Development of a method for profiling of volatile organic compounds to monitor heat stress in hot water dipped apples

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Abstract

Storage rot is a major contributor to losses of organic apples due to restrictions in spraying in orchards. Postharvest pre-treatments such as hot water dipping (HWD) have been proven successful to reduce storage rot. However, excessive heat during dipping easily induces heat stress and physiological disorders, which only show up after several weeks of cold storage. To develop further the HWD technology, a method for early detection of physiological disorders is desirable. As apples emit volatile organic compounds (VOCs) in response to stress and ripening, VOC profiling can be a useful tool for detecting the immediate heat stress and latent physiological disorders. In this study, a selected ion-monitoring program was developed for analyzing VOCs from apples (*Malus domestica* 'Ingrid Marie' and 'Pinova') dipped in water at 20 and 56°C for 3 min. VOCs were sampled by solid-phase microextraction and analyzed by GC-MS in total ion current mode. From these results, a selected ion-monitoring program was developed which enabled detection of trace VOCs collected by static headspace sampling. The results showed that all apples dipped in 56°C for 3 min developed visual signs of physiological disorders in cold storage. At the same time, the VOC profile of these apples differed from that of the 20°C treated apples. This work provides a solid basis for a future development of the VOC method to forecast physiological disorders and to improve the HWD technology for better control of storage rot.

Keywords: hot water dipping, physiological disorders, GC-MS, VOCs, static headspace sampling, solid-phase microextraction

INTRODUCTION

Research on organic production is becoming more important due to increasing consumer awareness towards the impact of pesticide application on the environment and on food safety. One of the main problems in organic apple production is the low yield of first class apples. Higher incidences of pest insects and diseases in the orchard result in smaller and irregular fruits (Pedersen, 2012). The yield of organic apples in Sweden has thus been reported to be almost half of the yield of conventional production (Ascard et al., 2010). Of the abovementioned factors, fungal infections are the most damaging factor causing yield reductions in orchards and during storage. Although most storage rots are already present as latent infections before harvest, the symptoms are often delayed until storage. Thus, a large proportion of the organic crop is lost after harvest due to development of storage rot caused by pathogenic fungi. As conventional fungicides are not allowed, there is a need for ecofriendly alternatives that are able to reduce storage rot in organic apple production.

Hot water dipping (HWD) is one of the most effective postharvest pre-treatments to control storage rot in apples. In northern Germany, HWD has proved to be effective against storage rots in apples with efficacies above 80% (Maxin et al., 2012a). HWD also reduces chilling injuries, delays ripening processes (Fallik, 2004), and controls pest insects (Smith and Lay-Yee, 2000). However, caution must be taken as excessive heat can cause heat stress

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and development of physiological disorders. Maxin et al. (2012b) found that the apple cultivar 'Elstar' developed physiological disorders when the fruit were dipped in hot water at temperatures above 53°C for 3 min. Browning of the peel, flesh darkening, pitting, elevated decay and earlier fruit softening are common symptoms of physiological disorders in hot water treated fruits (Lurie, 1998). As these symptoms do not show up until several weeks after treatment, new methods for early detection of physiological disorders are needed.

Apples produce a variety of volatile organic compounds (VOCs) during ripening (Fellman et al., 2003) and in response to multiple biotic (Llusià and Peñuelas, 2001; López et al., 2015; Spinelli et al., 2011) and abiotic stresses (Ebel et al., 1995; Vallat et al., 2005). VOC profiling might thus be a useful tool for the detection of heat stress in apples due to HWD. Several techniques have been developed for sampling of VOCs. Static headspace sampling (SHS) is by far the simplest method but the method lacks sensitivity. However, the sensitivity can be greatly improved by analyzing the gas samples in selected ion monitoring (SIM) mode. However, this requires that relevant compounds are included in the SIM program. To make a library of compounds and build the SIM program, the more sensitive, although time consuming, solid-phase microextraction (SPME) can be used (Luca et al., 2015).

The aim of this study was to develop a method for VOC profiling of hot water dipped apples as a tool for early detection of heat stress and development of physiological disorders during cold storage. The developed method is based on static headspace samples which are analyzed by GC-MS using a SIM program.

MATERIALS AND METHODS

Fruit

Apples (*Malus domestica* 'Ingrid Marie' and 'Pinova') were harvested from Aarhus University's orchard in Årslev. Only blemish free fruit were selected. The fruit were stored at 2°C until the day before HWD when they were moved to room temperature to acclimate overnight.

Hot water dipping

A plastic tank was filled with tap water and heated to 20 or 56°C before the apples were dipped. For dipping, the apples were placed in one box with an additional empty box on top to prevent apples from floating. The two boxes were submerged into the water for 3 min. The box containing the apples were then moved to a drying cabinet (Lytzen A/S, Herlev, Denmark) for 25 min at 24°C before it was moved to cold storage for 14 weeks at 2°C.

VOC analysis and assessment of physiological disorders

From each treatment, three 2-L jars were prepared, each with 5 apples with a combined weight of 900 g. The jars were closed with lids equipped with a septum for gas sampling and moved to climate chambers at 20°C. The remaining apples were moved to cold storage at 2°C and evaluated for visual physiological disorders after 8 and 14 weeks.

Solid-phase microextraction

To generate a broad list of VOCs emitted from the apples, the compounds were sampled by SPME after approximately 3 h of incubation from one jar of each combination of cultivar and treatment using a carboxen/polydimethylsiloxane fiber, which was exposed in the headspace for 5 min.

Separation of compounds was performed on an Agilent 7890A GC equipped with a HP-PLOT/Q (Agilent Technologies, Palo Alto, CA, USA) column (30 m × 320 µm × 20 µm). The GC was held at constant pressure (2.1215 psi) giving a flow of 1 mL min^{-1} using He as carrier gas. The SPME fiber was thermally desorbed in the injection port in splitless mode at 200 °C for 1 min. The oven temperature was initially held at 30°C for 1 min followed by heating to 250°C at 20°C/min and then kept at this temperature for 18 min. The fiber was reconditioned at 250°C for approximately 20 min after desorption.

The compounds were identified on an Agilent 5975C inertXL mass selective detector (Agilent Technologies, USA) operating in total ion current mode using electron ionization at 70 eV. Mass-to-charge ratios between 20 and 200 were scanned using a quadropole mass analyzer. Compounds suggested by the NIST/EPA/NIH Mass Spectral Database were verified by comparison of retention indices and mass spectra of authentic reference compounds when available.

A SIM program was constructed (Table 1) from the list of compounds generated using the SPME sampling procedure. The compounds were divided into 17 groups depending on retention time. Within each group, each compound were designated a target ion based on the purity of the individual peaks. In order to further validate the identity of compounds in Table 1, 1-2 additional qualifier ions were designated. Dwell time was set to 50 ms for target ions and 10 ms for qualifier ions.

Table 1. Full list of volatile organic compounds identified from hot water dipped apples using solid-phase microextraction sampling analyzed on GC-MS in total ion current mode. From this, a selected ion-monitoring (SIM) program was generated. Volatile organic compounds detected in the static headspace samples using this SIM program are also shown.

| RT (min) | Compound name[1] | SIM program[2] | | | | Detected by SHS |
		Start (min)	Target ion (m/z)	Q1 (m/z)	Q2 (m/z)	
7.18	Methanol	6.50	32	31		+
7.27	Acetaldehyde		43	29		+
8.07	2-Methylpropene[3]	7.70	41	56	39	+
8.66	Ethanol	8.30	31	45	46	+
9.15	Prop-2-enal[3]	9.00	56	55	26	
9.31	Propanal		58	29		+
9.42	Acetone		43	58		+
9.47	Methanedithione		76	78		
9.54	Methylthiomethane		47	62		+
9.76	Methyl acetate		43	74		+
10.06	Pentane	9.90	43	42	41	+
10.41	Propan-1-ol[3]	10.20	31	59		+
10.63	2-Methylpropanal		72	43		+
10.85	2-Methylfuran		82	81		
10.96	Butanal		41	43		+
10.98	3-Methylfuran		82	81		+
11.02	Butan-2-one		43	72		+
11.14	Ethyl acetate		43	61		+
11.26	Methyl propanoate[3]		57	88		
11.63	1-Methoxybutane[3]	11.50	45	56		
11.92	Butan-1-ol	11.75	56	43		

[1]Compounds were identified by library search (NIST/EPA/NIH Mass Spectral Database) and validated by comparing retention time (RT) and mass spectra to those of authentic gas samples.

[2]SIM program constructed from the list of compounds generated using a solid-phase microextraction sampling procedure. The program was used to analyze volatile organic compounds from samples collected by static headspace sampling (SHS). Dwell time were 50 ms for target ions and 10 ms for qualifier ion 1 and 2 (Q1 and Q2).

[3]Only tentative identified from library search.

Table 1. Continued.

| RT (min) | Compound name[1] | SIM program[2] | | | | Detected by SHS |
		Start (min)	Target ion (m/z)	Q1 (m/z)	Q2 (m/z)	
12.14	2-Methylbutanal[3]		58	57		
12.36	2-Pentanone[3]		86	71		
12.46	Ethyl propanoate		102	57		
12.53	Propyl acetate[3]		61	73		+
12.61	Methyl butanoate		71	74		
12.65	1-Methoxy-2-methylbutane[3]		45	70		+
13.21	2-Methyl-1-butanol	12.90	57	41		+
13.50	Ethyl 2-methylpropanoate[3]		43	71		
13.75	2-Methylpropyl acetate[3]		43	73		+
14.02	Ethyl butanoate[3]		88	43		+
14.09	Propyl propanoate[3]		75	57		+
14.22	Butyl acetate[3]		61	73		+
14.58	Butanoic acid[c]	14.35	60	73		
15.52	Ethyl 2-methylbutanoate	15.10	102	85		
15.62	Hexan-1-ol		43	56		
15.87	2-Methylpropyl propanoate[3]		57	29		
16.10	2-Methylbutyl acetate[3]		70	43		+
16.42	Propyl butanoate[3]		89	43		
16.59	Butyl propanoate[3]		75	56		+
16.78	Pentyl acetate[3]		61	70		
18.97	Propyl 2-methylbutanoate[3]	17.50	85	57		+
19.08	Butyl 2-methylpropanoate[3]		71	89		
19.34	2-Methylpropyl butanoate[3]		71	43		
19.68	1-Methylbutyl propanoate[3]		57	70		
19.92	6-Methyl-5-heptene-2-one		108	41		
20.53	Butyl butanoate[3]	20.25	89	71		
20.80	3-Methylbutyl propanoate[3]		57	70		
21.23	Hexyl acetate		43	56		+
24.87	Butyl 2-methylbutanoate[3]	24.40	57	103		
25.85	2-Methylbutyl butanoate[3]	25.30	71	43		
27.81	Propyl hexanoate[3]	26.50	99	43	61	
28.36	Hexyl propanoate[3]		75	57	56	
Termination of program after 29.10 min						

Static headspace sampling

A 2.4-mL headspace sample was taken with a gastight syringe from each jar after 2 h of incubation into a 3.0 mL depressurized, flat-bottomed soda glass vial (Labco Limited, Lampeter, UK) sealed with a double septum consisting of rubber and Teflon/silicon. The vials were stored at -80°C until GC-MS analysis.

Separation of VOCs was performed on the abovementioned GC-MS, using the same column and flow rate. From each vial, a gas sample of 500 μL was automatically withdrawn using a Combi PAL autosampler (CTC Analytics AG, Zwingen, Switzerland) and injected into the GC inlet in a 3:1 split ratio. Oven temperature was initially held at 30°C for 1 min followed by heating to 250°C at 20°C min^{-1} and kept at this temperature for 18 min. All compounds were identified by MS operating in SIM mode (70 eV) as described in Table 1.

RESULTS AND DISCUSSION

Apples treated with HWD at 56°C for 3 min showed signs of physiological disorders at weeks 8 and 14, respectively. Apples treated with HWD at 20°C for 3 min displayed no signs of physiological disorders. This is consistent with earlier findings that treatment with 53°C or warmer water for 3 min resulted in physiological disorders in 'Elstar' apples (Maxin et al., 2012b).

Sampling of VOCs using SPME resulted in the detection of 53 compounds when analyzed in total ion current mode. Based on this list, a SIM method was developed (Table 1) which was then used for the analysis of the static headspace samples. A total of 27 VOCs were identified in the static headspace samples using this SIM program.

VOCs associated with low temperature HWD

The esters were by far the largest group of compounds found in the static headspace samples (Table 1). These are important aroma compounds responsible for the fruity aromas of apples. Some of the esters, like butyl butanoate and hexyl acetate, were produced in higher quantities by both cultivars of apples treated with water at low temperature (Figure 1A). 'Ingrid Marie' also produced more butyl acetate at the low temperature but the same behavior was not observed for 'Pinova' (Figure 1B). This result underlines the importance of using more than one cultivar when developing a VOC based method to detect the immediate heat stress response in hot water dipped apples, especially if the method is to be applied on apples in general.

VOCs associated with high temperature HWD

The production of acetaldehyde was strongly linked to the high temperature HWD (56°C) of both apple cultivars (Figure 1C). Besides being released from plants as a result of fermentation reactions (Kozlowski and Pallardy, 2002; Kreuzwieser et al., 2001) acetaldehyde emission has also been linked to stress factors such as wounding, high intensity light and high temperature exposure (Loreto et al., 2006). Methanol was also produced in higher concentrations following high temperature HWD (Figure 1C). The source of methanol is most likely from demethylation of the pectin in the cell walls and middle lamellas (Galbally and Kirstine, 2002). A slight increase in the emission of acetone was observed in apples treated with high temperature HWD (Figure 1D). Acetone could be produced by fatty acid oxidation leading to acetoacetic acid and a spontaneous decarboxylation into acetone (Murphy, 1985). As all of these VOCs originate from universal metabolic processes, these compounds could possibly be used as biomarkers of heat stress response in various fresh produce subjected to HWD. Methylpropene also appears to be linked to apples treated at high temperature (Figure 1E). The existing literature offers no explanation as to the origin. However, as indicated in Table 1 the compound's identity has yet to be confirmed by a known standard.
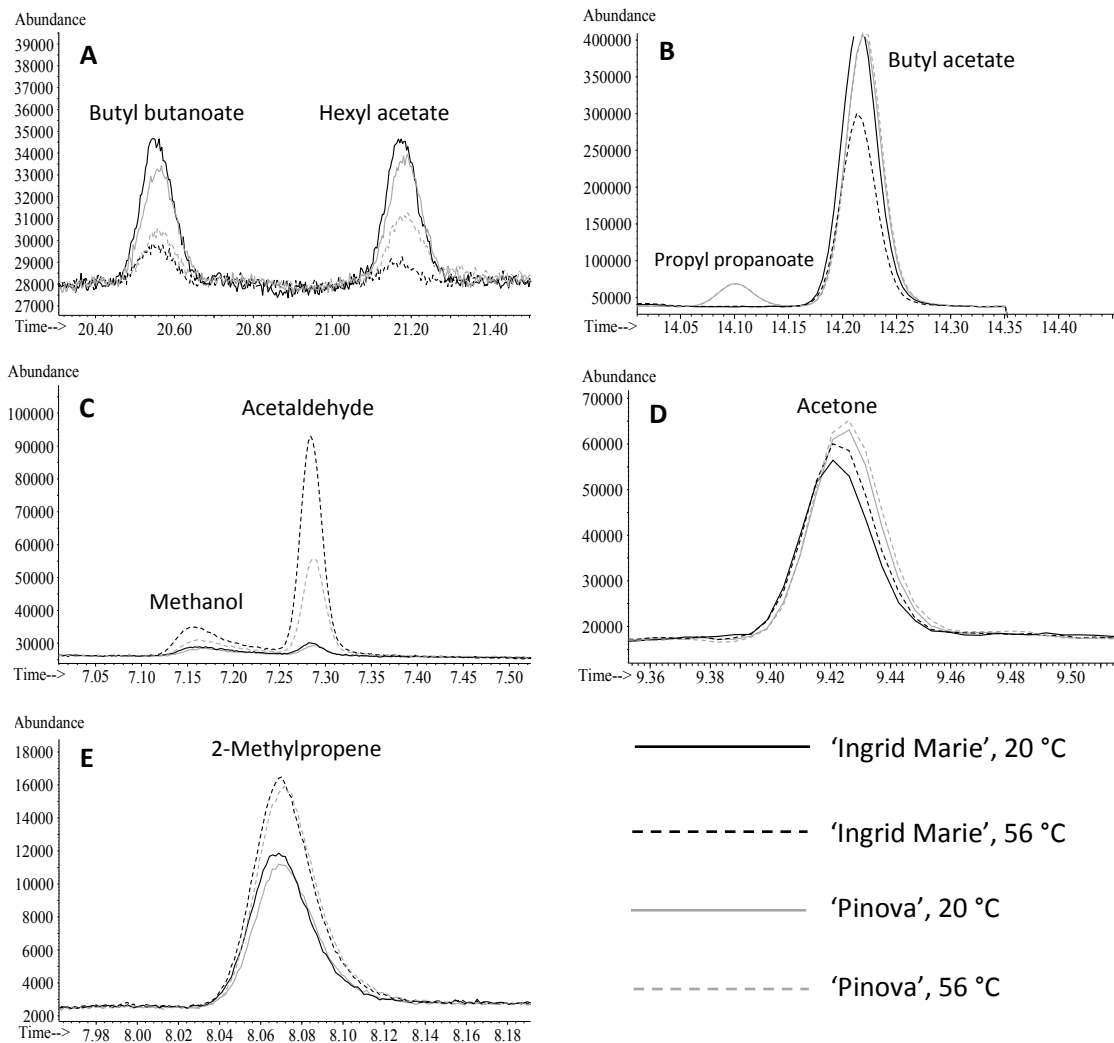
Figure 1. Selected volatile organic compounds produced by apples of the cultivars 'Ingrid Marie' and 'Pinova' treated with 20 and 56°C water for 3 min. The results were obtained using static headspace sampling in combination with GC-MS running in selected ion-monitoring mode.

CONCLUSIONS

A method for VOC profiling of hot water dipped apples based on SHS and analysis of compounds on GC-MS in SIM mode was developed. Several compounds were either characteristic for low temperature (butyl butanoate and hexyl acetate) or high temperature (acetaldehyde, methanol, and acetone) HWD of apples. These results show that the developed VOC profiling method could be a useful tool for early detection of heat stress response in apple due to HWD. Future studies on HWD should include a range of temperatures to find out the relations between VOCs and latent physiological disorders.

ACKNOWLEDGEMENTS

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348