Changes of apple fruit volatile profiles induced by fresh bruise at early stage

QING CHEN – WENHAO QIN – MENGQI SHE – SIYU CHEN – FANG WANG – ZUOJUN TAN

Summary

Fresh bruise of apple is a major postharvest problem, which is easily ignored at early stage and can develop into more serious external bruise as the time goes on. In this study, we investigated changes of volatile organic compounds (VOCs) profile of apples with fresh bruise during 0–24 h. For this purpose, headspace solid-phase microextraction (HS-SPME) combined with gas chromatography-mass spectrometry (GC-MS) was used. A total of 43 aroma compounds were identified in apple, consisting of 8 carbonyl compounds, 23 esters, 5 alcohols, 5 terpenes and 2 other compounds. The data of VOCs were subjected to partial least squares discriminant analysis, which showed that 12 h might be the optimum time for apple quality maintenance after fresh bruise. The content of VOCs in bruised tissues showed a significant increase, while they were basically unchanged in healthy tissues. The content of carbonyl compounds increased during 0–24 h, in particular C6 and C9 aldehydes. The content of (3*E*,6*E*)-3,7,11-trimethyldodeca-1,3,6,10-tetraene was increased. The results of this study may help top to understand the metabolic disorder and quality maintenance after fresh bruise in apple fruits.

Keywords

apple; fresh bruise; volatile organic compounds; partial least squares discriminant analysis; gas chromatography-mass spectrometry

Apple is one of the top four fruits cultivated in temperate regions all over the world, playing an important role in daily food consumption. It is a rich source of carbohydrates, vitamins, dietary fibre and trace elements. Bruise damage is the most common type of mechanical damage of apple fruits, resulting from the action of excessive external force on fruit surface during the impact, compression or vibration at harvesting, handling and transport. The fresh bruise is a type of mechanical damage, which is characterized by hidden damage and hence easily neglected, being difficult to detect at early stage [1]. As time passes, the apple tissue with fresh bruise is subject to bacterial and fungal infection, eventually leading to apple rot. The apple outer appearance defects directly affect consumer behaviour and decrease the fruit commercial value. Furthermore, the bruised, contaminated and infected apple can spread the contamination and infection to the sound products, with economic losses and food safety problems [2, 3]. Many research studies proved that presence of fresh bruise accelerates in apple the metabolic rate and moisture loss [4, 5]. The formation of dark brown pigments on apple peel of the bruised region is associated mainly with the contact between cytoplasmic oxidizing enzymes (polyphenol oxidase and peroxidase) and phenolic substances [1, 6]. Studies indicated that the respiration rate and weight loss of pomegranate were markedly increased after fresh bruise [5]. Li et al. [7] reported that firmness, titratable acid contents and total soluble solids contents were remarkably decreased after fresh bruise of apples. Metabolic changes have not been reported in apple with fresh bruise at the early stage.

The quality of fruits and vegetables is related to texture, appearance (colour, shape and surface character), flavour (aroma and taste), nutritional composition and safety [8]. Fruit aroma, com-

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posed from a complex of volatile organic compounds (VOCs), is usually considered a key factor in fruit quality [9]. VOCs of the apple fruit include alcohols, aldehydes, ketones, esters, terpenes and some other compounds [10]. Aldehydes, ketones and esters are the main compounds in fruit, which are mainly generated by oxidation of fatty acids and metabolism of amino acids. Phenylpropanoid and benzenoid compounds originate from the aromatic amino acid phenylalanine, and terpenoid compounds are generated by two independent compartmentally separated pathways from mevalonic acid and methylerythritol phosphate [11]. VOCs have been proven to defense the fruit against diseases, insect pests and environmental stresses [12]. The research about the fruit VOCs is critical to understand the metabolic changes in adversity stress. Gong et al. [10] investigated the changes of apple aroma profile after infection by *Penicillium expansum* and found that inoculated 'Delicious' apples released more volatile compounds. Zhu et al. [13] found that the volatiles related to the aroma in banana, in particular esters, significantly decreased due to low temperature. Gong et al. [14] found that hexanoic acid was an important aroma compound to distinguish the apple fruit infected by *P. expansum* and *Trichothecium roseum* from control. At present, most studies mainly focus on the effect of storage conditions, infection of pests and diseases on apple aromatic components [15, 16]. However, there has been no study to analyse the aroma compounds change after apple fresh bruise at early stage (0–24 h). From the quality standpoint, the apple aroma profile change is extremely significant to understand the apple quality change and maintenance after fresh bruise.

Gas chromatography-mass spectrometry (GC-MS) is widely applied in the general research to determine and understand the distribution of VOCs. It is a powerful and effective technology with high sensitivity, excellent repeatability and comparatively easy operation, which facilitates the qualitative and quantitative analysis of VOCs [17]. Routine analysis steps of volatile substances involve sampling, extraction, concentration and sample injection. The VOCs enrichment can be conducted by direct aqueous injection (DAI), purge and trap (P&T), liquid-liquid extraction (LLE), headspace (HS) and solid-phase microextraction (SPME) [18]. Although DAI looks like a convenient method, a special deactivated guard column is required for special equipment between the injector and the analytical column [19]. P&T is time-consuming and unsuitable for real-time and online monitoring, although it is a technique with high precision and reliability [20]. LLE is a common analytical method for VOCs, which is often replaced by alternative methods, because it needs large volumes of solvents for sample extraction. HS is a powerful and effective technique, by which VOCs of the sample can be directly analysed without any sample pre-treatment, avoiding matrix effects [18]. The combination of HS and SPME has the advantage of taking less time and of improved sensitivity and reproducibility at analysis of fruits and vegetables [21]. HS-SPME has been widely used in the field of VOCs analysis of fruits and vegetables. For example, Liu et al. [22] found that hexanal and *E*-2-hexenal were the most abundant aroma compounds in 'Fuji' and 'Delicious' apple varieties by using the technology of HS-SPME. Zhu et al. [23] determined the VOCs formed during ripening of two banana cultivars.

The objective of this study was to explore the influence of fresh bruise on aroma compounds in apples using HS-SPME coupled to GC-MS, and to compare the differences in VOCs profiles during 0–24 h.

MATERIALS AND METHODS

Samples

"Fuji" apples were obtained from a commercial orchard in Jingning, Gansu, China, hand-harvested on 25 July 2018. A total of 84 apples free of mechanical damage, disease or pests, and generally uniform in size and shape (weighing 285.6 ± 8.5 g) were selected for the experiment. All selected apples were washed with deionized water and wiped up with paper, then stored at 4 °C and 95% relative humidity (*RH*). Apples were divided into control group and experimental group, each group had 42 samples. The apple will form an invisible fresh bruise after absorbing 0.06 J energy. In the apple dropping impact test, the absorbed energy (E_a) can be approximately calculated by Eq. 1:

$$E_a = M \cdot g \cdot (h_1 - h_2) \quad (1)$$

where M is the apple's weight in grams, g is the gravitational acceleration in metres per second squared, h_1 is the initial height of dropping in metres, h_2 is the second rebound height in metres.

In our study, the apple fresh bruise was simulated by freely falling from the height of 0.2 m to a steel plate, being timely held by hand to prevent secondary damage. The fresh bruised samples were randomly divided into seven groups ($n = 6$) and designated 0B, 4B, 8B, 12B, 16B, 20B and 24B. Unbruised ("healthy") samples were

used as control, being also randomly divided into seven groups ($n = 6$) and designated 0H, 4H, 8H, 12H, 16H, 20H and 24H. Samples were stored at 4 °C and *RH* of 95%. After 0 h, 4 h, 8 h, 12 h, 16 h, 20 h and 24 h, the apple pulp tissue within 2.5 mm from the pericarp was sampled, flash frozen in liquid nitrogen, cryogenically milled to a fine powder and stored at –80 °C until further analysis for a maximum of 1 month.

Analysis of volatile compounds

A portion of 3 g of the apple pulp tissue was accurately weighed and transferred to a 15 ml glass vial with 3 g NaCl (1 mol·l^{-1}) in the vial. The vial was incubated in a water bath at 50 °C for 10 min and then SPME fibre (divinylbenzene-carboxen-polydimethysiloxane 50/30 μm; Supelco, Bellefonte, Pennsylvania, USA) was exposed to the headspace at 50 °C for 30 min. Before analysis, SPME fibres were pre-conditioned in accordance with manufacturer's instructions. VOCs were desorbed from the SPME fibre in the injection port of the gas chromatograph (Trance 1300; Thermo Fisher Scientific, Waltham, Massachusetts, USA) at 250 °C for 5 min in splitless mode.

GC-MS analysis

VOCs were separated on a 5% diphenyl–95% dimethyl polysiloxane non-polar capillary column (TG-5MS; 30 m, inner diameter 0.25 mm, film thickness 0.25 μm; Thermo Fisher Scientific). The GC oven initial temperature was 35 °C, then raised up to 250 °C at 0.086 °C·s^{-1} and held at 250 °C for 1 min. The helium carrier gas was used at constant flow at 0.2 ml·s^{-1}. The temperature of the mass transfer line and electron impact ion source was set to 250 °C and 230 °C, respectively. The measurements were performed under the condition of electron impact ionization (70 eV) and full scan (*m*/*z* from 50 to 450).

Identification and quantification of volatile compounds

VOCs with similarities of more than 70 % was identified by the comparison of mass spectra with NIST 2014MS database (National Institute of Standards and Technology, Gaithersburg, Georgia, USA). In addition, a series of *n*-alkanes from C_7 to C_{40} (50 mg·l^{-1} in *n*-hexane; o2si, Charleston, South Carolina, USA) was used to determine linear retention indices (*LRI*) under the same GC-MS conditions. *LRI* was calculated by Eq. 2:

$$LRI = 100Z + \frac{t(x) - t(z)}{t(z+1) - t(z)} \quad (2)$$

where Z and $(z+1)$ are the numbers of carbon atoms, $t(x)$ is the retention time of unknown compound (in minutes), $t(z)$ is the retention time of *n*-alkane eluting immediately before the unknown compound (in minutes) and $t(z+1)$ is the retention time of *n*-alkane eluting immediately after the unknown compound (in minutes) [24]. The amount of volatiles was expressed as peak area in the chromatogram of total ion current (*TIC*).

Statistical analysis

The GC-MS raw data were converted to netCDF format using the file convert tool in Xcalibur (version 2.0; Thermo Fisher Scientific), then were imported to XCMS online platform (Feline Laboratory, La Jolla, California, USA) for feature detection, retention time correction, alignment, annotation, statistical analysis and data visualization [25]. The corrected GC-MS data were subjected to square root transformation via Microsoft Excel (version 2013; Microsoft, Redmond, Washington, USA) [26].

Partial least squares discriminant analysis (PLS-DA) was used to visualize the significant differences between unbruised and bruised tissues, as well as the trend with time, using SIMCA-P (version 14.0; Umetrics, Umea, Sweden). The parameters of R^2 and Q^2 were used to assess the quality and reliability of the PLS-DA model. Here, R^2 reflected the explained variation in data and represented the goodness of fit, Q^2 was the result of cross-validation and represented the predictability of the model. The model is considered excellent when the values of R^2 and Q^2 approach 1, and $(R^2_Y - Q^2) < 0.3$, where R^2_Y represents the explanatory power of Y matrix. In addition, the statistical significance as well as the robustness and reliability of the PLS-DA model were assessed by a permutation test ($n = 200$). This test can detect whether the specific classification of samples in data set is remarkably better than any other random classification in two groups [27]. The model is validated as a robust and reliable when it corresponds to a negative intercept in the Q^2 regression line. In order to find the major VOCs perturbations, the variable importance in the projection (*VIP*) was subjected to the PLS-DA model. The compound with high *VIP* value (> 1) is considered important for discrimination in the PLS-DA, whereas those with low *VIP* value reflect poor discriminatory power. All peak data were scaled by unit variance (*UV*) before the multivariate analysis.

Tab. 1. Volatile compounds identified in apple tissues using solid-phase microextraction coupled to GC-MS.

No.	Compound	CAS	*VIP*	*RT* [min]	*LRI*	
					Theoretical	Experimental
Carbonyl compounds						
C1	Hexanal	66-25-1	1.83	4.722	800	805.52
C2	(*E*)-Hex-2-enal	6728-26-3	1.77	5.923	854	855.77
C3	Nonanal	124-19-6	1.21	12.950	1104	1107.27
C4	Decanal	112-31-2	1.20	15.888	1206	1209.31
C5	6-Methylhept-5-en-2-one	110-93-0	1.05	9.532	986	990.18
C6	(*E*)-Non-2-enal	18829-56-6	0.69	14.579	1162	1163.63
C7	Oct-1-en-3-one	4312-99-6	0.55	9.288	979	981.55
C8	(*E*)-Oct-2-enal	2548-87-0	0.06	11.631	1060	1062.15
Esters						
E1	2-Methylbutyl acetate	624-41-9	1.72	6.616	880	884.77
E2	Butyl acetate	123-86-4	1.72	5.043	812	818.95
E3	Pentyl acetate	628-63-7	1.40	7.552	911	920.21
E4	Methyl hexanoate	106-70-7	1.36	7.804	925	929.12
E5	Hexyl 2-methylbutanoate	10032-15-2	1.27	16.756	1236	1240.65
E6	Hexyl butanoate	2639-63-6	1.26	15.546	1192	1197.09
E7	Butyl butanoate	109-21-7	1.21	9.843	995	1001.13
E8	2-Ethylhexyl acetate	103-09-3	1.19	14.312	1129	1154.39
E9	Hexyl hexanoate	6378-65-0	0.98	20.802	1384	1391.68
E10	Butyl 2-methylbutanoate	15706-73-7	0.84	11.185	1043	1046.93
E11	Methyl 2-methylbutanoate	868-57-5	0.79	4.271	774	–
E12	Propyl hexanoate	626-77-7	0.74	12.743	1094	1100.10
E13	2-Methylbutyl butanoate	51115-64-1	0.74	11.617	1056	1061.67
E14	Pentyl 2-methylbutanoate	68039-26-9	0.74	14.022	1142	1144.36
E15	3-Methylbutyl hexanoate	2198-61-0	0.69	17.192	1252	1256.39
E16	Butyl (*E*)-2-methylbut-2-enoate	7785-66-2	0.65	13.894	1134	1139.93
E17	2-Methylbutyl hexanoate	2601-13-0	0.54	17.248	1247	1258.41
E18	Butyl hexanoate	626-82-4	0.54	15.536	1189	1196.75
E19	Pentyl hexanoate	540-07-8	0.50	18.217	1287	1293.39
E20	2-Ethylhexyl 2,2-dimethylpropanoate	16387-18-1	0.38	20.244	1332	1370.38
E21	Methyl octanoate	111-11-5	0.37	13.603	1126	1129.86
E22	Hexyl acetate	142-92-7	0.30	10.358	1011	1018.70
E23	(4-tert-butylcyclohexyl) acetate	32210-23-4	0.30	19.424	1368	1339.08
Alcohols						
A1	2-Methyl-5-prop-1-en-2-ylcyclohexan-1-ol	38049-26-2	1.46	10.807	1192	1034.03
A2	2-Methylbutan-1-ol	137-32-6	1.41	3.602	739	–
A3	Nonan-3-ol	624-51-1	1.34	12.793	1095	1101.83
A4	Hexan-1-ol	111-27-3	1.28	6.398	868	875.65
A5	6-Methylhept-5-en-2-ol	1569-60-4	0.88	9.774	994	998.73
Terpenes						
T1	(3*E*,6*E*)-3,7,11-Trimethyldodeca-1,3,6,10-tetraene	502-61-4	1.68	23.831	1508	1514.03
T2	1-Methyl-4-(6-methylhept-5-en-2-yl)benzene	644-30-4	1.18	23.235	1483	1489.31
T3	(4*Z*)-1-methyl-4-(6-methylhept-5-en-2-ylidene)cyclohexene	495-62-5	1.03	25.303	1515	1553.00
T4	1-Methyl-4-(6-methylhept-5-en-2-ylidene) cyclohexene	53585-13-0	0.95	25.295	1533	1551.00
T5	(5*E*)-6,10-Dimethylundeca-5,9-dien-2-one	3796-70-1	0.70	22.500	1453	1459.68
Other Compounds						
O1	2-Pentylfuran	3777-69-3	1.31	9.677	1040	993.00
O2	Nitrosobenzene	586-96-9	0.66	24.620	–	–

CAS – code by Chemical Abstracts Service, *VIP* – variable importance in the projection, *RT* – retention time, *LRI* – linear retention index. Theoretical retention index was obtained from the NIST database ((National Institute of Standards and Technology, Gaithersburg, Georgia, USA). Experimental retention index was determined using *n*-alkanes.

RESULTS AND DISCUSSION

Characterization of volatile compounds in bruised and unbruised apples

Forty-three volatile components were identified using HS-SPME coupled to GC–MS in apple tissues (Tab. 1). The volatiles encompassed 5 chemical groups, comprising 8 carbonyl compounds, 23 esters, 5 alcohols, 5 terpenes and 2 other compounds.

PLS-DA is a simple and effective multivariate statistical analysis method to classify and differentiate samples in many fields, such as the metabolomics and proteomics research [28]. Using this method, the unbruised apple samples were divided into four regions according to their storage time in PLS-DA score plots (Fig. 1A). Groups 1, 2 and 3 contained the samples 0H, 4H and 8H, respectively, which were mainly distributed in the positive region of PC1. Group 4 contained the samples 12H to 24H, which were distributed mainly in the negative region of PC1. The GC-MS data of unbruised samples stored for 0–8 h were more scattered than the data of higher storage time samples (12–24 h), which might have been the result of stress response to the environment change at early storage period.

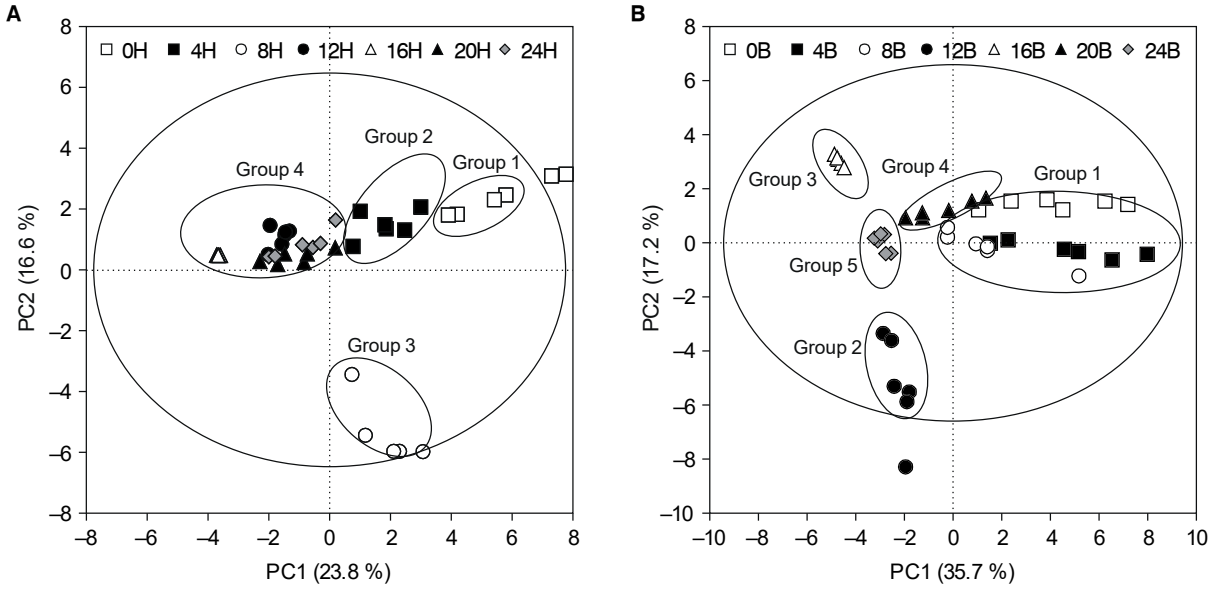

Fig. 1. Partial least squares discriminant analysis score scatter plot.

A – unbruised apples, B – fresh bruised apples.
0H, 4H, 8H, 12H, 16H, 20H, 24H – unbruised samples stored 0 h, 4 h, 8 h, 12 h, 16 h, 20 h and 24 h, respectively. 0B, 4B, 8B, 12B, 16B, 20B, 24B – bruised samples stored 0 h, 4 h, 8 h, 12 h, 16 h, 20 h and 24 h, respectively.

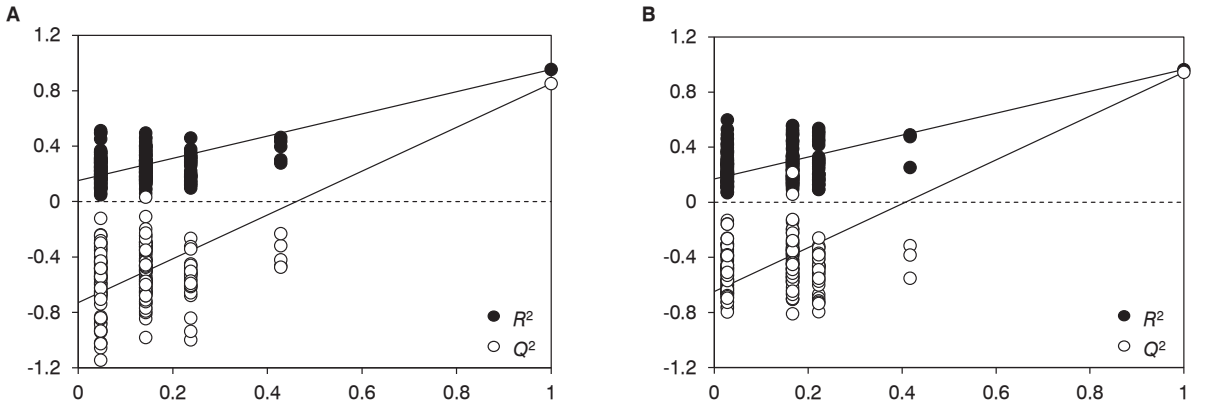

Fig. 2. Permutation test.

A – unbruised apples, B – fresh bruised apples.
R^2 – prediction rate of the model, Q^2 – interpretation of the model.

Tab. 2. Partial least squares discriminant analysis model parameters.

	n_{PC}	R^2_X	R^2_Y	Q^2	$R^2_Y - Q^2$
Healthy samples	8	0.913	0.933	0.898	0.0350
Bruised samples	7	0.924	0.918	0.890	0.0280

n_{PC} – number of principal components of the partial least squares discriminant analysis model, R^2_X – explanatory ability to *X* matrix, R^2_Y – explanatory ability to *Y* matrix, Q^2 – predictive ability.

The GC-MS data for bruised apple samples were divided into five regions according to their storage time in PLS-DA score plots (Fig. 1B). Group 1 contained the samples 0B, 4B and 8B, which were distributed mainly in the first and fourth quadrants in score plots. Groups 2, 3, 4 and 5 contained samples 12B, 16B, 20B and 24B, respectively, which were distributed mainly in the second and third quadrants in score plots. The bruised apple samples exhibited opposite trends compared with unbruised samples in score plots. Tab. 2 lists the parameters of the PLS-DA model describing this situation. The parameter values indicate that the model possessed good fitness with good predictability. As shown in Fig. 2A and Fig. 2B, Q^2 regression lines corresponded to a negative intercept, which validated the PLS-DA model's robustness and reliability. This result indicated that the metabolic disturbance occurred in bruised samples and it became more severe over time. Although the distribution of GC-MS data of unbruised and bruised samples showed an opposite tendency in PLS-DA score plots, we observed that they both significantly changed at 12 h. This finding was consistent with our previous results from an untargeted metabolomics research of apples [29] and suggested that 12 h might be an optimum time for checking apple quality regarding fresh bruise.

Carbonyl compounds

Carbonyl compounds are an important group of volatiles in the apple VOC profile. They are formed from many precursors from sugar, amino acid and fatty acid metabolism [30]. Total aldehydes content showed a significant increase in bruised tissues Fig. 3A. C_6 and C_9 aldehydes are the most important carbonyl compounds in apple, which are usually formed by oxidation of C_{18} unsaturated fatty acids (linoleic or linolenic) by

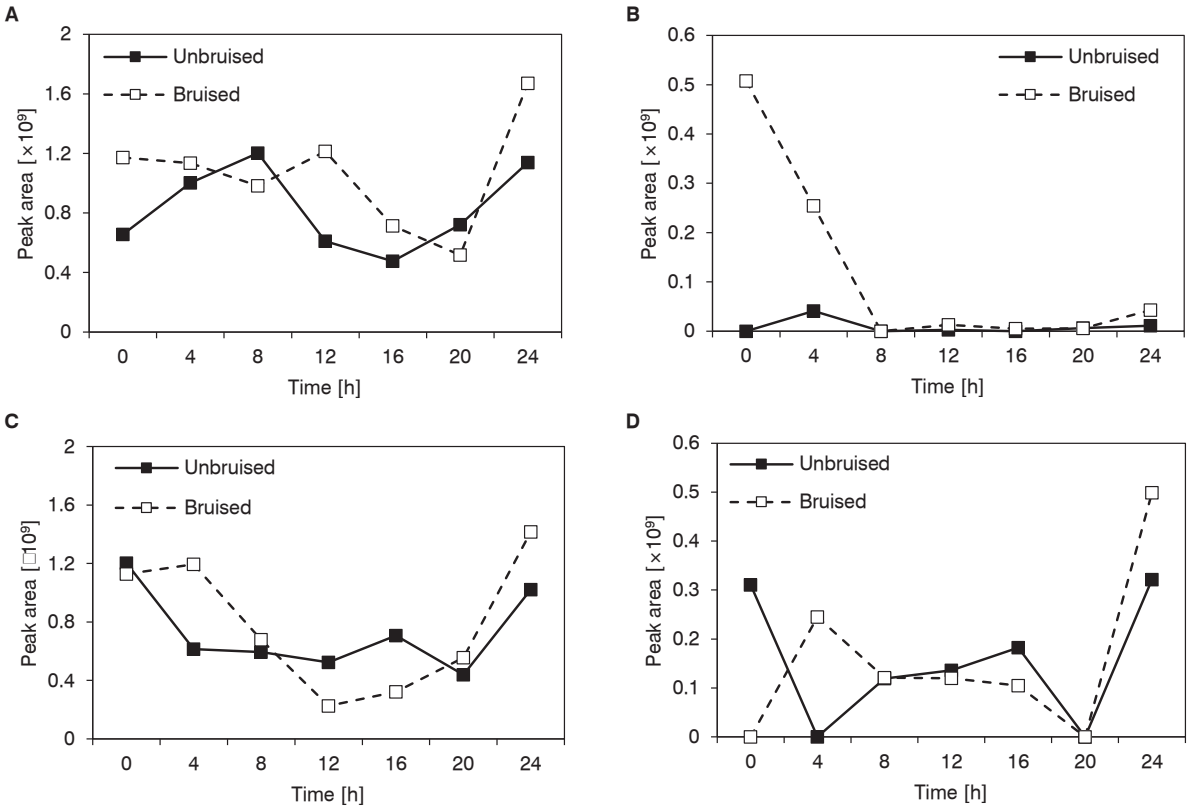

Fig. 3. Changes of relative contents of volatile compounds in bruised and unbruised apples.

A – carbonyl compounds, B – alcohols, C – esters, D – terpenes.

lipoxygenase [11]. The increase in the content of carbonyl compounds in our study was probably the result of the increased oxidation rate of unsaturated fatty acids.

Among carbonyl compounds, hexanal had the highest *VIP* value (1.827), followed by (*E*)-hex-2-enal, nonanal, and decanal (Tab. 1). Hexanal (Fig. 4A) and (*E*)-hex-2-enal (Fig. 4B) are primary oxidation products of linoleic acid [31]. The level of reactive oxygen species (ROS) and membrane lipid peroxidation are known to be increased after apple fresh bruise. The level of malondialdehyde (MDA) is an important marker of membrane lipid peroxidation. TANG and ZANG [32] studied the MDA content change after kiwifruit fresh bruise, and they observed that the content of MDA in bruised tissue was increased. The hexanal and (*E*)-hex-2-enal contents decreased during 12–20 h. This may be the result of competition between hexanal and MDA for the same substrate, the linoleic acid being more susceptible to oxidation to form MDA during 12–20 h. As the peroxidation degree of unsaturated fatty acids continued to accelerate, hexanal and (*E*)-hex-2-enal contents increased during 20–24 h.

Decanal (Fig. 4C) is the primary oxidation product of oleic acid [31]. The decanal content descended during 0–24 h in unbruised tissue. Oleic acid tended to be further desaturated, while the synthesis of decanal was inhibited in unbruised apple tissue. Linoleic acid and linolenic acid are more easily damaged with ROS than oleic acid, because of their higher degree of unsaturation. The content of decanal was principally unchanged during 0–20 h in bruised tissue, which might have been the result of preferential oxidation of linoleic acid and linolenic acid. As the aggravation of membrane lipid peroxidation, decanal could be synthesized via oxidation of oleic acid during 20–24 h.

Alcohols and esters

The content of alcohols and esters in bruised tissue was higher than that in inbruised tissue during 0–24 h (Fig. 3B, Fig. 3C). C_6 and C_9 aldehydes are often converted to alcohols by alcohol dehydrogenases [33], followed by further conversion to esters by alcohol acetyltransferase [34]. At the moment of bruise, it may be that the lipoxygenase pathway was activated and the synthesis of al-

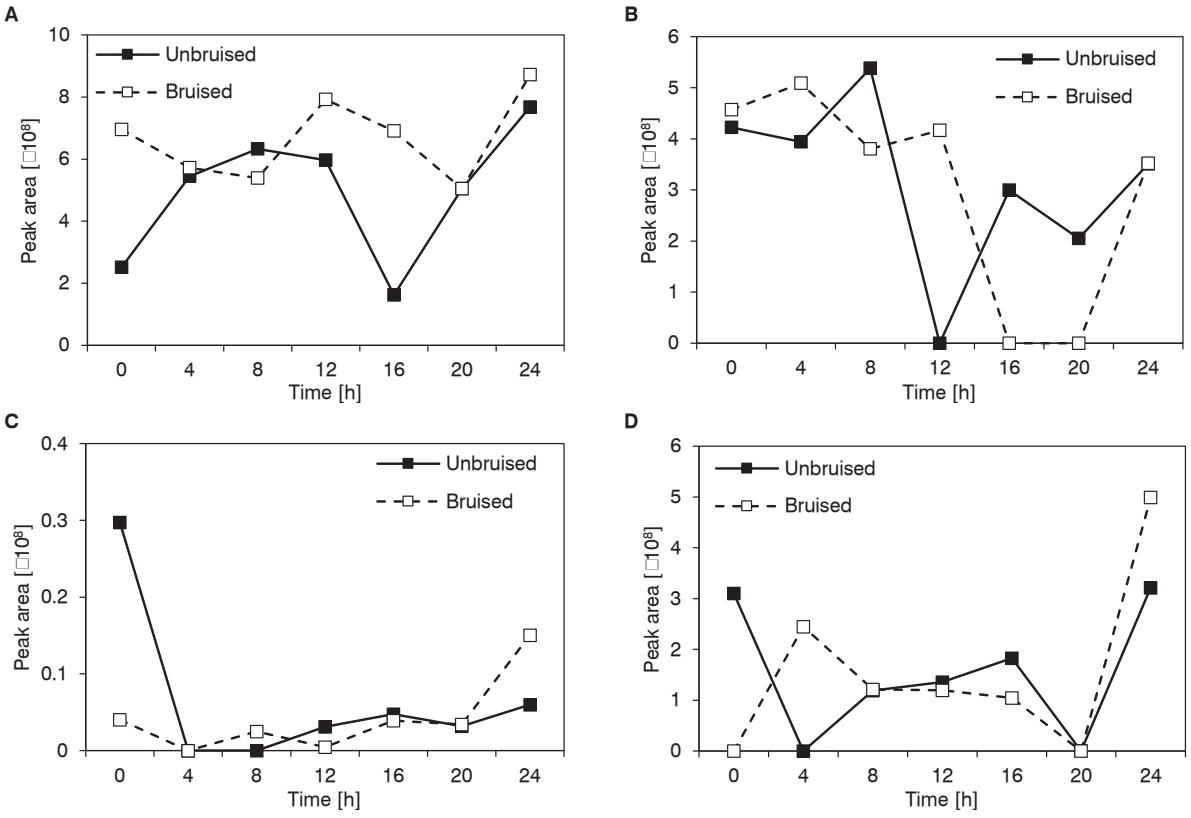

Fig. 4. Changes of relative contents of selected carbonyl compounds in bruised and unbruised apples.

A – hexanal, B – (*E*)-hex-2-enal, C – decanal, D – (3E,6E)-3,7,11-trimethyldodeca-1,3,6,10-tetraene.

cohols as well as esters was significantly increased. The contents of alcohols and esters decreased and then increased during 0–24 h in bruised tissue. The decrease in alcohols and esters during 0–20 h might have been associated with the competition between alcohols, esters and MDA for the same substrate [32]. With the development of bruise, the relative contents of alcohols and esters increased, which may indicate that the activity of alcohol dehydrogenase and alcohol acetyltransferase were increased, and the synthesis of alcohols and esters were activated [35].

Terpene compounds

Terpene compounds are a group of secondary metabolites, which play an important role in plant growth and development, environmental adaptation, as well as protection against pests and diseases [36]. In apples, precursors of terpene compounds, isopentenyl diphosphate and dimethylallyl diphosphate, are synthesized via two compartmentally separated and independent pathways from mevalonic acid and methylerythritol phosphate [11]. The relative content of terpenes increased during 0–24 h in bruised tissue (Fig. 3D). (3*E*,6*E*)-3,7,11-trimethyldodeca-1,3,6,10-tetraene is an important terpene compound in apple tissue, making up to 98.7 % of terpene compounds. Under abiotic stress, the synthesis of terpenoids is activated, being related to a defense-related function [37]. Acetyl-CoA is the precursor of isopentenyl diphosphate and dimethylallyl diphosphate. The fluctuation of the content of (3*E*,6*E*)-3,7,11-trimethyldodeca-1,3,6,10-tetraene was very small during 0–20 h in bruised tissue (Fig. 4D). Sesquiterpene synthase is an important enzyme for (3*E*,6*E*)-3,7,11-trimethyldodeca-1,3,6,10-tetraene synthesis. It is located in cytoplasm and in the junction of cytoplasm and endoplasmic reticulum [38]. It can be hypothesized that with the further destruction of the membrane structure, the activity of sesquiterpenesynthase was increased and the content of (3*E*,6*E*)-3,7,11-trimethyldodeca-1,3,6,10-tetraene increased during 20–24 h.

CONCLUSION

In this study, we investigated the apple fruit volatile compound change after fresh bruise in 24 h by using HS-SPME coupled to GC-MS. A total of 43 compounds were identified in apple tissue, belonging to five chemical groups, comprising 8 carbonyl compounds, 23 esters, 5 alcohols, 5 terpenes and 2 other compounds. Carbonyl compounds and esters were the most important VOCs in apple fruit. In this study, we found a clear-cut distinction between bruised and unbruised samples stored for different time, by characterizing the VOCs profile and analysing data by PLS-DA. Although there were obvious differences between unbruised and bruised samples in PLS-DA scores plots, they both exhibited significant changes at the storage time of 12 h. Combined with our previous study, we proved that 12 h might be an optimal time to check the quality of appples after bruise. The content of VOCs in bruised tissue was higher than that in unbruised tissue after 24 h. The content of hexanal, (*E*)-hex-2-enal, decanal, hexyl ester and (3*E*,6*E*)-3,7,11-trimethyldodeca-1,3,6,10-tetraene significantly changed after fresh bruise. The content of carbonyl compounds increased during 0–24 h, in particular C6 and C9 aldehydes. Contents of esters and alcohols decreased during 0–20 h and the content of terpene compounds increased after fresh bruise. Results of this study may be of importance for the description of the metabolic disorder and the quality maintenance after apple fresh bruise at early stage (0–24 h). Next, we will analyse the characteristics of individual stages to determine the role of these VOCs in apple bruise, and further confirm 12 h as an optimal quality control time by other analytical methods.

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