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Determination of volatile compounds by headspace-solid phase microextraction – gas chromatography / mass spectrometry: Quality evaluation of Fuji apple

Yun-Yeol Lee, Moon-Cheol Jeong and Hae Won Jang★
Korea Food Research Institute, Seongnam 13539, Korea
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Abstract: The volatile components in 'Fuji' apple were effectively determined by a headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS). A total of 48 volatile components were identified and tentatively characterized based on National Institute of Standards and Technology (NIST) MS spectra library and the Kovats GC retention index I (RI). The harvested Fuji apples were divided into two groups: 1-methylcyclopropene (1-MCP) treated and non-treated (control) samples for finding important indicators between two groups. The major volatile components of both apples were 2-methylbutyl acetate, hexyl acetate, butyl 2-methylbutanoate, hexyl butanoate, hexyl 2-methylbutanoate, hexyl hexanoate and farnesene. No significant differences of these major compounds between 1-MCP treated and non-treated apples were observed during 1 month storage. Interestingly, the amount of off-flavors, including 1-butanol and butyl butanoate, in 1-MCP treated apples decreased over 5 months, and then increased after 7 months. However, non-treated apples did not show significant changes for off-flavors during 7 month storage ($p<0.05$). The non-treated apples also contained the higher levels of two off-flavors than 1-MCP treated apples. These two compounds, 1-butanol and butyl butanoate, can be used as quality indicators for the quality evaluation of Fuji apple.

Key words: Fuji apples, volatile compounds, off-flavors, HS-SPME/GC-MS

1. Introduction

Apple is one of the most widely consumed fruit in worldwide and 'Fuji' apple (*Malus* × *domestica* Borkh.) is mainly cultivated in several producing areas such as Korea, Japan, China and United States.[1,2] A good relationship between consumer's preference and sensory characteristics has been reported.[3] The flavor, texture and appearance are major sensory characteristics of apples.[3,4] In particular, flavor plays the most important role in the consumer choice and perception on apple freshness.[4,5]

Recently, the volatile compounds in apple have received much attention because those compounds contribute to the overall sensory quality from different varieties and storage conditions after harvest.[5-7] Many studies demonstrate that apples consist over 300 volatile compounds (such as carboxylic esters,

★ Corresponding author
Phone : +82-(0)31-780-9377 Fax : +82-(0)31-780-9280
E-mail : hwjkfri@kfri.re.kr

alcohols, aldehydes, ketones and ethers) and the majority of volatile compounds are ester (78-92 %), including ethyl 2-methylbutanoate, 2-methylbutyl acetate and hexyl acetate, and alcohols (6-16 %), including hexanol and butanol.[5-8] The amounts and composition of volatile compounds in apples are significantly affected by storage time and conditions, harvest time, and cultivar.[8] Especially, post-harvest apple treated with 1-methylcyclopropene (1-MCP), ethylene action inhibitor has been conducted to delay fruit ripening and reduce softening, therefore 1-MCP treatment is considered as a critical technology in apple storage.[9,10] During long term storage of apples, typical technologies are cold storage under regular atmosphere (RA) or controlled atmosphere (CA).[11]

Several analytical methods are available for the analysis of the volatile compounds in plants and fruits. Notably, gas chromatography (GC) and GC/mass spectrometry (GC/MS) have been frequently used as analytical tools due to their high separation capacity and detection sensitivity for mixtures of volatile compounds. In general, GC and GC/MS methods have employed several extraction methods including purge-trap or dynamic headspace techniques.[4,13-15] Recently, solid-phase microextraction (SPME) using various adsorbents is being widely used for rapid extraction of volatile compounds from aromatic plants and fruits with a complicated sample matrix.[15-17] One advantage of the SPME method is that several types of fibers can be used, based on the polarity of the analytes, for the extraction of volatiles in herbal plants that have a complex matrix. In particular, headspace (HS) SPME combined with a GC/MS method, has improved the analytical performance in terms of the elimination of interfering substances and enhancement of chromatographic separation, sensitivity, selectivity, and measurement precision and accuracy. Consequently, SPME-GC/MS methods have been successfully adapted to a variety of applications, including the quality evaluation[18-20] and the discrimination of geographical origins for apple species [21-23] with subparts per billion (ppb) level sensitivity. The chemical compositions of volatile components in various apples have been reported using different extraction methods combined with GC or GC/MS.[5-8] To the best of our knowledge, however, no direct comparison of chemical compositions has yet been performed for Fuji apples with different treatments during storing 7 months.

In the present study, the volatile compounds in Fuji apples were extracted using HS-SPME method and then characterized by GC-MS. Changes in the volatile compounds during 7 month storage period were discovered to identify off-flavors as potential indicators to assess the quality of Fuji apples under different storage conditions, 1-MCP treated and non-treated (control).

2. Experimental

2.1. Apple samples

'Fuji' apple (*Malus* × *domestica* Borkh.), including non-treated and 1-MCP (1 μL/L, SmartFresh, AgroFresh Inc., Springhouse, PA, USA) treated samples, were harvested and provided from a farm in Chungju in South Korea. All samples were stored in regular atmosphere (RA) at 1 ºC until it was used for experiment. Apples were analysed during 1, 3, 5 and 7 months in regular atmosphere storage at 4 °C.

2.2. Volatile extraction procedures

A headspace solid-phase micro-extraction (HS-SPME) manual holder and a fibers with a 50/30 μm divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) were purchased from Supelco (Bellefonte, PA, USA). Before analysis, fiber was conditioned as recommended by the manufacturer's instructions. An apple was placed into a 0.7 L glass desiccators (Duran, Wertheim, Germany) and the desiccator was tightly sealed with desiccator lids, stopcocks, mininert valves (screw top, 20 mm i.d., Supelco, Bellefonte, PA, USA). After sealing, the desiccator was equilibrated at 25 °C for 24h in the vacuum oven (OV-12, Jeio Tech, Korea). For HS-SPME, the desiccator was maintained at 25 °C for 30 min in the vacuum oven. After extraction, the fiber was pulled into the needle sheath and the SPME device was removed from the desiccator and then inserted directly into the injection port of the GC for thermal desorption at 280 °C for 1 min.

2.3. Gas chromatography-mass spectrometry (GC/MS) analysis of volatile compounds

GC-MS analysis was performed on a 6890A gas chromatograph and 5973C mass-selective detector (Agilent Technologies, Palo Alto, CA, USA). The gas chromatograph was equipped with 30 m HP-5ms column with 0.25 mm i.d. and 0.25 μm thickness (Agilent Technologies, Palo Alto, CA, USA). The temperature of injection port was maintained at 250 °C and extracts of sample was manually injected in split mode (split ratio, 10:1) at flow rate 1.0 mL/min. The helium was used as carrier gas. The oven temperature was held at 40 °C for 3 min, increased to 165 °C at 5 °C/min. The temperature of MS source, transfer line and quadrupole was maintained at 230 °C, 280 °C and 150 °C, respectively. The mass spectra were recorded in the full scan range from m/z 35 to 300. The volatile compounds were identified using the MS fragmentation, National Institute of Standards and Technology (NIST) MS spectra library and verified by the Kovats GC retention index I (RI), which were calculated as described by the peak areas of each identified compound in Fuji apple.

2.4. Statistical analysis

The mean and standard deviation were calculated for all experimental data. Significant differences between variance of off-flavors were evaluated by One-way ANOVA Duncan's test (p<0.05) in the SPSS software program.

3. Results and Discussion

3.1. Volatile composition in 'Fuji' apple for 1 month storage

Aroma volatile compounds identified and quantified in Fuji apple are shown in *Table* 1. The detected compounds were similar for two groups, 1-MCP treated and non-treated apple. A total of 48 Aroma chemicals was detected, namely 40 esters (15 butanoates, 8 propanoates, 7 hexanoates, 6 acetates, and 4 octanoates), 4 hydrocabons, 3 alcohols, 1 acids. The peak area of each compound indicate the mean±standard deviation (n=15). Although 8-9 apples are previously needed for headspace technique to obtain the volatiles from intact apples,[15-16] an apple was used in this study.

In the case of 1-MCP treated apples stored for 1 month, almost 67 % of the total volatile compounds originated from three compounds such as 2-methylbutyl acetate (35.7 %), hexyl 2-methylbutanoate (20.3 %) and hexyl acetate (11.4 %). From the non-treated apples for 1 month storage, almost 53 % of the total volatiles also originated from 2-methylbutyl acetate (21.2 %), hexyl 2-methylbutanoate (18.2 %) and hexyl acetate (13.4 %).

The major volatile compounds obtained from 1-MCP treated apples for 1 month storage were 2-methyl butanol, butyl acetate, butyl propanoate, butyl butanoate, butyl-2-methylbutanoate, butyl hexanoate, pentyl acetate, hexyl acetate, hexyl butanoate, hexyl 2-methylpropanoate, hexyl hexanoate, 2-methylbutyl acetate, 2-methylbutyl butanoate and E,E-farnesene. There are no important differences of those major compounds between 1-MCP treated and non-treated apples for 1 month storage. These volatile compounds obtained in this study were in agreement with previous studies in which volatile compounds were isolated by dynamic headspace or solvent extraction.[14-15] When the dynamic headspace technique on apples was compared to SPME, similar volatiles were detected and quantified.[17]

3.2. Changes of Volatile compounds of 'Fuji' apple for 7 months storage

Fig. 1 shows total ion chromatogram of Fuji apple with or without 1-MCP treatment after 7 months of storage. In *Table* 1, total volatile compounds of Fuji apples stored in cold room for 1, 3, 5 and 7 months of storage with or without 1-MCP treatment. After 7 months storage, over 65 % of the total volatiles quantified in 1-MCP treated apples originated from six volatiles such as hexyl 2-methylbutanoate (23.3 %), E,E-farnesene (11.8 %), hexyl butanoate (10.0 %), *Table* 1. Identified volatile compounds from Fuji apples with or without 1-MCP treatment by HS-SPME-GC-MS

Peak No.	R.T (min)	Quant Ion	Compound	RI[b]	1-MCP				Control			
					1 months	3 months	5 months	7 months	1 months	3 months	5 months	7 months
1	3.00	56	1-butanol	676	66±20[a]	14±5	nd[c]	97±15	76±19	46±16	57±32	66±18
2	3.89	43	Propyl acetate	711	nd	nd	nd	22±6	nd	11±10	nd	11±7
3	3.99	74	Methyl butanoate	715	nd	nd	nd	19±17	nd	nd	nd	12±8
4	4.27	57	2-methylbutanol	717	292±45	163±57	81±34	89±12	229±75	106±54	129±46	166±49
5	4.93	91	Methyl benzene	760	nd	26±11	31±16	10±2	nd	44±24	78±93	49±25
6	5.15	43	2-methylpropyl acetate	770	nd	23±9	1±3	nd	nd	11±9	nd	nd
7	5.24	88	Methyl 2-methylbutanoate	774	nd	nd	nd	25±17	nd	nd	nd	10±6
8	5.53	60	Butanoic acid	788	nd	nd	nd	12±9	nd	nd	nd	51±27
9	5.85	71	Ethyl butanoate	802	11±41	3±13	nd	89±50	33±41	nd	302±228	72±66
10	6.10	57	Propyl propanoate	809	nd	nd	nd	35±12	nd	10±9	66±73	26±16
11	6.22	43	Butyl acetate	814	632±174	137±70	11±13	382±61	714±283	537±313	256±112	230±96
12	7.29	57	Ethyl 2-methylbutanoate	850	7±28	7±26	nd	51±23	16±50	nd	249±259	45±47
13	7.84	56	1-hexanol	867	135±52	55±22	19±7	333±88	196±51	107±50	235±96	296±103
14	8.18	43	2-methylbutyl acetate	878	3837±868	1868±639	252±145	549±126	2537±610	1871±816	605±159	583±227
15	8.50	104	Ethenyl benzene	888	nd	12±8	19±6	9±3	nd	16±11	39±36	12±4
16	8.78	89	Propyl butanoate	894	27±10	1±3	nd	147±42	37±23	46±21	215±169	101±40
17	9.12	57	Butyl propanoate	905	106±64	30±18	6±11	162±44	208±139	233±101	130±94	153±68
18	9.31	43	Pentyl acetate	911	195±48	52±17	3±7	48±8	143±50	108±44	60±21	45±15
19	9.65	74	Methyl hexanoate	922	nd	nd	nd	26±21	nd	nd	nd	14±9
20	10.33	103	Propyl 2-methylbutanoate	943	33±17	2±5	2±5	152±26	19±13	36±17	225±193	114±55
21	10.50	71	Butyl 2-methyl propanoate	953	nd	nd	nd	nd	nd	2±5	nd	nd
22	10.58	71	2-methylpropyl butanoate	955	nd	1±4	nd	12±3	nd	5±7	21±12	17±7
23	11.16	57	3-methylbutyl propanoate	972	nd	59±30	20±14	38±12	nd	104±40	69±58	70±28
24	11.93	71	Butyl butanoate	994	251±86	46±20	12±14	631±198	545±232	382±175	407±232	442±129
25	12.05	88	Ethyl hexanoate	998	5±18	nd	nd	84±53	8±22	21±12	79±63	37±31
26	12.30	57	Pentyl propanoate	1007	nd	nd	nd	8±3	nd	10±11	11±14	13±5
27	12.50	43	Hexyl acetate	1012	1227±321	358±143	45±28	1192±235	1598±607	1253±528	795±298	594±170
28	13.40	103	Butyl 2-methylbutanoate	1041	250±90	49±23	7±13	725±162	311±114	313±109	269±179	359±129
29	13.92	71	2-methylbutyl butanoate	1062	125±31	53±23	15±12	135±42	203±62	106±39	149±85	193±70
30	15.01	89	Pentyl butanoate	1092	23±7	nd	nd	47±14	40±13	23±9	38±22	50±16
31	15.05	99	Propyl hexanoate	1097	nd	nd	1±3	76±14	1±4	7±11	72±55	45±18
32	15.12	57	Undecane	1099	nd	21±6	22±11	nd	nd	18±17	19±10	nd
33	15.33	85	2-methylbutyl 2-methylbutanoate	1103	144±66	43±24	8±10	120±34	132±94	66±35	52±36	123±47
34	15.37	57	Hexyl propanoate	1105	88±56	31±12	9±12	319±86	200±129	219±110	253±231	249±90
35	16.37	103	Pentyl 2-methylbutanoate	1138	62±28	13±5	2±4	75±17	52±24	38±14	47±30	63±23
36	16.66	89	Hexyl 2-methylpropanoate	1148	24±7	8±4	nd	41±9	36±5	34±15	22±10	28±7
37	17.91	117	Butyl hexanoate	1190	107±52	48±20	17±18	540±88	190±88	223±90	153±103	232±73
38	17.95	71	Hexyl butanoate	1191	225±69	66±23	24±15	1339±322	634±180	370±148	515±273	810±230
39	19.23	103	Hexyl 2-methylbutanoate	1236	2183±886	356±166	105±63	3110±604	2174±1209	1135±359	1277±868	1790±550
40	19.65	99	2-methylbutyl hexanoate	1254	51±28	44±23	21±11	89±14	56±23	51±24	40±26	64±24
41	20.63	117	Pentyl hexanoate	1291	18±8	11±5	2±4	38±6	22±8	20±8	14±12	27±9
42	20.75	145	Propyl octanoate	1295	nd	nd	nd	10±4	nd	nd	nd	nd
43	23.22	71	2,4,4,trimethylpentyl ester 3-hydroxy 2-methyl propanoate	1373	nd	2±5	2±4	nd	nd	5±8	nd	nd
44	23.22	117	Hexyl hexanoate	1385	186±43	125±42	43±21	768±118	314±85	265±101	165±91	288±72
45	23.27	145	Butyl octanoate	1390	23±7	15±6	nd	109±33	47±22	50±25	18±18	34±10
46	24.82	70	2-methylbutyl octanoate	1451	14±5	20±9	3±5	25±7	16±5	16±9	7±8	12±4
47	26.30	93	E,E-farnesene	1508	417±152	141±35	268±100	1582±155	1173±297	1349±254	1067±424	728±239
48	27.90	43	Hexyl octanoate	1585	nd	nd	nd	nd	7±4	1±3	nd	nd

[a]Values represent means±standard deviation (n = 15). [b]Retention Index (HP-5MS column). [c]nd, not detected.

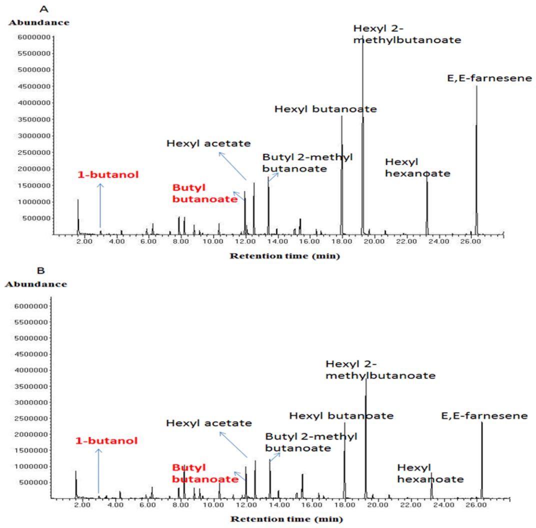

Fig. 1. Total ion chromatogram of Fuji apple treated with 1-MCP (A) and control (B) for 7 months.

hexyl acetate (8.9 %), hexyl hexanoate (5.7 %) and butyl 2-methylbutanoate (5.4 %). In the non-treated apples after 7 month storage, over 53 % of the total volatiles originated from hexyl 2-methylbutanoate (21.5 %), E,E-farnesene (8.78 %), hexyl butanoate (9.7 %), hexyl acetate (7.1 %), hexyl hexanoate (3.5 %) and butyl 2-methylbutanoate (2.7 %). These major compounds were also reported to contribute to apple aroma.[2]

All of those major volatile compounds, which obtained from 1-MCP treated and non-treated apples after 1 month storage, dramatically decreased from 3 to 5 months of storage time. The cold storage in regular atmosphere after 3 months inhibited production and concentration of volatile compounds in apples.[24] The level of the major compounds in 1-MCP treated was considerably lower than in non-treated apples from 3 to 5 months of storage time. It has been reported that post-harvest apple treatment with 1-MCP can reduce the production of volatiles that contribute to the character impact volatiles of apples.[9] On the other hand, the level of several compounds in 1-MCP treated apples increased after 7 months of storage than after 3 to 5 months of storage, which are ethyl butanoate, ethyl 2-methylbutanoate, propyl 2-methylbutanoate, butyl butanoate, butyl 2-methylbutanoate, hexyl propanoate, hexyl 2-methylbutanoate, and hexyl hexanoate, and butyl octanoate. Those volatile compounds in non-treated apples did not show important differences between 3 months and 5 months storage.

During long-term storage, the level of the esters such as ethyl butanoate, propyl 2-methylbutanoate, butyl butanoate, butyl 2-methylbutanoate, hexyl propanoate, butyl hexanoate, and hexyl butanoate, quantitatively increased while the alcohols decreased such as 2-methylbutanol. These results are consistent with previous reports that the volatile ester production can be affected by its precursors, especially by alcohol precursors.[7] In this study, the production of 2-methylbutyl acetate was higher for 7 months stored apples than for 5 months stored apples. 2-methylbutyl acetate are major ester compound in Fuji apples, which is formed from the alcohol precursors, 2-methylbutanol.[4] The ultimate levels of volatile esters in apples after harvest are influenced by the levels of alcohol precursors.[5]

3.3. Off-flavor compounds in Fuji apples

Table 2 shows the major volatile compounds from

Table 2. Major apple volatile compounds and their sensory description

Peak No.[a]	Major compound	Odour description
1	1-butanol	chessy[6 b]
9	Ethyl butanoate	fruity[19]
11	Butyl acetate	red apple aroma[6]
12	Ethyl 2-methylbutanoate	apple like[18]
14	2-methylbutyl acetate	characteristic apple solvent[6], banana like[18]
17	Butyl propanoate	apple, fuity[18]
24	Butyl butanoate	rotten apple, chessy[6]
27	Hexyl acetate	red apple aroma[6]
28	Butyl-2-methylbutanoate	apple, fuity[18]
36	Hexyl-2-methylpropanoate	apple, grapefruit[18]
38	Hexyl butanoate	apple, fuity[18]
44	Hexyl hexanoate	apple peel[18]
47	E,E-farnesene	green harbaceous[20]

[a]Values show the number of peak detected from Fuji apples in this study and is listed in Table 1.
[b]The number is appeared in the reference of this report.

Fuji apples in this study and their sensory description in previous studies.[6, 24-26] Although the most volatile compounds are characterized by apple-like or fruits-like odour, 1-butanol and butyl butanoate are described as cheesy or rotten apple odour. The flavors can be recognized as the characteristic off-flavor in apples associated with the sensory evaluation. In the present study, volatile analysis of two different flavor ingredients of apple used in food products was investigated. The result showed that apple flavor ingredients did not contain the off-flavor, 1-butanol and butyl butanoate because consumer preference could be strongly affected by the off-flavor.

It has been reported that ethanol and acetaldehyde are the source of off-flavor in apple.[27] However, the compounds were not detected in all 1-MCP treated and non-treated apples in this study. This result may explain that their concentration decreased during harvest maturity in previous study.[27] In addition, Fuji apples produce different volatiles in various conditions such as harvest date, storage atmosphere, storage period, temperature, seasons and ripening period.[5]

In 1-MCP treated apples, 1-butanol and butyl butanoate significantly decreased from 1 to 3 months of storage, while the amounts of the volatile compounds were much higher after 7 month storage than after 3 months (*Fig.* 2). In contrast, non-treated apples had no significant effect on the amounts of 1-butanol and butyl butanoate. Indeed, the amounts of those compounds were significantly lower in 1-MCP treated apples than in non-treated app for 5 months of storage, however no significant difference after 7 months storage was found between 1-MCP treated and non-treated apples. This may be due to 1-MCP treatment after apple harvest. Therefore, the impact of 1-MCP treatment on Fuji apples is to reduce the amounts of off-flavors, 1-butanol and butyl butanoate, for 5 months storage.

4. Conclusions

Volatile compound is one of the most important indicators to assess fruit quality. Fuji apples also produced a lot of volatile compounds and displayed different production of volatiles under different storage conditions. In present study, the volatile analysis from the intact Fuji apples was investigated by HS-SPME-GC-MS. During long period (7 months) of storage, alcohols such as 2-methylbutanol, decreased, however more esters such as ethyl butanoate, propyl 2-methylbutanoate, butyl butanoate, butyl 2-methylbutanoate, hexyl propanoate, butyl hexanoate, and hexyl butanoate, were produced. It seemed that the production of esters may be affected by the amounts of emitted alcohol precursor in Fuji apples. Especially, the production of 1-butanol and butyl

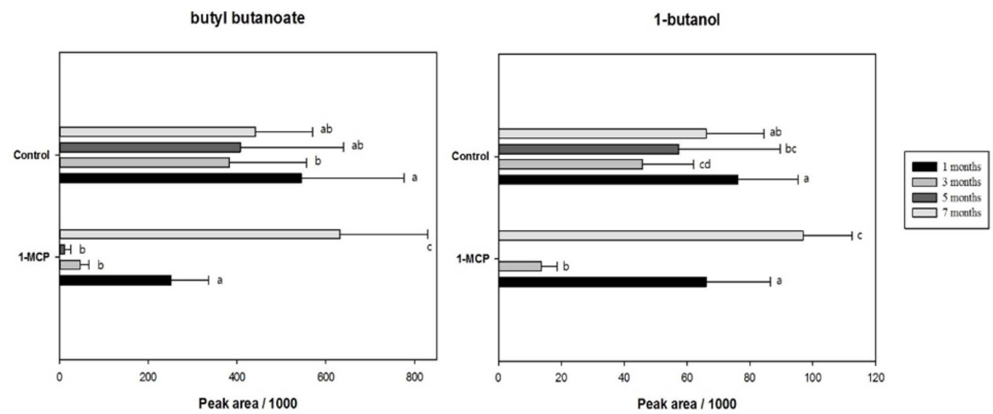

Fig. 2. Comparison of peak area of off-flavors, 1-butanol and butylbutanoate, detected from 1-MCP treated and non-treated apples (control) during the 7 month storage. There are significant differences ($P < 0.05$) on off-flavors throughout entire 7 months storage using Duncan's multiple comparison test between those apple samples having the different letter.

butanoate was significantly higher in non-treated apples than in 1-MCP treated apples, which were described as cheesy or rotten apple odour. It is very important to control the off-flavor to provide fresh apples to consumers. Therefore, given the search for volatile indicators to evaluate apple quality, 1-butanol and butyl butanoate may be an appropriate way to optimize apple flavor quality in the market place for both consumers and the apple industry.

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