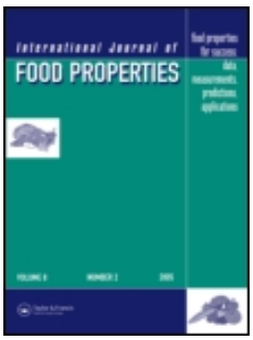

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Determination of Terpene Profiles in Potential Superfruits

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The aim of this work was to characterize and compare the profiles of volatile terpenes in four potential superfruits. These profiles were determined using headspace solid-phase microextraction coupled with comprehensive two-dimensional gas chromatography linked to time-of-flight mass spectrometry. The proposed technique allowed the separation and identification of 79 terpenes present in cape gooseberry, crabapple, cherry silver berry, and scarlet hawthorn. The preliminary compound identification was based on the analysis of deconvoluted mass spectra and a comparison of the calculated linear retention indices with their values reported in the scientific literature. The compound identification was performed using the available standards. Also, a semi-quantitative total ion chromatogram-based analysis was performed. The richest terpene profile was identified in cape gooseberry (62 terpenes), where the terpene fraction constituted about 14% of total volatile fraction.

Keywords: Superfruits, Terpenes, Headspace solid-phase microextraction, Comprehensive two-dimensional gas chromatography, Composition profiles, Bioactive compounds, Time-of-flight mass spectrometry.

INTRODUCTION

The word "superfruit" has been recently introduced to the nomenclature.[1] It comprises 13 natural products, including fruits, vegetables, corn, and tea. Once introduced into the human diet, the previously mentioned food components may bring many health benefits and can easily enhance our well-being. A large group of nutrient-rich fruits have played an important role in folk medicine in Asia (China, Tibet) and Africa for thousands of years. Today, a superfruit is treated more as a marketing term than a science. This is the reason why food and medicinal preparations based on these kinds of fruits have been gaining increasing attention among consumers. The present globalization of world markets results in the world-wide availability of even the most exotic fruits that are used to enrich the human diet with new flavors while, at the same time, providing many health-enhancing natural ingredients.[2] The term superfruit is used in new marketing approach to promote the demand for rare fruits which can be consumed as foodstuffs or used as ingredients by manufacturers of functional foods, nutraceuticals, and beverages.

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However, an increase in the popularity of health-enhancing superfruits on the market depends heavily on both pertinent research results and appropriate marketing. Fruits which contain powerful bioactive compounds, characterized by high antioxidant capacity, such as polyphenols, anthocyanins, or procyanidins may be classified as superfruits. Considerable interest led to the increase in the number of research projects and publications focusing on the health benefits of superfruits.[3–6]

Superfruits contain a great number of bioactive compounds, especially terpenes.[7] Terpenes are the group of fruit-based compounds, with more than 40,000 known molecules from among which more than 400 are monoterpenes.[8] Many terpenes have bioactive properties, and they often determine the flavor and taste of fruits. Moreover, terpenes and terpenoids are the main components of essential oils.[9–12]

The volatile fraction of fruits was investigated by means of solid-phase microextraction (SPME), which is considered a suitable analytical technique for the extraction of flavor compounds. SPME meets the requirements of green analytical chemistry as developed by Arthur and Pawliszyn.[13] In recent years, solvent-free SPME has gained widespread acceptance regarding the analysis of semi-volatile and volatile food components, including bioactive compounds. Several authors conducted SPME experiments aimed at analyzing the composition of various fruits and investigated the volatile fruit components via SPME by using fused-silica fibres coated with divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) stationary phases.[14–19]

Due to the large number of volatile compounds and the complexity of investigated fruits, a novel tool was used to measure and compare the fruits' aromas, namely, comprehensive multidimensional gas chromatography (GC×GC). The time-of-flight mass spectrometry (TOFMS) was applied to identify the components (e.g., terpenes) present in complex fruit samples (see Fig. 1). In the past few years, GC×GC has been shown to provide the capability to considerably improve the analysis of complex samples.[20–24]

In this article, the profiles of volatile terpenes identified in four different potential superfruits are presented for the first time, which is the element of scientific novelty. In order to determine the highest possible number of bioactive compounds in potential superfruits, the innovative technique of headspace solid-phase microextraction coupled with comprehensive two-dimensional gas chromatography with time-of-flight mass spectrometry (HS-SPME/GC×GC-TOFMS) was

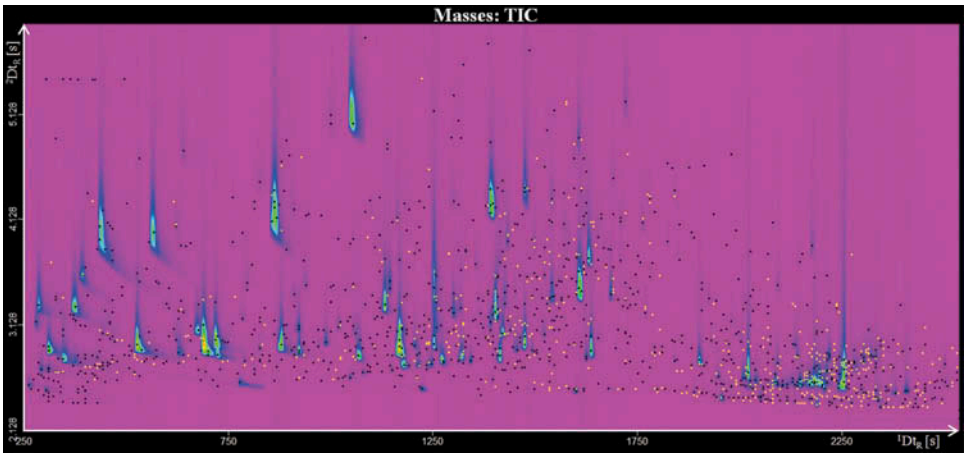

FIGURE 1 Chromatogram of volatile organic compounds of cape gooseberry obtained by GC×GC-TOFMS.

performed. The study demonstrated the potential of GC×GC-TOFMS method regarding the trace-level determination of terpene compounds in fruit extracts.

EXPERIMENTAL

Chemicals

All standards, i.e., (-)-β-pinene (≥99%), (+)-α-pinene (≥99%), geraniol (≥97%), eucalyptol (≥99%), (+)-α-terpineol (97%), (-)-menthol (≥99%), myrcen (≥90%), (-)-linalool (≥98%), (-)-camphor (≥99%), citral (≥96%), (R)-(+)-limonene (≥99%), eugenol (≥98%), *p*-cymene (99%), ocimene (≥90%), terpinolene (≥90%), γ-terpinene (97%), camphene (analytical standard), β-cyclocitral (≥90%), (-)-fenchone (≥98%), α-phellandrene (halal grade), and alkane standard solution C8-C20) were purchased from Sigma-Aldrich. Sodium chloride (ACS grade), methanol (99.8%) and ethanol (96%) were obtained from Avantor Performance Materials Poland S.A.

Identification of Analytes

For the standard mixture of terpenes, a 10 mL or 10 mg aliquot of the standard substance (depending on its physical phase) was transferred to a flask, and then topped up to 10 mL with methanol. The obtained solution was diluted to the needed concentrations of 10 and 0.1 ppm. A stock solution of *n*-alkanes was prepared in a 10-mL flask in methanol which was subsequently diluted to obtain the solutions with the following concentrations: 100, 1, and 0.1 ppm. The standard solution of terpenes was prepared in methanol in a 10-mL flask.

Samples

The samples of four fruit species harvested in 2012, namely, cape gooseberry (*Physalis peruviana*; imported from Chile) cherry silver berry (*Elaeagnus multiflora*), crabapple (*Malus baccata*), and scarlet hawthorn (*Crategus coccinea*), delivered from the northern Poland (Osielsko), were analyzed. Prior to analysis, the samples of fruits were stored in the freezer at –35°C. Before the analyte extraction step, the fruits were pureed (calyx removed) by using a hand-held blender. All fruit samples reached the room temperature before proceeding with the SPME extraction (Fig. 2).

Isolation of Volatile Compounds from Fruit Samples by Using HS-SPME

Automated HS-SPME was performed with an autosampler (MPS, Gerstel). The SPME was fiber coated with DVB/CAR/PDMS, (50/30 µm, 2 cm long), was purchased from Supelco (Bellefonte, PA, USA). Prior to analysis, 50 g of frozen fruit was thawed at 4°C and then blended for 1 min with the addition of 2 g NaCl. NaCl was added during the blending stage in order to prevent possible enzymatic reactions that can lead to the conversion of some volatile compounds to their derivatives.[25] Furthermore, the addition of NaCl causes the transport of analytes from the liquid phase to the sample headspace. The extraction of analytes was performed after a 25 min incubation (at 50°C) of 8 g samples of pureed fruits with 2 g of sodium chloride added in the 20 mL headspace vials crimped with Teflon-coated silicon rubber septa. Prior to sample extraction, the fibers were conditioned in a GC injector, according to the manufacturer's recommendations. The extraction parameters were chosen on the basis of published scientific reports.[14–16,26] Considering the results reported by other researchers, e.g., the determination of *Physalis peruviana* profile by Berger,[27] a 50/30 µm DVB/CAR/PDMS fiber coating was chosen for the extraction procedure.[24] Samples were

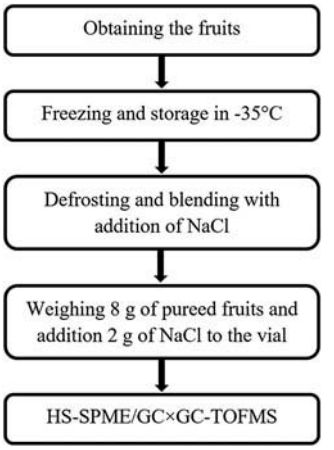

FIGURE 2 Diagram of the sample preparation procedure for the analysis of terpene profiles in fruits.

incubated at 50°C for 25 min with agitation at 700 rpm and then extracted at 50°C for 30 min. The fiber was thermally desorbed in the GC injector at 250°C for 3 min.

GC×GC Analysis

Experimental parameters for the blue honeysuckle berry analysis were established according to the methodology previously developed by the authors of this article.[24] The GC×GC system consisted of Agilent 7890A gas chromatograph coupled with time-of-flight mass spectrometer (TOF-MS, LECO, Pegasus 4D) equipped with an autosampler (Gerstel), splitless injector and a liquid nitrogen-based quad jet cryogenic modulator as a connector between two orthogonal columns. A nonpolar 1D column (Equity-1; 30 m × 0.25 mm, I.D. 0.25 μm) purchased from Supelco (Bellefonte, PA, USA), with 100% PDMS stationary phase, and a 2D column (BPX-50; 1.5 m × 0.1 mm, I.D. 0.1 μm) manufactured by SGE Analytical Science (Austin, TX, USA), with the stationary phase consisting of 50% diphenyl and 50% dimethyl polysilphenylenesiloxane, were used. The previously mentioned GC columns were placed in two different ovens. The temperature program for the 1D column was set as follows: a 3 min hold at 40°C; from 40–150°C at 5°C/min; from 150–250°C at 10°C/min; a 2 min hold at 250°C. The program of 2D column was as follows: a 3 min hold at 45°C; from 45 to 155°C at 5°C/min; from 155 to 255°C at 10°C/min; a 2 min hold at 255°C. Ultra-high purity (UHP) helium (6.0) was used as the carrier gas at a constant flow rate of 1.0 mL/min (the column head pressure 27.7 psi) with the modulation time set to 6s (1.2 s hot pulse). The TOFMS was operated at a spectrum storage rate of 125 Hz, using a mass range of *m/z* 33–400 and a voltage of –1600 V. The MS transfer line and ion source temperature was 250°C. The chromatograph and detector control, data collection, and processing were performed using LECO ChromaTOF software (4.44 version). The identification of terpenes was carried out by comparing the acquired spectra with the NIST 2.0 (2011) spectra and linear retention indices (LRI) database.

Data Analysis

Total ion chromatograms (TIC) were processed using the automated data processing software ChromaTOF (LECO version 4.44) at the S/N threshold of 100. The obtained contour plots were

used to evaluate the overall quality of separation and for manual peak identification. Mass spectral match factor (similarity > 700) was used to decide whether a given peak has been identified correctly. The tentative identification of terpenes was verified by comparing their LRI with the values found in the published literature. The GC×GC analysis of C8 – C20 *n*-alkanes series was performed in order to calculate LRI by using the van den Dool and Kratz equation.[28] Moreover, the identification of some terpenes was confirmed by comparing their LRI and mass spectra with those of applied standards. All samples were analyzed four times. The calculations were performed using Excel 2010, Microsoft Office 2010.

RESULTS AND DISCUSSION

The preliminary identification of terpenes was based on the comparison of the obtained spectra with the NIST spectra database and by comparing the experimental LRI with the pertinent values found in NIST webbook database. The final identification of some compounds was performed by using 20 standards. Many of the identified terpenes were present in all analyzed fruits, although the volatile components unique to specific samples were also observed. Two-dimensional GC allows for obtaining a complete separation of components present in the food samples characterized by complex composition and co-elution ability of compounds with similar chemical properties. Figure 1 presents a chromatogram of compounds with the same retention time in one dimension, but quite another in the second dimension. It proves the necessity of GC×GC application instead of classical GC technique in which such compounds cannot be separated.

Identification of Terpenes

The terpene profiles were determined for four potential superfruits. The obtained results are summarized in Table 1. A total of 79 terpene compounds were separated and identified, including 18 monoterpene hydrocarbons, 23 monoterpenols, 8 monoterpene ketones, 3 monoterpene oxides, 1 monoterpene phenol, 5 monoterpene aldehydes, 1 monoterpene acids, 9 sesquiterpenes, 8 sesquiterpenoids, 2 sesquiterpen oxides, and 1 hemiterpene. A semi-quantitative TIC-based analysis was performed.

Comparison of Terpene Profiles of Four Analyzed Fruits

Overall, 80 terpenes were identified of which 62 were found in cape gooseberry, 38 in crabapple, 27 in cherry silver berry, and 29 in scarlet hawthorn. Thirteen of 62 terpenes identified in cape gooseberry fruits have already been reported;[27] i.e., α-pinene; β-pinene; myrcene; limonene; terpinolene; eucalyptol (as 1,8-cineol); terpinene-4-ol; α-terpineol; geraniol; β-cyclocitral; geranial; camphor; β-ionone. Also, in 2014, Yilmaztekin[29] determined 11 terpene compounds in the same fruit species. Information about the unique flavor, texture, and color of cape gooseberry and its potential use by the food, nutraceutical, and pharmaceutical industries can be found in the published literature.[30] The wide spectrum of applications in which *Physalis peruviana* can be used makes this fruit very important.

In 1996, Loughrin et al.[31] analyzed volatile compounds extracted from crabapple, however, the presence of only few terpenes in leaves was reported. Li et al.,[32] who analyzed volatile aroma compounds in crabapple, did not find any terpenes in the investigated material. In the reviewed literature no information was found about the chemical profiles of two other fruits that had been analyzed in this article.

Eleven of 79 identified terpenes were found in four analyzed fruits (Table 2). Moreover, 38 out of 79 identified terpenes were found only in one of the analyzed fruits (22 in cape gooseberry, 11

TABLE 1
Terpenes determined in fruits of cape gooseberry, crab apple, cherry silver berry, and scarlet hawthorn

Formula	*Name*	$LRI_{lit.}$	$LRI_{calc.}$	$^{1}Dt_R[s]$	$^{2}Dt_R[s]$	*CG*	*CA*	*CSB*	*SH*
Monoterpene hydrocarbons									
$C_{10}H_{16}$	β-Pinene*	981	**980**	1170	2,7	1,52%	—	—	0,33%
$C_{10}H_{16}$	α-Pinene*	937	**937**	1074	2,7	0,86%	0,30%	0,85%	0,49%
$C_{10}H_{16}$	Limonene*	1026	**1025**	1254	2,9	1,20%	0,16%	0,06%	—
$C_{10}H_{14}$	1,3,8-p-Menthatriene	1105	**1103**	1404	3,1	0,52%	<0,01%	—	0,02%
$C_{10}H_{18}$	β-Myrcene*	985	**985**	1176	2,8	0,16%	0,09%	1,20%	0,40%
$C_{10}H_{14}$	p-Cymene*	1036	**1026**	1356	3,2	1,01%	0,02%	0,01%	0,02%
$C_{10}H_{16}$	β-trans-Ocimene*	1045	**1041**	1284	2,8	—	0,17%	0,14%	—
$C_{10}H_{16}$	α-Phellandrene*	1003	**1003**	1212	2,8	0,25%	0,03%	2,72%	0,09%
$C_{10}H_{16}$	Terpinolene*	1084	**1084**	1368	3,0	0,09%	0,02%	0,07%	—
$C_{10}H_{16}$	γ-Terpinene*	1051	**1053**	1308	2,9	0,20%	0,01%	0,01%	—
$C_{10}H_{16}$	Camphene*	952	**954**	1110	2,7	0,54%	—	—	—
$C_{10}H_{16}$	α-Thujene	921	**923**	1176	2,7	<0,01%	—	0,08%	—
$C_{10}H_{16}$	β-Thujene	968	**951**	1104	2,8	0,34%	—	0,53%	—
$C_{10}H_{16}$	β-Phellandrene	1021	**1025**	1254	3,0	0,25%	—	—	—
$C_{10}H_{16}$	Cyclohexene, 1,5,5-trimethyl-3-methylen-	992	**991**	1188	2,8	0,26%	0,04%	0,03%	0,03%
$C_{10}H_{16}$	3-Carene, 2-(acetylmethyl)-	1390	**1399**	1896	2,7	—	0,02%	—	—
$C_{10}H_{16}$	4-Carene, 2-(acetylmethyl)-	1382	**1383**	1782	2,8	—	—	—	0,02%
$C_{10}H_{16}O$	β-Cyclocitral*	1215	**1208**	1584	3,2	0,41%	0,08%	0,17%	0,09%
Monoterpenols									
$C_{10}H_{18}O$	Eucalyptol*	1030	**1028**	1260	3,0	0,18%	0,01%	—	0,05%
$C_{10}H_{20}O$	Menhtol*	1150.4	**1169**	1518	3,0	0,35%	—	0,02%	—
$C_{10}H_{20}O$	Isomenthol	1164	**1166**	1512	3,1	—	0,01%	—	—
$C_{10}H_{18}O$	4-Terpineol	1172	**1172**	1524	3,2	0,29%	0,02%	0,09%	0,03%
$C_{10}H_{18}O$	Linalool*	1087	**1087.5**	1374	3,0	0,49%	0,14%	0,49%	0,41%
$C_{10}H_{18}$	Geraniol*	1237	**1240**	1632	2,9	0,05%	0,05%	—	—
$C_{10}H_{18}O$	α-Terpineol*	1182	**1183**	1542	3,3	0,01%	0,04%	0,24%	<0,01%
$C_{10}H_{12}O_2$	Eugenol*	1345	**1337**	1764	3,0	—	0,07%	—	—
$C_{10}H_{18}O$	Isocamphol	1175	**1186**	1548	3,2	0,01%	—	—	—
$C_{10}H_{18}O$	Isoborneol	1162	**1157**	1548	3,2	0,38%	—	—	—
$C_{10}H_{16}O$	Myrtenol	1190	**1190**	1554	3,2	0,04%	0,04%	—	—
$C_{10}H_{18}O$	cis-Thujan-4-ol	1098	**1094**	1386	3,2	0,08%	—	—	—
$C_{10}H_{18}O$	cis-Myrtanol	1238	**1252**	1650	3,1	0,01%	—	—	—
$C_{10}H_{16}O$	Verbenol	brak	**1162**	1506	3,2	—	0,04%	—	—
$C_{10}H_{16}O$	cis-Verbenol	1125	**1087.5**	1374	3,2	0,07%	—	—	—
$C_{10}H_{16}O$	Carveol	1192	**1197**	1566	3,2	—	0,10%	—	—
$C_{10}H_{20}O$	Citronellol	1212	**1212**	1590	2,9	0,06%	—	—	—
$C_{10}H_{18}O$	endo-Borneol	1162	**1162**	1506	3,3	0,38%	—	—	—
$C_{10}H_{18}O$	Isopulegol	1152	**1152**	1488	3,2	0,02%	—	—	—
$C_{10}H_{14}O$	p-Cymene-7-ol	1260	**1262**	1680	3,2	0,72%	—	—	—
$C_{10}H_{18}O$	trans-p-Menth-2-en-7-ol	1254	**1256**	1656	3,0	0,08%	—	—	—
$C_{15}H_{24}O$	cis-Lanceol	1480	**1470**	1992	2,7	0,02%	—	—	—
$C_{10}H_{18}O_2$	Lilac alcohol C	968	**963**	1128	2,7	—	0,17%	—	—
Monoterpene ketones									
$C_{10}H_{16}O$	β-Thujone	1089	**1069**	1338	2,7	0,34%	—	—	—
$C_{13}H_{20}O$	α-Ionone	1428	**1421**	1860	2,7	0,03%	0,60%	—	0,07%
$C_{10}H_{16}O$	Pinocamphone	1143	**1148**	1482	3,4	0,02%	0,02%	0,02%	0,01%
$C_{10}H_{16}O$	Camphor*	1127	**1131**	1452	3,6	0,29%	<0,01%	0,03%	0,03%
$C_{10}H_{14}O$	Carvone	1212	**1228**	1614	3,2	0,02%	—	0,02%	0,03%

(*Continued*)

TABLE 1
(*Continued*)

Formula	*Name*	$LRI_{lit.}$	$LRI_{calc.}$	$^{1}Dt_R[s]$	$^{2}Dt_R[s]$	*CG*	*CA*	*CSB*	*SH*
$C_{10}H_{16}O$	Pulegone	1211	**1222**	1506	3,2	<0,01%	—	—	—
$C_{10}H_{14}O$	Pinocarvone	1150	**1148**	1482	3,6	<0,01%	0,01%	—	<0,01%
$C_{10}H_{16}O$	Fenchone*	1080	**1075**	1350	3,3	0,17%	—	—	0,05%
Monoterpene oxides									
$C_{10}H_{16}O_2$	p-Mentha-2,8-diene, 1-hydroperoxide	1324	**1337**	1764	2,9	0,02%	—	—	—
$C_{10}H_{12}O_2$	Linalool oxide	1078	**1078**	1356	3,0	0,12%	0,14%	0,02%	—
$C_{10}H_{18}O$	trans-Rose oxide	1115	**1117**	1428	3,0	0,02%	—	0,09%	0,04%
Monoterpene phenols									
$C_{10}H_{14}O$	Thymol	1267	**1272**	1680	3,0	—	—	—	0,02%
Monoterpene aldehydes									
$C_{10}H_{16}O$	Citral*	1222	**1224**	1608	3,0	0,02%	0,01%	0,17%	—
$C_{10}H_{16}O$	Geranial	1270	**1272**	1650	3,0	0,19%	0,03%	0,69%	—
$C_{10}H_{16}O$	α-Campholenal	1115	**1110**	1416	3,3	0,01%	—	—	—
$C_{10}H_{18}O$	Citronellal	1132	**1134**	1458	3,1	—	0,01%	—	—
$C_{10}H_{16}O$	Carvenone	1277	**1277**	1638	3,2	0,06%	—	—	0,04%
Monoterpene acids									
$C_{10}H_{16}O_2$	Geranic acid	1333	**1332**	1758	2,8	—	0,01%	—	—
Sesquiterpenes									
$C_{15}H_{22}$	β-Vatirenene	1452	**1441**	1926	2,6	0,09%	—	0,02%	—
$C_{15}H_{24}$	β-Copaene	1418	**1420**	1938	2,8	0,01%	0,01%	—	—
$C_{15}H_{24}$	β-Curcumene	1510	**1451**	1950	2,5	0,02%	—	—	—
$C_{15}H_{24}$	α-Muurolene	1480	**1478**	1944	2,6	—	0,01%	—	—
$C_{15}H_{20}$	α-Calacorene	1517	**1465**	1980	2,7	0,34%	0,02%	—	<0,01%
$C_{15}H_{22}$	trans-Calamenene	1450	**1457**	1962	2,7	—	0,01%	—	—
$C_{15}H_{24}$	trans-α-Bergamotene	1405	**1394**	1830	2,5	—	—	—	0,11%
$C_{15}H_{24}$	Aristolene	1423	**1389**	1824	2,5	—	—	0,01%	—
$C_{15}H_{24}$	Aromadendrene	1447	**1484**	2022	2,6	0,01%	0,05%	—	—
Sesquiterpenoids									
$C_{15}H_{24}$	β-Caryophyllene	1412	**1416**	1872	2,6	0,12%	—	—	0,02%
$C_{15}H_{24}$	γ-Caryophyllene	1419	**1411**	1860	2,6	0,04%	—	—	—
$C_{15}H_{24}$	β-Ylangene	1442	**1441**	1926	2,5	0,01%	—	—	0,02%
$C_{15}H_{24}$	α-Ylangene	1380	**1389**	1824	2,5	<0,01%	—	—	—
$C_{15}H_{24}$	α-Farnesene	1490	**1490**	1938	2,5	0,27%	—	0,01%	0,02%
$C_{15}H_{24}$	β-Farnesene	1445	**1440**	1950	2,6	—	0,53%	—	—
$C_{15}H_{24}$	Humulene	1429	**1432**	1908	2,6	0,47%	—	—	—
$C_{15}H_{24}$	α-Cubebene	1349	**1363**	1794	2,5	0,14%	—	—	—
Sesquiterpen oxides									
$C_{15}H_{24}O$	Ledene oxide-(II)	1490	**1481**	2016	2,7	0,10%	—	<0,01%	—
$C_{15}H_{24}O$	Caryophyllene oxide	1547	**1505**	2016	2,7	0,04%	—	—	0,02%
Hemiterpenes									
$C_{10}H_{16}$	Santolina triene	1089	**1084**	1368	3,0	—	—	—	0,01%
Total						13,79%	3,08%	7,81%	2,49%

*Terpenes confirmed by using authentic standards.

LRI_{calc}: Linear Retention Index calculated; LRI_{lit}: Linear Retention Index reported in the literature for 100% poly (dimethyl siloxane) phase GC column or equivalents; $1Dt_R[s]$: retention time in first dimension; $2Dt_R[s]$: retention time in second dimension; CG: cape gooseberry; CA: crab apple; CSB: cherry silver berry; SH: scarlet hawthorn.

TABLE 2
Determined terpenes characteristics for each fruits and common to all

Terpenes \ *Fruits*	*Cape gooseberry*	*Crab apple*	*Cherry silver berry*	*Scarlet hawthorn*
Camphor	+	+	+	+
p-Cymene				
β-Cyclocitral				
Cyclohexene, 1,5,5-trimethyl-3-methylen-				
Linalool				
β-Myrcene				
α-Phellandrene				
α-Pinene				
trans-3-Pineone				
α-Terpineol				
4-Terpineol				
Aristolene			+	
trans-α-Bergamotene				+
4-Carene, 2-(acetylmethyl)-				+
Santolina triene				+
Thymol				+
trans-Calamenene		+		
3-Carene, 2-(acetylmethyl)-		+		
Carveol		+		
Citronellal		+		
Eugenol		+		
β-Farnesene		+		
Geranic acid		+		
Isomenthol		+		
Lilac alcohol C		+		
α-Muurolene		+		
Verbenol		+		
endo-Borneol	+			
Camphene	+			
α-Campholenal	+			
γ-Caryophyllene	+			
Citronellol	+			
α-Cubebene	+			
β-Curcumene	+			
p-Cymene-7-ol	+			
Humulene	+			
Isoborneol	+			
Isocamphol	+			
Isopulegol	+			
cis-Lanceol	+			
trans-p-Menth-2-en-7-ol	+			
p-Mentha-2,8-diene, 1-hydroperoxide	+			
cis-Myrtanol	+			
β-Phellandrene	+			
Pulegone	+			
cis-Thujan-4-ol	+			
β-Thujone	+			
cis-Verbenol	+			
α-Ylangene	+			

in crabapple, 1 in cherry silver berry, and 4 in scarlet hawthorn). It is noticeable that the chemical profile of cape gooseberry is the most diverse as 22 out of 79 terpenes were identified only in this fruit. The above findings allow us to assume that the identified compounds may be a characteristic feature in the case of fruits of defined origin and species; however, further studies in this field are necessary.

Based on the obtained results, it can also be assumed that the fruits of boreal zone (such as crabapple, cherry silver berry, and scarlet hawthorn) have a poorer profile of compounds from the class of terpenes than tropical fruits (such as cape gooseberry). However, in order to confirm this hypothesis, further research on a larger number of fruits from different climate zones is needed.

Among all the determined terpenes, five were found at the concentration level above 1% (based on relative peak areas), namely, α-phellandrene, β-myrcene, ρ-cymene, β-pinene, and limonene. Information about health-enhancing properties of these compounds can be found in the published literature.[33–48] Although α-phellandrene and β-myrcene were found in all the investigated fruits, the highest content of these compounds was measured in cherry silver berry fruits. The two previously mentioned terpenes have proven antioxidant properties.[33] Moreover, α-phellandrene displays antimicrobial, antifungal, and anti-inflammatory activities.[34,35] In the case of ρ-cymene, β-pinene, and limonene,the highest contents of these compounds were determined in cape gooseberry fruits, while β-pinene was only found in cape gooseberry and scarlet hawthorn. All the of the previously mentioned terpenes display antioxidant properties. Additionally, β-pinene has antimicrobial, antibacterial, and anticancer properties,[36–39] while limonene is characterized by antimicrobial, antidiabetic, and antifungal activities.[40–44] On the other hand, ρ-cymene displays antibacterial, antinociceptive, and anti-inflammatory[45–48] properties.

Comparison of Terpene Contents in Other Fruits

Information about the profiles of volatile compounds in various fruits, including some terpenes, can be found in the published literature. Shashikeira et al.[49] analyzed the flavor compounds present in custard apple. In the case of nine terpenes identified in custard apple (i.e., α-pinene; β-pinene; eucalyptol [as 1,8-cineol]; limonene; linalool; α-cubebene; caryophyllene; aromadendrene; α-farnesene), their presence was also determined in the fruits analyzed in this study. Ferreira et al.[50] determined α-pinene, β-pinene, linalool, caryophyllene, and α-terpineol in cherimoya fruits. In comparison to terpene profiles of peaches and nectarines,[51] eight determined terpenes were the same (limonene; eucalyptol; ocimene; linalool; camphor; α-terpineol; β-cyclocitral; α-ionone). Pereira et al.[52] determined β-myrcene, limonene, ocimene, α-bergamotene, β-caryophyllene, α-cubebene, and β-farnesene in the samples of avocado. Twelve terpenes (i.e., myrcene; α-phellandrene; β-phellandrene; α-terpinene; γ-terpinene; *p*-cymene; limonene; terpinolene; terpineol; caryophyllene; aromadendrene; α-humulene) were determined in the fruits of *Mangifera indica var. coquinho*.[53] Also, Pereira et al.[54] analyzed selected fruits originating from Madeira such as, kiwi, plum, papaya, and lemon. The identified terpenes, which were mainly detected in lemon, included β-pinene, β-myrcene, limonene, γ-terpinene, terpinolene, *p*-cymene, linalool, bergamotene, α-terpineol, and geranial. The volatile constituents of exotic fruits from Brazil were determined by Bicas et al.[55] The analyzed fruits and identified terpenes (listed in brackets) are as follows: Brazilian cherry (trans-β-ocimene and β-pinene), acerola (limonene), starfruit (α-limonene), and the fruits of the genera *Annona* (eucalyptol as 1,8-cineole, linalool, α-pinene, limonene, α-phellandrene, β-ocimene, β-pinene), and *Sponolias* (caryophyllene, myrcene, β-phellandrene, limonene, *p*-cymene, α-pinene). The main components determined in *Myristica fragrans* were sabinene, α-pinene, β-pinene, and terpene-4-ol.[56] Because of different selectivity and sensitivity of the methods applied by the cited

authors, it is difficult to compare the overall terpene profile obtained in this work with the profiles of other fruits reported in the scientific literature published until now.

CONCLUSIONS

GC×GC-TOFMS with ChromaTOF software is a promising tool for the determination of terpene profile of superfruits. The HS-SPME technique offers the advantages of shorter isolation time and meeting the requirements of "green chemistry." HS-SPME was shown to be a fast and reliable extraction process and a valuable alternative for traditional methods for establishing the volatile profile of four selected fruits by using the HS-SPME/GC×GC-TOFMS methodology. Furthermore, this method enables the composition analysis of samples with complex matrix and is characterized by improved sensitivity and selectivity, i.e., the methodology allowed the identification of 79 terpenes. The analysis of four potential superfruits showed that their terpene profiles are very diverse. Moreover, most of the terpenes identified in this study should be considered novel volatiles found in those fruits, while the terpene profiles presented here are published for the first time. The obtained results allow us to classify, on preliminary basis, the four analyzed fruits as superfruits, which is a novel approach to the subject of research. Based on the obtained data, it can be assumed that cape gooseberry has the greatest potential to be classified as superfruit because the largest number of terpenes had been determined in this fruit species. Also, another advantageous properties of this fruit are its exotic origin, interesting taste, smell, and appearance. The growing market for healthy fruits and fruit juices stimulates the scientific community to deliver to the general public the complete chemical characterization of pertinent fruits. In this context, the composition of terpene fraction of fruits that had not yet been chemically analyzed is important with regard to their application in the food, pharmaceutical, and cosmetic industries.

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