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Direct SPME/GC-MS analyzes of small citrus fruits cultivated in Turkey

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Abstract

A headspace-solid phase microextraction (HS-SPME) procedure for isolation and investigation of volatile compounds in hybrid “limequat” and parents “Mexican lime and kumquat” fruits using Gas Chromatography-Mass Spectrometry (GC-MS) for the separation and identification of the volatiles was employed in the present study. Each of mature, fresh and whole fruits was freeze-dried, and each sample was powdered. In all samples, limonene (29.9-46.6%) was the most abundant monoterpene hydrocarbon among the identified monoterpenes. The common characteristic compounds are critically discussed.

Keywords

GC-MS, HS-SPME, kumquat, limequat, limonene, Mexican lime, volatile compounds.

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INTRODUCTION

Essential oils are aromatic oily liquids characterized by strong odors and produced by different methods such as distillation and expression in the case of citrus fruits (Baser and Demirci, 2011). Essential oils are low volume-high value products used in different industrial applications such as food, food ingredients, cosmetic and pharmaceutical preparations. Essential oils, their fractions and natural and/or synthetic aromachemicals are primarily used in the perfumery, flavor and fragrance industries (Palazzolo *et al.,* 2013; Gonzalez-Mas *et al.,* 2019).

Distillation (steam, water and water/steam distillation), cold-pressing (for essential oils), organic solvent extraction (pentane, diethyl ether, *n*-hexane *etc*), simultaneous distillation and extraction (Likens-Nickerson), microwave-assisted distillation/extraction, ultrasonic extraction and supercritical fluid extraction can be employed to isolate essential oils and/or aromatic extracts from plant matrices. European Pharmacopoeia 9.0 defines essential oils as products of distillation or cold-pressing (EP, 2016). Headspace trapping techniques are also used to extract volatile compounds from live plants. One of the most frequently used techniques is headspace-solid phase microextraction (HS-SPME). This technique is employed to isolate volatiles and semi-volatile compounds from materials. It is a versatile, efficient and frequently-used technique in the last few decades for sampling volatiles without using any solvents. Sample preparation is based on sorption of analytes from a sample onto a coated fused silica fiber that is mounted in a specially designed GC syringe. After introducing the coated fiber into a sample, the compounds to be analyzed are enriched according to their distribution coefficients and can be subsequently desorbed thermally from the coating after introducing the fiber into the hot injector port of a gas chromatograph (Kubeczka, 2010). In summary, it integrates sampling, extraction, concentration and sample introduction into a single solvent free-step. Combinations with gas chromatography (GC) and gas chromatography/mass spectrometry (GC-MS) are successfully applied to a wide variety of compounds from environmental, biological and food samples (Kusch, 2017). In addition to its applications (HS-SPME), may enhance the detection limits in GC-MS and is favorable for comparative studies of samples that have similar matrices (Spietelun *et al.,* 2013). In the literature, SPME is the subject of numerous studies and is widely applied for sampling a broad spectrum of analytes from gaseous, liquid, and solid media with diverse matrix compositions (Pini *et al.,* 2004; Stashenko *et al.,* 2006; Perestrelo *et al.,* 2009; Xu *et al.,* 2016; Raza *et al.,* 2019; Song *et al.,* 2019).

Hybridization plays an important role in the evolution of many lineages. In natural populations, hybridization may act in opposition to divergence, introduce adaptive variation into a population, drive the evolution of stronger reproductive barriers, or generate new lineages (Goulet *et al.,* 2017). The study of hybridization in plants has a long history. Among the plant kingdom, *Citrus* products have become one of the world's most economically important fruit crops through a largely obscure history of evolution and domestication. Diversity among *Citru*s and related genera seems to offer opportunities to create new and improved types of *Citrus*. The genus originated in a wide area spanning North-Eastern India to South China and South East Asia is thought to have been cultivated for thousands of years (Butelli *et al.,* 2019). Most of the current cultivars must have been originated from natural or induced mutations rather than from sexual breeding that is considered difficult for most *Citrus* species (Bona *et al.,* 2011). Hybridization of *Citrus* affects secondary metabolites' numbers, diversities and quantities (Kamiya *et al.,* 1979; Shaw *et al.,* 2001; Borges and Pio, 2003; Barboni *et al.,* 2009; Bassene *et al.,* 2009). Essential oils are specific to varieties and taxonomic groups. These oils are often used in comparative studies to assess the genetic diversity of a species, quantify the relationships between varieties or species, and classify the unknown on the basis of discriminating compounds (*e.g.,* in mandarin, kumquat, grapefruit, lemon, lime and citron) (Lota *et al.,* 2002; Luro *et al.,* 2012; Liu *et al.,* 2013; Guney *et al.,* 2015; Sutour *et al.,* 2016). Moreover, essential oils are used to characterize hybrid fruits from their parents' fruits. A few studies found in the literature concluded that the essential oil composition of a hybrid differed from those of the parents. The hybrid fruits were found to have characteristic volatile compounds (Shaw *et al.,* 2001; Barboni *et al.,* 2009; Bassene *et al.,* 2009).

Because of the worldwide economical and nutritional importance of *Citrus* fruits, farmers in Turkey started to cultivate many citrus varieties. As a result of hybridization studies, new citrus varieties are increasingly cultivated. Most of the commonly available and consumed citrus fruits, among others like lemon, orange, mandarin and grapefruits are hybrid fruits. Recently, Mexican lime (*Citrus aurantifolia* (Christm.) Swingle) and kumquat (*Fortunella japonica* Swingle) fruits have started to be cultivated in Turkey. Kumquats (*Fortunella* spp.) are the smallest species compared to the other citrus species and grow naturally in China. Kumquat essential oils have flavor characters of typical citrus (Guney *et al.*, 2015). *Citrus aurantifolia* is a polyembriyonic species cultivated across the globe, mostly in hot subtropical or tropical regions, such as southern Florida, India, Mexico, Egypt, Iran and the West Indies. The juice and essential oil are the major commercial products (Spadaro *et al.*, 2012). In Cukurova Region, Turkey limequat has been produced as a bigeneric hybrid of *C. aurantifolia* and *F. japonica*. Limequat is similar in size and shape to kumquat but it is closer to Mexican lime in smell and taste. It can be consumed whole or its juice and rind can be used to flavor drinks and foods. It can be made into jams, jellies and preserves (Lim, 2012).

Evaluation of the composition of citrus hybrids may be more challenging because of the contribution of the traits of both parents. In the present study, headspace-SPME coupled with gas chromatography/mass spectrometry was used to characterize the volatile compound of the hybrid fruit “limequat” as well as its parent fruits “Mexican lime” and “kumquat” that were cultivated in Turkey.

MATERIALS AND METHODS

Plant material

Mature fruits were harvested from Subtropical Fruits Research and Experimental Center at Cukurova University, Adana, Turkey, in December 2017.

Fruit powder preparation

The sliced fresh and mature fruits were directly freeze dried for 48 hours. The obtained powders were passed through a 100 mesh sieve.

Isolation of the fruit volatiles

To trap volatile compounds in the fruit powders, Headspace-SPME (Headspace-Solid Phase MicroExtraction) technique was employed. The manual SPME device (Supelco, Bellafonte, PA, USA) with a fiber precoated with a 65 μm thick layer of polydimethylsiloxane/divinylbenzene (PDMS/DVB-blue) was used for extraction of the volatiles. The vial containing each fruit powder was sealed with parafilm. The fiber was pushed through the film layer for exposure to the headspace of the powder for 15 minutes at 40°C. The fiber was then inserted immediately into the injection port of the GC-MS for desorption of the adsorbed volatile compounds for analysis.

Analyzes of the fruit volatiles by GC-MS

The GC-MS analyses were carried out using an Agilent 5975 GC-MSD system. An Innowax fused silica capillary (FSC) column (60 m × 0.25 mm, 0.25 µm film thickness) was used with helium as the carrier gas (0.8 mL/min). Oven temperature was kept at 60°C for 10 minutes, then programmed to 220°C at a rate of 4°C/min, and maintained constant at 220°C for 10 minutes. Finally, oven was programmed to 240°C at a rate of 1°C/min. Injector temperature was set at 250°C. Split flow was adjusted at 50:1. Mass spectra were recorded at 70 eV with the mass range *m/z* 35–450.

Identification of volatile compounds

Individual components were identified by computer matching with commercial mass spectral libraries (Wiley GC-MS Library, MassFinder 3 Library) and in-house “Baser Library of Essential Oil Constituents”, which includes over 3200 authentic compounds with Mass Spectra. Retention data of pure standard compounds and components of known oils as well as MS literature data were also used for the identification (McLafferty and Stauffer, 1989; Joulain and Koenig, 1998; ESO 2000; Koenig *et al.,* 2004). These identifications were accomplished by comparing the retention times with authentic samples or by comparison of their relative retention indices (RRI) to a series of *n*-alkanes (Curvers *et al.,* 1985).

RESULTS AND DISCUSSION

Totally 16 monoterpenes, 42 sesquiterpenes, 23 oxygenated monoterpenes, 10 oxygenated sesquiterpenes and 10 miscellaneous compounds were characterized by GC-MS analysis. Table 1 shows the volatile compounds that were characterized in all of the fruits. As expected, all of the fruits were found to be rich in monoterpenes. In general, the groups in the fruits were as follows: Monoterpenes (48.3-72.0%) > sesquiterpenes (11.6-32.1%) > oxygenated monoterpenes (5.6-13.0%) > oxygenated sesquiterpenes (<1.0). Terpenoids are important due to their wide range of chemical and biological properties. Sesquiterpenes and their oxygenated analogues act as flavors and fragrances, pheromones, plant defense chemicals, or antibiotics (Schmidt *et al.,* 1999).

The genus *Citrus* has important crops like orange, lemons, grapefruits, limes and pummelos. *Citrus* essential oils that have been used widely in many applications over the years, include some major terpenes with biological activities like limonene, linalool, α-/β-pinene, sabinene, β-myrcene, humulene and α-terpineol. *Citrus* fruits are accepted as a major source of limonene (Mahato *et al.,* 2019; Simeone *et al.,* 2020).

The present study agreed with the literature as limonene was the most abundant monoterpene hydrocarbon among the identified monoterpenes. D-limonene is one of the most common monoterpenes in nature and a major constituent in several citrus oils. Limonene is a colorless liquid and exists as two enantiomers (D- or L-limonene) or as a racemic mixture. It possesses a pleasant lemon-like odor. Therefore, it is used widely as a flavor and fragrance additive in common food products. Besides its safety, it is an inexpensive fragrance for use in many products. Limonene was found to exert anti-inflammatory, anti-diabetic, anti-oxidant, anti-cancer, anti-allergic and anti-stress activities (Vieira *et al.,* 2018). Limonene containing essential oils and extracts may be promising dietary supplements. The present study suggested that kumquat, limequat and Mexican lime whole fruit powders may be used for different industrial purposes due to their rich limonene contents.

Other major compounds identified in fruits were germacrene D (6.5% of kumquat), linalool (4.9% of kumquat and 6.1% of limequat), β-bisabolene (4.6% of limequat), γ-terpinene (9.1% of Mexican lime) and *p*-cymene (10.4% of Mexican lime). Germacrene D is predicted as a precursor of many sesquiterpene hydrocarbons. It was shown that germacrene D undergoes acid-catalyzed cyclization yield cadinane, muurolane and amorphane sesquiterpenes (Bülow and König, 2000). Germacrene D's biological activity is on insects and other organisms (Silva *et al*., 2010). β-bisabolene, a sesquiterpene compound, has shown cytotoxic effects in recent studies. Yeo et al. (2015) found that β-bisabolene possessed tumour-specific pro-apoptotic properties in mouse and human breast cells both *in vitro* and *in vivo*. As a result, the researchers suggested further investigation of the use of β-bisabolene in the treatment of breast cancers (Yeo *et al.*, 2015).

When compared with conventional isolation techniques, HS-SPME is considered effective as it saves time and energy. HS-SPME is an eco-friendly technique because of its solvent free nature. For isolation and comparison of the volatiles of *Citrus* species and cultivars and/or detection of changes in volatile compounds during fruit growth, HS-SPME is a preferred technique. In addition, it is used to identify potential markers to that will help to distinguish geographical origin of fruits. The fingerprint analyzes of Pummelo cultivars in China (Zhang *et al.,* 2017), characterization of lemon wax volatiles in Italy (Costa *et al.*, 2019), characterization of orange cultivars including 137 samples from three different geographical origins, such as Italy, South Africa, and Spain (Centonze *et al.*, 2019), volatiles of Persian lime (*C. latifolia*) from Mexico (Ledesma-Escobar *et al.*, 2019), stability of orange essential oil/β-cyclodextrin inclusion complex (Kringel *et al.*, 2017) and characterization of chemical markers in satsuma mandarin (*C. unshiu* Marc.) honey (Jerkovic *et al.*, 2016) include the examples of which HS-SPME was used for the isolation of volatiles.

Table 1: The volatile compounds of kumquat, limequat and Mexican lime.

RRI	Compound	Kumquat	Limequat	Mexican lime	IM
1032	α-Pinene	tr	0.9	4.1	RRI, MS
1076	Camphene	nd	nd	1.6	RRI, MS
1118	β-Pinene	nd	0.6	5.2	RRI, MS
1174	Myrcene	2.1	1.2	2.0	RRI, MS
1188	α-Terpinene	nd	0.4	1.6	RRI, MS
1203	**Limonene**	46.6	37.0	29.9	RRI, MS
1218	β-Phellandrene	0.3	0.5	nd	RRI, MS
1224	*o*-Mentha-1(7)-5,8-triene	nd	nd	3.8	MS
1246	*(Z)*-β-Ocimene	tr	nd	0.2	MS
1255	γ-Terpinene	tr	3.3	9.1	RRI, MS
1266	*(E)*-β-Ocimene	0.6	nd	nd	MS
1280	*p*-Cymene	0.3	3.4	10.4	RRI, MS
1290	Terpinolene	tr	0.4	1.0	RRI, MS
1327	3-Methyl-2-butenol	tr	0.2	nd	MS
1348	6-Methyl-5-hepten-2-one	0.7	0.3	1.0	MS
1382	Alloocimene	0.1	tr	0.3	MS
1413	Rosefurane	nd	nd	0.2	MS
1450	*trans*-Linalool oxide (furanoid)	nd	0.4	0.1	MS
1452	α-*p*-Dimethylstyrene	0.1	0.6	2.6	MS
1466	α-Cubebene	2.0	0.8	nd	MS
1478	Furfural	nd	tr	0.1	MS
1478	*cis*-Linalool oxide (furanoid)	nd	0.4	nd	MS
1479	δ-Elemene	0.9	0.5	nd	MS
1483	Octyl acetate	1.0	nd	nd	RRI, MS
1493	α-Ylangene	0.6	1.3	0.2	MS
1495	Bicycloelemene	0.8	nd	nd	MS
1497	α-Copaene	1.5	0.5	0.1	MS
1506	Decanal	tr	tr	0.1	RRI, MS
1535	β-Bourbonene	0.1	nd	nd	MS
1544	α-Gurjunene	0.1	nd	nd	MS
1549	β-Cubebene	1.0	tr	nd	MS
1553	Linalool	4.9	6.1	1.4	RRI, MS
1562	Isopinocamphone	nd	nd	0.1	MS
1565	Linalyl acetate	1.9	0.4	0.2	RRI, MS
1568	1-Methyl-4-acetylcyclohex-1-ene	tr	0.1	nd	MS
1572	α-Bergamotene	tr	0.4	0.2	MS
1589	β-Ylangene	3.6	0.8	nd	MS
1594	*trans*-β-Bergamotene	tr	3.8	2.2	MS
1600	β-Elemene	0.5	0.4	nd	MS
1610	β-Copaene	2.7	0.7	nd	MS
1611	Terpinen-4-ol	tr	1.5	0.9	RRI, MS
1612	β-Caryophyllene	0.8	3.2	2.4	RRI, MS
1616	Hotrienol	nd	0.2	nd	MS
1617	6,9-Guaiadiene	tr	nd	nd	MS
1628	Aromadendrene	0.3	nd	nd	MS
1645	Cadina-3,5-diene	0.2	nd	0.2	MS
1650	γ-Elemene	0.9	1.6	nd	MS

1659	γ-Gurjunene	1.0	nd	nd	MS
1661	Alloaromadendrene	tr	nd	nd	MS
1661	*trans*-Pinocarvyl acetate	nd	nd	tr	MS
1664	*(Z)*-β-Santalene	nd	0.3	0.2	MS
1668	Citronellyl acetate	0.2	0.1	nd	RRI, MS
1668	*(Z)*-β-Farnesene	nd	0.3	tr	MS
1670	*trans*-Pinocarveol	nd	nd	tr	MS
1687	α-Humulene	0.3	0.6	0.3	RRI, MS
1688	Selina-4,11-diene	nd	nd	0.1	MS
1692	Bicyclosesquiphellandrene	0.8	0.1	nd	MS
1695	*(E)*-β-Farnesene	nd	0.2	0.1	MS
1704	γ-Muurolene	2.1	1.6	nd	MS
1706	α-Terpineol	0.5	3.3	1.5	RRI, MS
1707	δ-Selinene	0.3	0.9	0.6	MS
1719	Borneol	nd	tr	0.2	RRI, MS
1720	4,6-Guaiadiene	0.1	0.2	0.1	MS
1726	Germacrene D	6.5	0.6	nd	MS
1737	Neryl acetate	0.3	tr	nd	RRI, MS
1740	α-Muurolene	0.8	nd	nd	MS
1741	β-Bisabolene	nd	4.6	2.4	MS
1741	Geranial	nd	nd	0.5	RRI, MS
1742	β-Selinene	0.8	0.4	0.4	MS
1744	α-Selinene	0.6	0.6	0.6	MS
1751	Carvone	0.3	0.2	nd	RRI, MS
1757	Dihydrocarveol	0.1	nd	nd	MS
1758	*(E,E)*-α-Farnesene	nd	nd	1.0	MS
1765	Geranyl acetate	2.6	0.2	0.7	RRI, MS
1773	δ-Cadinene	1.3	0.7	0.1	MS
1776	γ-Cadinene	0.9	0.5	tr	MS
1784	*(E)*-α-Bisabolene	nd	0.1	tr	MS
1796	Selina-3,7(11)-diene	0.1	1.2	0.3	MS
1799	Cadina-1.4-diene	0.1	nd	nd	MS
1807	Perilla aldehyde	0.6	0.1	tr	MS
1811	α-Cadinene	0.2	0.1	nd	MS
1837	Eudesma-5,7(11)-diene	tr	0.2	0.1	MS
1853	Calamenene	0.2	0.1	nd	MS
1857	Geraniol	tr	tr	tr	RRI, MS
1864	*p*-Cymen-8-ol	nd	nd	tr	MS
1871	*p*-Mentha-1,8-dien-10-yl acetate	0.2	nd	nd	MS
1900	*epi*-Cubebol	tr	nd	nd	MS
1916	Perilla acetate	0.1	nd	nd	MS
1941	α-Calacorene	tr	0.1	nd	MS
1957	Cubebol	tr	nd	nd	MS
2037	Salvial-4(14)-en-1-one	0.1	tr	nd	MS
2050	*(E)*-Nerolidol	tr	0.1	nd	MS
2096	Elemol	tr	0.1	nd	MS
2144	Spathulenol	0.2	nd	nd	MS
2185	γ-Eudesmol	nd	tr	nd	MS
2232	α-Bisabolol	nd	tr	nd	MS
2250	α-Eudesmol	tr	0.1	nd	MS
2257	β-Eudesmol	tr	0.2	nd	MS
2300	Tricosane	tr	0.1	tr	RRI, MS
2500	Pentacosane	tr	0.3	tr	RRI, MS
2600	Hexacosane	tr	0.3	tr	RRI, MS
2700	Heptacosane	tr	0.4	tr	RRI, MS
2800	Octacosane	tr	0.4	tr	RRI, MS
2900	Nonacosane	tr	0.4	tr	RRI, MS
	TOTAL	95.9	91.6	90.4	

RRI: Relative retention indices calculated against *n*-alkanes; %: calculated from TIC data; tr: Trace (< 0.1 %)

nd: not detected. IM: Identification method based on the relative retention indices (RRI) of authentic compounds on the HP Innowax column; MS, identified on the basis of computer matching of the mass spectra with those of the Wiley and MassFinder libraries and comparison with literature data.

Kumquat fruits and volatile compounds

In kumquat fruits, 80 volatile compounds were identified representing 95.9% of the sample. The percentages of monoterpenes, sesquiterpenes, oxygenated mono- and sesquiterpenes were calculated as 50.1%, 32.1%, 11.6% and 0.3%, respectively. Limonene (46.6%), germacrene D (6.5%) and linalool (4.9%) were the main terpenic constituents followed by β-ylangene (3.6%), β-copaene (2.7%), geranyl acetate (2.6%), γ-muurolene (2.1%), α-cubebene (2.0%), linalyl acetate (1.9%), α-copaene (1.5%) and δ-cadinene (1.3%). Other characteristic compounds included octyl acetate (1.0%), γ-gurjunene (1.0%), α-muurolene (0.8%), bicycloelemene (0.8%), *(E)*-β-ocimene (0.6%), aromadendrene (0.3%), *p*-mentha-1,8-dien-10-yl acetate (0.2%), spathulenol (0.2%), β-bourbonene (0.1%), α-gurjunene (0.1%), dihydrocarveol (0.1%), cadina-1,4-diene (0.1%), and trace compounds (cubebol, alloaromadendrene, 6,9-guaiadiene).

The essential oil compositions of peel and fruit were reported by previous studies in different countries. The essential oil was characterized with very high limonene content.

Kumquat fruits cultivated in Italy were hydrodistilled by a Clevenger-type apparatus. The essential oil contained 31 compounds. Among them, limonene (91.51%) was the most abundant. Also, myrcene (2.9%), *cis*-muurola-4(14,5)-diene (1.6%), α-thujene (0.8%) and linalool (0.7%) were the other major compounds (Schirra *et al.*, 2008).

Nouri and Shafaghatlonbar (2016) reported that the peel essential oil comprised 51% of limonene and 12.1% of germacrene D.

Limequat fruits and volatile compounds

In limequat fruits, 75 volatiles were detected comprising 91.6% of the sample. General groups were classified as monoterpenes (48.3%), sesquiterpenes (27.4%), oxygenated monoterpenes (13.0%) and oxygenated sesquiterpenes (0.5%). Limonene (37.0%), linalool (6.1%) and β-bisabolene (4.6%) were found to be the major constituents. The other volatiles more than 1% were *trans*-β-bergamotene (3.8%), *p*-cymene (3.4%), γ-terpinene (3.3%), α-terpineol (3.3%), β-caryophyllene (3.2%), γ-elemene (1.6%), terpinen-4-ol (1.5%) and selina-3,7(11)-diene (1.2%). *cis*-linalool oxide (furanoid) (0.4%), hotrienol (0.2%), γ-eudesmol (<0.1%) and α-bisabolol (<0.1%) were only found in limequats. Bigeneric hybrid limequat has common volatile compounds with its parents. Ten compounds were common in limequat and Mexican lime. Among these compounds, β-pinene (0.6%, 5.2%), α-terpinene (0.4%, 1.6%), as β-bisabolene (4.6%, 2.4%) were major compounds. Twenty-four common volatile compounds were detected in limequat coming from kumquat. Germacrene D (6.5%, 0.6%), γ-muurolene (2.1%, 1.6%), γ-elemene (0.9%, 1.6%), β-copaene (2.7%, 0.7%), β-ylangene (3.6%, 0.8%) and α-cubebene (2.0, 0.8%) were the common major constituents.

In a study carried by Guney *et al.* (2015) investigated on five kumquat species (*F. hindsii, F. crassifolia, F. obovata, F. margarita, C. aurantifolia* x *F. japonica*) cultivated in Turkey, each fruit was homogenized with a blender and an automatic headspace technique was used to trap volatile compounds. Limequat was characterized with its high limonene (88.7%) content and β-myrcene (2.5%). Interestingly, when compared to the present study, linalool was not detected and the amount of β-bisabolene (0.8%) was very low. The present study agreed with Guney *et al.* that limequat fruits were rich in terpenic compounds.

Eun-Jin *et al.* (2010) analyzed the peel essential oil of *F. japonica* var. *margarita* native to Island of Jeju, Korea. Limonene (61.6%) and carvone (6.4%) were found to be major compounds.

Mexican lime and volatile compounds

In Mexican lime fruits, 58 volatile compounds were detected representing 90.4% of the sample. Monoterpene content (72.0%) was high in the fruits followed by sesquiterpenes (11.6%) and oxygenated monoterpenes (5.6%) while no oxygenated sesquiterpenes were detected. Monoterpenes like limonene (29.9%), *p*-cymene (10.4%), γ-terpinene (9.1%) and β-pinene (5.2%) were the main constituents. α-pinene (4.1%), *o*-mentha-1(7)-5,8-triene (3.8%), α-*p*-dimethylstyrene (2.6%), β-bisabolene (2.4%), β-caryophyllene (2.4%), *trans*-β-bergamotene (2.2%), α-terpineol (1.5%) and linalool (1.4%) were among the major constituents. *o*-Mentha-1(7)-5,8-triene (3.8%), camphene (1.6%), *(E,E)*-α-farnesene (1.0%), geranial (0.5%), rosefurane (0.2%), isopinocamphone (0.1%), selina-4,11-diene (0.1%), *p*-cymen-8-ol (<0.1%), *trans*-pinocarveol (<0.1%) and *trans*-pinocarvyl acetate (<0.1%) were only detected in Mexican lime fruits.

CONCLUSION

In conclusion, the volatile compounds of kumquat, limequat and Mexican lime fruit powders isolated by HS-SPME coupled with GC-MS were identified. Totally, a hundred and four volatile compounds comprising mono-/sesquiterpenes, oxygenated mono-/sesquiterpenes were identified in the fruits. Monoterpenes were dominant groups in the fruits with different percentages. Limonene was the abundant monoterpene with 46.6%, 37.0% and 29.9% of the kumquat, limequat and Mexican lime fruits, respectively.

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