juicing is added as a reference. Through HS-SPME and GCMS analysis of the samples processed under the above conditions, the spectral library search and retention index are
Table 1, and the total content of each type is shown in Table 2.

A. Whole fruit enzyme peeled without sterilization
B. Whole fruit artificial peeling without sterilization
C. Whole fruit artificial juicing without sterilization

Figure 1. Total ion chromatogram of volatile components of whole peeled citrus.

Table 1. Semi quantitative results of whole peeled citrus fruits with different treatments.

| No. | Retention index | Composition name | Molecular formula | Whole fruit enzyme peeled without sterilization | Whole fruit artificial peeling without sterilization | Artificial juice not sterilized |
|-----|-----------------|-------------------|-------------------|-----------------------------------------------|-----------------------------------------------|---------------------------------|
| 1   | 944             | (1S)-2,6,6-TrimethylBicyclo[3.1.1]hept-2-ene | C10H16            | 2.33                                          | -                                            | 4.89                            |
| 2   | 982             | 6,6-dimethyl-2-methylene-Bicyclo[3.1.1]heptane | C10H16            | 0.52                                          | 0.16                                          | 1.50                            |
| 3   | 1011            | α-Phellandrene    | C10H16            | -                                             | -                                            | 1.02                            |
| 4   | 1022            | 1-methyl-4-(1-methylethyl)-1,3-Cyclohexadiene | C10H16            | 0.33                                          | 0.22                                          | 1.17                            |
| 5   | 1036            | D-Limonene       | C10H16            | 175.22                                        | 103.34                                        | 377.98                          |
| 6   | 1048            | (Z)-3,7-dimethyl-1,3,6-Octatriene | C10H16            | -                                             | -                                            | 1.11                            |
| 7   | 1062            | γ-Terpinepine     | C10H16            | 0.62                                          | -                                            | 1.68                            |
| 8   | 1098            | Terpinolene       | C10H16            | 0.64                                          | 0.42                                          | 2.84                            |
| 9   | 1148            | Trans-Limonene oxide | C10H16O          | 0.14                                          | 0.06                                          | -                               |
| 10  | 1172            | 1-Decene         | C10H20            | 0.06                                          | 0.12                                          | 0.25                            |
| 11  | 1352            | 2,6-dimethyl-2,6-Octadiene | C10H18          | -                                             | -                                            | 0.27                            |
| 12  | 1358            | α-Cubebene        | C15H24            | -                                             | -                                            | 0.27                            |
| 13  | 1384            | Copaene          | C15H24            | 0.14                                          | 0.10                                          | 0.58                            |
| 14  | 1396            | [1S-(1α,α,2,β,4,β,α,3)-1-ethenyl-1-methyl-2,4-bis(1-methylethyl)]-Cyclohexane | C15H24           | 1.25                                          | 0.89                                          | 1.93                            |
| 15  | 1433            | Caryophyllene     | C15H24            | 0.52                                          | 0.32                                          | 0.86                            |
| 16  | 1442            | [3αS-(3α,α,3b,β,α,7α,7αS*)]-octahydro-7-methyl-3-methylene-4-(1-methylethyl)-(1H-Cyclopenta[1,3]cyclopropa[1,2]benzene | C15H24           | -                                             | -                                            | 0.32                            |
| 17  | 1466            | Alloromandrene    | C15H24            | 0.67                                          | 0.46                                          | 1.04                            |
| 18  | 1471            | Humulen          | C15H24            | 0.11                                          | 0.08                                          | 0.22                            |
| 19  | 1492            | δδ-Selinene       | C15H24            | 0.78                                          | 0.53                                          | 1.36                            |
| 20  | 1512            | [1R-(1α,α,7,β,8α,α,8α,α,7)-1,2,3,5,6,7,8α-octahydro-1,β,8-methyl-7-(1-methylethyl)-Naphth[a] | 1eR-(1α,α,7,β,8α,α,7)-1,2,3,5,6,7,8α-octahydro-1,β,8-methyl-7-(1-methylethyl)-Naphth[a] | 28.22                                         | 19.36                                          | 39.04                           |
| 21  | 1521            | 1α,2,3,5,6,7,7α,7b-octahydro-1,1,4,7-tetramethyl-1H-Cyclopropene| C15H24           | 0.59                                          | 0.39                                          | 0.89                            |
| 22  | 1537            | (–)-α-Panacisien  | C15H24            | 1.85                                          | 1.23                                          | 3.16                            |
| 23  | 1552            | Bicyclo[10.1.0]pridec-1-ene | C16H28O2         | -                                             | 0.09                                          | 0.48                            |
The results show that 55 kinds of volatile flavor compounds were identified in the three treatment methods, including 25 terpenes, 6 aldehydes, 7 alcohols, 11 esters, 3 ketones, 3 others. The main components detected are terpenes, aldehydes, alcohols, esters, ketones and other oxygenated compounds.

### 3.2. Analysis of volatile flavor components

| No. | Mass (m/z) | Compound Name                                           | Class      | Relative Abundance | Relative Intensity |
|-----|-----------|--------------------------------------------------------|------------|--------------------|--------------------|
| 24  | 1600      | Caryophyllene oxide                                     | Aldehydes  | -                  | -                  |
| 25  | 1680      | [4aR-(4a,alpha,7alpha,8a,beta,3)-decahydro-4a-methyl-1-methylene-7-(1-methylhexyl)]-Naphthalen | Ketones    | 0.05               | 0.12               |
| 26  | 853       | 3-methyl-Butanal                                        | Aldehydes  | 2.21               | 1.64               |
| 27  | 1202      | Decanal                                                | Ketones    | 0.25               | 0.30               | 0.67               |
| 28  | 1284      | (S)-4-(1-methylhexyl)-1-Cyclohexene-1-carboxaldehyde   | Ketones    | -                  | -                  | 0.38               |
| 29  | 1297      | 2,4-Decadienal                                         | Ketones    | -                  | -                  | 0.21               |
| 30  | 1830      | 5,9,13-trimethyl-4,8,12-Tetradecatrienyl                | Ketones    | -                  | -                  | 0.05               |
| 31  | 1066      | 1-Octanol                                              | Alcohols   | 0.57               | 0.60               | 2.42               |
| 32  | 1105      | 3,7-dimethyl-1,6-Octadien-3-ol                          | Alcohols   | 2.92               | 1.05               | 9.89               |
| 33  | 1226      | Citronellol                                            | Alcohols   | 0.21               | 0.25               | 1.61               |
| 34  | 1184      | Terpinen-4-ol                                          | Alcohols   | 0.33               | 0.22               | 1.15               |
| 35  | 1256      | Geraniol                                               | Alcohols   | -                  | 0.07               | 0.47               |
| 36  | 1272      | 1-Decanol                                              | Alcohols   | -                  | 0.07               | 0.60               |
| 37  | 1625      | Cedrol                                                 | Alcohols   | -                  | -                  | 0.23               |
| 38  | 798       | Butanoic acid, ethyl ester                             | Esters     | 5.99               | 1.51               | 1.36               |
| 39  | 999       | Hexanoic acid, ethyl ester                              | Esters     | -                  | 8.04               | -                  |
| 40  | 1130      | Octanoic acid, methyl ester                             | Esters     | 0.18               | -                  | 0.10               |
| 41  | 1133      | 3-hydroxy-Hexanoic acid, ethyl ester                    | Esters     | 0.19               | 0.14               | 0.25               |
| 42  | 1189      | Butanoic acid, hexyl ester                              | Esters     | -                  | 0.07               | -                  |
| 43  | 1193      | Octanoic acid, ethyl ester                              | Esters     | 0.29               | 0.98               | 1.86               |
| 44  | 1207      | Acetic acid, octyl ester                                | Esters     | -                  | -                  | 0.20               |
| 45  | 1363      | (Z)-acetate, 3,7-dimethyl-2,6-Octadien-1-ol             | Esters     | -                  | -                  | 0.27               |
| 46  | 1375      | 4-tert-Butylcyclohexyl acetate                          | Ketones    | 0.14               | 0.15               | 0.33               |
| 47  | 1380      | Geranyl acetate                                         | Ketones    | -                  | -                  | 0.11               |
| 48  | 1390      | Decanoic acid, ethyl ester                              | Ketones    | 0.07               | 0.06               | 0.31               |
| 49  | 1251      | D-Carvone                                              | Ketones    | -                  | -                  | 0.25               |
| 50  | 1454      | (E)-6,10-dimethyl-5,9-Undecadien-2-one                  | Ketones    | 0.16               | 0.38               | 0.58               |
| 51  | 1822      | Nootkatone                                              | Ketones    | -                  | -                  | 0.10               |
| 52  | 1234      | 4-methylene-Spiro[2.4]heptane                           | Ketones    | -                  | -                  | 0.13               |
| 53  | 1502      | 2-(Isopropenyl)-4a,8-dimethyl-1,2,3,4a,5,6,7-octahydroxalalene | Ketones    | 2.16               | 4.95               | 8.43               |
| 54  | 1685      | 1a,2,3,4,4a,5,6,7-octahydro-1,14,7-tetramethyl-1H-Cycloprop[e]enulene | Ketones    | 0.07               | 0.08               | 0.21               |

Aldehydes: 3-methyl-Butanal
Alcohols: Decanal, (S)-4-(1-methylhexyl)-1-Cyclohexene-1-carboxaldehyde, 2,4-Decadienal, 1-Octanol, 3,7-dimethyl-1,6-Octadien-3-ol, Citronellol, Terpinen-4-ol, Geraniol, 1-Decanol, Butanoic acid, ethyl ester, Hexanoic acid, ethyl ester, Octanoic acid, methyl ester, 3-hydroxy-Hexanoic acid, ethyl ester, Butanoic acid, hexyl ester, Octanoic acid, ethyl ester, Acetic acid, octyl ester, (Z)-acetate, 3,7-dimethyl-2,6-Octadien-1-ol, 4-tert-Butylcyclohexyl acetate, Geranyl acetate, Decanoic acid, ethyl ester, D-Carvone, (E)-6,10-dimethyl-5,9-Undecadien-2-one, Nootkatone, 4-methylene-Spiro[2.4]heptane, 2-(Isopropenyl)-4a,8-dimethyl-1,2,3,4a,5,6,7-octahydroxalalene, 1a,2,3,4,4a,5,6,7-octahydro-1,14,7-tetramethyl-1H-Cycloprop[e]enulene.
Table 2. Total volatile flavor of peeled citrus fruits with different treatments.

| Category   | Whole fruit enzyme peeled without sterilization | Whole fruit artificial peeling without sterilization | Artificial juice not sterilized |
|------------|-----------------------------------------------|--------------------------------------------------|--------------------------------|
|            | Relative content (%) | Content (μg/g) | Relative content (%) | Content (μg/g) | Relative content (%) | Content (μg/g) |
| Terpenes   | 92.47% | 214.04 | 85.70% | 127.77 | 92.46% | 443.10 |
| aldehydes  | 1.79%  | 4.13   | 1.80%  | 2.69   | 1.01%  | 4.85 |
| alcohols   | 1.74%  | 4.03   | 1.52%  | 2.27   | 3.51%  | 16.37 |
| esters     | 2.97%  | 6.88   | 7.34%  | 10.95  | 1.00%  | 4.78 |
| Ketones    | 0.07%  | 0.16   | 0.25%  | 0.38   | 0.19%  | 0.93 |
| Others     | 0.96%  | 2.23   | 3.38%  | 5.04   | 1.83%  | 8.76 |
| Total      | 100.00% | 231.47 | 100.00% | 149.08 | 100.00% | 478.81 |

In terms of content, the terpene content of enzymatic peeling is 67.52% higher than that of artificial peeling (the traditional way), and 51.70% lower than that of fresh juice of the whole fruit. The main differences are from d-limonene, Valencia tangerine, α-pinene, β-elemene, terpine, basilene, α-ginseng, γ-terpinene, which may be caused by the heating process of enzymatic peeling and artificial peeling. The results show that the content of aldehydes in enzymatic peeling is 53.74% higher than that in artificial peeling and 14.79% lower than that in fresh pressing. The main differences are isovaleraldehyde, cyanophyllal and decanal. The results show that the content of isovaleraldehyde decreased 38.65%, decanal decrease 62.81% and geraldehyde increase 140.86% compared with that of artificial peeling, which indicate that the volatile components are closely related to the peeling methods. The alcohol content of enzymatic peeling is 77.76% higher than that of artificial peeling, and 75.39% lower than that of the whole fruit. In the comparison of the content of specific components in enzymatic peeling and whole fruit fresh pressing, 1-octanol decreased by 76.41%, linalool decrease by 70.53%, citronellol decrease by 86.95%, and 4-terpineol decreased by 71.05%. Among them, the change of linalool content is different from the report that linalool concentration will increase after pasteurization in the literature [9], which may be related to the effect of enzymatic peeling on the outer epidermis of whole fruit, which needs further study. In addition, cedrol only exists in the whole fruit fresh pressing, but not detected in enzymatic peeling and artificial peeling, indicating that the substance is volatile and easy to be distributed in the process of processing; in the ester part, enzymatic peeling is 37.16% lower than artificial peeling, 43.76% higher than the whole fruit fresh pressing. The content of ethyl butyrate in enzymatic peeling is 297.84% and 341.71% higher than that in artificial peeling and whole fruit fresh pressing respectively, with a significant increase, but ethyl caproate shows a significant decrease trend. Octyl acetate, neryl acetate and vanillin acetate are not detected in the whole fruit enzymatic method and artificial peeling, so it can be considered that the way of peeling also has different effects on them; in terms of ketones, D-carvone and nocardione are detected in the whole fruit fresh pressing, but not in the enzymatic hydrolysis and artificial peeling. The content of geranyl acetone is the highest in the whole fruit fresh pressing, and it decrease after different peeling treatments.

4. Conclusion
By headspace solid phase microextraction and gas phase mass spectrometry, 50 volatile flavor compounds are identified, including limonene (69.32%-78.88%), Valencia tangerine (8.14%-12.98%), α-pinene (1.01%-1.02%), β-elemene (0.40%-0.60%), terpine (0.28%-0.59%), basilene (0.23%), α-ginseng (0.66%-0.82%), γ-terpinene (0.27%-0.35%), isovaleraldehyde (0.75%-1.10%), cyanophyllal (0.47%-0.73%), decanal (0.11%-0.20%), 1-octanol (0.25%-0.50%), linalool (0.70%-2.16%), 4-terpineol (0.14%-0.24%), ethyl butyrate (0.28%-2.59%), ethyl hexanoate (5.39%), ethyl octanoate
(0.29%-1.86%), geranyl acetone (0.07%-0.25%) and so on. The way of peeling has an important effect on the type and content of volatile flavor, the volatile flavor components of the three treatments are 34, 36 and 50, respectively. The contents of terpenes, aldehydes, alcohols and ketones decrease by 51.70%, 14.79%, 75.39% and 82.49% respectively, and ketones increase by 43.76% respectively. The flavor compounds such as oxidized limonene and cyanobaldehyde are newly detected. The contents of terpenes, aldehydes, alcohols and ketones decrease by 71.17%, 44.57%, 86.16% and 59.44% respectively, while ketones increase by 128.78%. The flavor compounds such as limonene oxide, cyanophyllal, acacia acetaldehyde, ethyl hexanoate and hexyl butyrate are newly detected.

As a minimum processing product, peeled whole fruit can become a nutritious, edible and convenient terminal product on the market, and can also be used as an intermediate preparation for orange juice cells, breaking the seasonal constraints and achieving annual supply. By comparing the volatile flavor of the whole fruit under different peeling methods, the effect of peeling methods on the volatile flavor of the fruit ball was understood, which has reference significance for the development of the whole fruit can combining sensory threshold, GC-O and electronic nose.

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