Headspace-SPME Analysis of Volatiles from Quince Whole Fruits

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Abstract

Solid-phase microextraction (SPME) combined with GC and GC/MS was used for analysis of the volatile compounds of quince (*Cydonia oblonga* Mill.). The whole fruits, representing two different ripening stages, were stored and analyzed one day after collection. More than 40 volatile compounds were identified. The relative percentage of acetates, with exception of (Z)-3-hexenyl acetate, increased in a parallel way with ripeness. Of the esters of higher organic acids, ethyl hexanoate and ethyl octanoate were present in the greatest quantity. Ethyl octanoate, which was present in the highest quantity, showed the highest increase with ripeness (from 6–8% to 15–18%), while ethyl hexanoate increased 3% to >4%. In contrast, the relative percentages of two sesquiterpenes, α-bergamotene and α-farnesene, clearly decreased with ripeness.

Key Word Index

Cydonia oblonga, Rosaceae, headspace volatiles.

Introduction

The study of particular flavor notes in uncommonly used vegetables and fruits often receives the interest of the flavorist, who often finds inspiration in natural sources that are not widely available. A flavor note that has been rarely used is that of a fruit of the Rosaceae family, known in Italy as the "mela-cotogna" ("quince" apple), and properly identifiable as *Cydonia oblonga* Miller (syn. *Cydonia vulgaris* Pers.). However, quince fruit is too astringent to be consumed fresh. It is a seasonal fruit and is frequently processed in the home into a jam or jelly during October and November.

The fruit has also recently received interest for its phenolic profile (1,2), which is responsible for its antioxidant and, in turn, free radical scavenging activities. In the pulps and peels from quince fruit derived from different geographical origins in Portugal (1), the main factors contributing to the variability in phenolic distribution included cultivar and genetics, geographical origin, maturity, climate. The phenolic profile determination was found to be most useful in the discrimination of the different parts of quince fruit (2). However, the antioxidant activities of the analyzed samples were not attributed to their phenolic and/or organic acid contents, but resulted from the action of different compounds present in quince fruit and jams and to possible synergistic effects.

In quince fruit, procyanidins are the most abundant of the proanthocyanidins, which are polymers constituted of a variable number of flavan-3-ol units (3). Proanthocyanidin oligomers and polymers, called condensed tannins, have also been studied in Chinese quince (*Pseudocydonia sinensis* Schneid.), quince (*Cydonia oblonga* Mill.), and apple (*Malus domestica* Mill.) fruits.

In steam-distilled quince fruit oil, a number of C13 norisoprenoids, as isomeric theaspiranes, vitispiranes, and theaspirones, were identified (4,5). They originated from carotenoids by oxygenase systems, photo-oxygenations, and other nonenzymatic oxidations. The role of free and bound C13 norisoprenoids in quince fruit as potential precursors of industrially attractive quince fruit constituents (6) and the search for C15 carotenoid metabolites in the same fruit (7) were the focus of successive studies conducted by Winterhalter and colleagues. The results indicated that for quince fruit a less site-specific oxidase seems to be present for cleavage of the carotenoid chain, which justifies the presence of marmelo oxides, marmelo lactones, and quince oxepine.

The aim of the present work was to investigate the volatile compounds characterizing the head space of the overall quince fruit. It is well know that the crushing process introduces flavor changes, presumably due first to the difference in volatility of compounds such as esters and the monoterpene and sesquiterpene hydrocarbons. Furthermore, monoterpenes and oxygenated compounds are vulnerable to chemical changes under steam distillation conditions, and the exact chemical

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Table I. Headspace composition (relative %) by HS-SPME-GC-MS of volatile compounds in whole quince fruits at two ripening stages

Compound	RI	October 2007	November 2007
ethyl acetate	628	0 - t	0.60 – 0.80
2-methylbutanol	739	0.03 – 0.1	0.14 – 0.23
isobutyl acetate	776	0.09 – 0.13	0.14 – 0.18
ethyl butyrate	804	0.05 – 0.07	0.07 – 0.11
butyl acetate	816	0.32 – 0.48	0.54 – 0.65
ethyl crotonate	832	0 - t	0.01 – 0.03
ethyl 2-methylbutyrate	846	0.01 – 0.04	0.08 – 0.14
(Z)-3-hexen-1-ol	857	0.03 – 0.06	0.01 – 0.03
hexanol	867	0.22 – 0.28	0.19 – 0.22
isoamyl acetate	876	0.14 – 0.20	0.48 – 0.63
2-methylbutyl acetate	880	0.57 – 0.64	0.97 – 1.20
2-heptanone	895	0 - t	0.02 – 0.04
amyl acetate	947	0 - t	0.07 – 0.12
prenyl acetate	998	0.06 – 0.09	0.22 – 0.30
methyl hexanoate	1000	0.18 – 0.24	0.11 – 0.16
ethyl tiglate	1001	0.11 – 0.18	0.25 – 0.36
ethyl hexanoate	1002	3.14 – 3.30	4.11 – 4.64
5-hexenyl acetate	1003	0 - t	0.23 – 0.34
(Z)-3-hexenyl acetate	1004	1.65 – 1.84	0.31 – 0.41
hexyl acetate	1014	1.97 – 2.14	2.53 – 3.14
limonene	1033	0.04 – 0.07	0.35 – 0.64
(E)-β-ocimene	1048	0.05 – 0.08	0 - t
γ-terpinene	1062	0 - t	0.01 – 0.03
octanol	1070	0.05 – 0.07	0.04 – 0.06
isobutyl tiglate	1086	0 - t	0.03 – 0.06
propyl hexanoate	1097	0 - t	0.04 – 0.07
ethyl heptanoate	1103	0.40 – 0.54	0.40 – 0.67
nonanal	1123	0.03 – 0.05	0.02 – 0.04
heptyl acetate	1134	0 - t	0.03 – 0.07
methyl octanoate	1147	0.09 – 0.14	0.28 – 0.34
isobutyl hexanoate	1152	0.01 – 0.03	0.06 – 0.12
benzyl acetate	1163	0.06 – 0.08	0.02 – 0.04
ethyl 4-octenoate*	1174	0.38 – 0.46	0.36 – 0.46
ethyl octanoate	1195	6.58 – 8.35	15.32 – 18.62
hexyl 2-methylbutyrate	1227	0.01 – 0.03	0.06 – 0.10
ethyl 2-octenoate	1239	0 - t	0.20 – 0.42
propyl octanoate	1341	0 - t	0.03 – 0.06
theaspirane (isomer) *	1354	0.10 – 0.21	0.05 – 0.07
theaspirane (isomer) *	1365	0.06 – 0.08	0.02 – 0.04
isobutyl octanoate	1376	0 - t	0.03 – 0.05
ethyl decanoate	1398	0 - t	0.23 – 0.29
α-bergamotene*	1436	1.31 – 2.18	0.88 – 1.12
α-farnesene*	1508	82.26 – 87.34	70.33 – 74.48

The results represent the min-max values obtained for seven samples for each ripening stage.
RI: retention indices calculated against n-alkanes
t: trace (< 0.02%)
* : corrected isomer not identified

nature of all these changes is not clear. However, many studies used steam distillation as the isolation method for volatile compounds and the severity of this process has the potential to introduce changes in volatile composition.

HS-SPME (head-space solid-phase microextraction) has proved to be an alternative method for the determination of volatiles. This study was finalized to describe the results of the SPME/GC/MS characterization of head space of overall quince fruits, corresponding to two ripening conditions (October and November 2007). The fruits were derived from the same group of trees growing in an experimental ground of the Apulia region (South of Italy), where these trees are traditionally cultivated.

Experimental

Materials: Two series of samples, consisting of seven fruits of *C. oblonga,* were collected at the first half of October and thirty days later in November of the year 2007 in an experimental field in Matino, province of Lecce, southern Italy. The fruits, representing two different ripening stages, were stored in glass containers and analyzed one day after collection and submitted to a solid-phase microextraction (SPME).

SPME sampling: The SPME fibers (2 cm of 50/30 DVB/CAR/PDMS) and the holder were obtained from Supelco, Italy, and were first conditioned according to the manufacturer's instructions. The intact fruits, unbroken and not washed, were submitted to SPME extraction, one at a time, and were placed in a glass container with a silicone septum coated with a Teflon film. The containers were kept for 30 min in a water bath at 30°C to achieve partition equilibrium between the sample and the air in the container, and then the SPME fiber was exposed to the headspace to adsorb the analytes. After 10 min exposure time, the fibers were retracted into the needle and introduced into the GC/MS injector for desorption and analysis of the volatiles.

GC Analysis: The released volatiles were analyzed by GC-FID using a Shimadzu GC 17A model gas chromatograph, equipped with a SPB-5 capillary column (60 m x 0.32 mm, 0.25 µm film thickness; Supelco, Italy). Hydrogen was used as carrier gas (1.5 mL/min). The column oven temperature was programmed as follows: starting temperature 60°C (1 min), then a linear temperature ramp of 60–240°C at a 3°C/min heating rate, after which it was held for 10 min.

The relative composition of each SPME sample was calculated from the GC peak area by normalization, without using correction factors. Relative retention indices were determined using C8–C32 n-alkanes as reference points in the calculation of retention indices (RI).

GC/MS: The capillary GC/MS were carried out using a gas chromatograph Shimadzu GC-QP2010 model coupled to a Shimadzu MS – QP2010. An Equity-5 capillary silica column (30 m x 0.25 mm, 0.25 µm film thickness) was used for compound separation. The column oven temperature was programmed as follows: starting temperature 60°C (1 min), then 60–240°C at 3°C/min heating rate, and 10 min hold.

The carrier gas was He (1.0 mL/min), injection in split mode (1/5); injector and detector temperatures were set to 220°C and 240°C, respectively. The MS ran in electron impact (EI) mode at 70 eV electron energy, electron multiplier 1010 V, and ion source temperature 200°C. Mass spectral data were acquired in scan mode (m/z range 40–300). Identification was carried out by calculating retention indices and comparing mass

spectra with those in databanks: in-house TB L.R.A. library, NIST 147 (Shimadzu), and Adams (8).

Results and Discussion

The efficiency of SPME for analysis of volatile compounds depends both on the properties of the fiber coating and on experimental conditions. The two-phase coated fiber included a carbon sorbent layer (Carboxen) that was more suitable for quince fruits volatiles than were other tested coatings. PDMS (polydimethylsiloxane), which is a non-polar phase, efficiently extracts non-polar terpenes and the DVB (divinylbenzene) porous microsphere phase increases the absorption of small organic molecules. In the conditions used in present work, the SPME method was very reproducible, with only small variability (< 5%) in peak areas. The fiber used allowed extraction of more than 40 volatile compounds, most of which were identified and there were several compounds that occurred only in trace amounts (< 0.02%).

The composition of volatiles in fruits at the two different stages of ripeness (October and November) showed evident differences for some compounds (Table I). The relative percentage of acetates, with the exception of (Z)-3-hexenyl acetate, increased in a parallel way with ripeness. In contrast, the main content of (Z)-3-hexenyl acetate decreased three- to fivefold with the increase of ripeness. Less pronounced was the increase of methyl octanoate (from 0.1% to 0.3%).

Of the esters of higher acids, ethyl octanoate was present in the greatest concentration, and its levels showed the highest increase with progression of ripeness (from 6–8% to 15–18%). Ethyl hexanoate was the second most concentrated ester, and its level increased from 3% to > 4% between the October and November samples.

The relative percentages of two sesquiterpenes, α-bergamotene and α-farnesene, clearly decreased with ripeness. The percentage of α-farnesene was the highest of all compounds (more than 80% in the sample group of October and more than 70% in the sample group of November), whereas the α-bergamotene reached a maximum value of 2% in the samples of October, and decreased to 1% in the samples of November.

More than 20% of the volatile compounds that were either not identified or were found in trace quantities in the October group of samples were clearly quantitatively identifiable in the samples collected in November. The majority of the compounds were esters, principally ethyl decanoate (0.2–0.3%), ethyl 2-octenoate (0.2–0.4%), 5-hexenyl acetate (0.2–0.3%), and ethyl acetate (0.6–0.8%).

The relative percentage of the two theaspirane isomers that were detected did not increase with ripeness, and the main content fell from 0.06–0.10% to 0.02–0.07%.

In conclusion, HS-SPME has been confirmed to be a simple and non-destructive method useful for the fast characterization of organic volatile compounds. This technique holds promise for monitoring the degree of ripeness of fruits, which is an important quality index for transformation technology.

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