

High-Quality Essential Oils Extracted by an Eco-Friendly Process from Different Citrus Fruits and Fruit Regions

Rosaria Ciriminna,[†] Alexandra Fidalgo,[‡] Riccardo Delisi,[†] Diego Carnaroglio,^{§,||} Giorgio Grillo,^{||} Giancarlo Cravotto,^{||} Alfredo Tamburino,[⊥] Laura M. Ilharco,^{*,‡} and Mario Pagliaro^{*,†} 

[†]Istituto per lo Studio dei Materiali Nanostrutturati, CNR, via U. La Malfa 153, 90146 Palermo, Italy

[‡]Centro de Química-Física Molecular and IN-Institute of Nanoscience and Nanotechnology, Instituto Superior Técnico, University of Lisboa, Complexo I, Avenida Rovisco Pais 1, 1049-001 Lisboa, Portugal

[§]Milestone Srl, via Fatebenefratelli 1, 24010 Sorisole (BG), Italy

^{||}Dipartimento di Scienza e Tecnologia del Farmaco, Centre for Nanostructured Interfaces and Surfaces, Università degli Studi di Torino, 10124 Torino, Italy

[⊥]Legambiente, via Salaria 403, 00199 Rome, Italy

Supporting Information

ABSTRACT: Essential oils (EOs) were extracted from three types of citrus fruits (orange, lemon, and grapefruit) grown in Sicily and harvested in December or early January, considered to be the best picking season. Different fruit parts were used as sources for EO isolation: outer skin (exocarp), peel (exo- and mesocarp), and waste (exo-, meso-, and endocarp). The extraction was performed by a solvent-free microwave extraction (SFME) process. Very high yields were obtained, in comparison to other industrial methods (0.4% vs 0.05–0.25%). Diffuse reflectance infrared Fourier transform (DRIFT) spectroscopy was used to assess the distribution of the main constituents of the EOs. A number of compounds were identified in the EOs, with limonene being the major constituent (50–80%) in most cases. Orange oils proved to be richer in β -myrcene, whereas lemon oils have γ -terpinene and higher contents of β -pinene. Lemon provides more valuable EOs, with higher amounts of oxygenated compounds, although orange and grapefruit oils are richer in citral isomers. Generally, EOs extracted from the exocarp contain higher amounts of oxygenated monoterpenes, whereas those extracted from the exo-/mesocarp are richer in flavone and furanocoumarin derivatives.

KEYWORDS: Citrus, Essential oil, Microwave-assisted extraction, Green extraction



■ INTRODUCTION

We have recently reported a scalable eco-friendly process for the simultaneous extraction of pectin and essential oils (EOs) from lemon and orange peel.¹ Compared to current conventional processes, the new method affords significantly better yields and less degradation of pectin, a valued heteropolysaccharide with plenty of applications,² better time scale (microwave heating is much faster), and higher energy efficiency. Furthermore, water is the only solvent used. Along with pectin, valued EOs are obtained, consisting mainly of *d*-limonene, a monoterpene in high demand due to numerous and increasing commercial uses.³

In detail, the solvent-free microwave extraction (SFME) process to obtain pectin and citrus EOs consists of microwave hydrodistillation (to recover the EOs), followed by freeze-drying with or without a hydrodiffusion and gravity separation step (to recover pectin). The new SFME process is ready for kilogram-scale production, converting 20 kg of waste lemon peel (mixed in 36 L of water) into 3 kg of pectin and 10 mL of

EO. The yield relative to the peel is 0.4 wt % for EO. Common yields for EOs by conventional extraction methods are in the range 0.05–0.25 wt %.^{4,5}

In the present study, we have applied this new microwave-assisted extraction to different citrus grown in Sicily and harvested in December or early January, which is considered to be the best picking period of the year. We use the term “essential oil”, which according to ISO standards for EOs developed by ISO Technical Committee 54 can be used only if the EO is obtained by hydrodistillation, steam-distillation, or cold pressing, since solvent-free microwave extraction is essentially a sort of hydrodistillation, in which the heating source is represented by a microwave system.

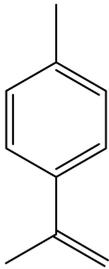
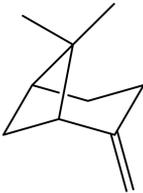
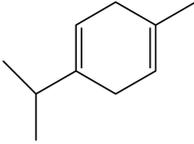
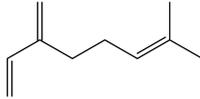
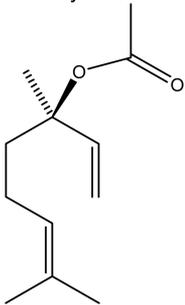
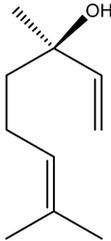
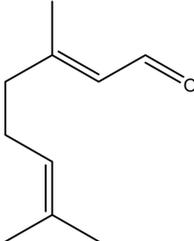
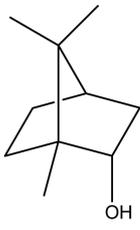
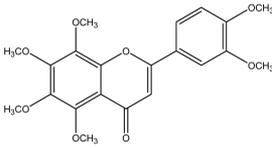
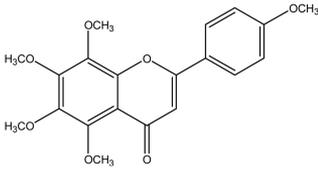
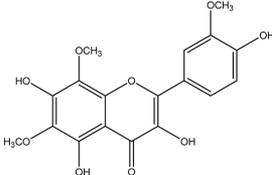
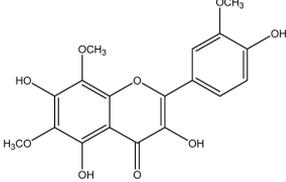
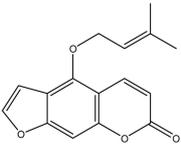
The composition of EOs from citrus peels has been extensively studied mostly by gas chromatography and mass

Received: April 6, 2017

Revised: May 2, 2017

Published: May 8, 2017

Table 1. 2D Structures of Some of the More Common Components Found in the EOs from Citrus

Examples of monoterpenes:			
Limonene 	β -Pinene 	γ -Terpinene 	β -myrcene 
Examples of oxygenated monoterpenes (terpenoids):			
Linalyl acetate 	Linalool 	Trans-Citral 	Borneol 
Examples of Polymethoxyflavones (PMFs) and hydroxylated PMFs:			
Sinensetin 	Nobiletin 	Tangeretin 	Limocitrol 
Other oxygen heterocyclic compounds (furanocoumarins):			
Isoimperatorin 			

spectrometry analysis.⁶ It was shown that both the qualitative and quantitative compositions depend on the type of citrus (orange, lemon, lime, grapefruit, tangerine, mandarin, etc.),⁷ the species and variety,^{8,9} whether the fruit is sweet or bitter,¹⁰ the origin,¹¹ the harvesting season,¹² the fruit maturity,¹³ the pretreatment of peels, and the extraction procedure.¹⁴ Still, there is no question that an ever-present component of all the citrus EOs is the cyclic monoterpene *d*-limonene.¹⁵ Other major constituents include cyclic and acyclic monoterpene hydrocarbons and oxygenated monoterpenes, such as linalyl acetate and linalool. According to Lota et al.'s statistical study,⁹ *d*-limonene content in most lemon peel oils varies from 38.1 to

95.8%. Appreciable amounts of linalyl acetate, linalool, γ -terpinene, and α -/ β -pinene (up to 31.2, 23.3, 18.0, and 15.8%, respectively) may also be present,⁹ as well as polymethoxyflavones (PMFs), hydroxylated polymethoxyflavones (HPMFs),¹⁶ and other oxygen heterocyclic compounds, such as furanocoumarins (e.g., isoimperatorin).¹⁰ In the EOs from orange peel, limonene has been reported as the predominant component (61%), followed by small amounts of citral (7.7%), borneol (7.6%), and capraldehyde (5.6%);⁷ polymethoxyflavones up to 40% were quantified by FTIR analysis.¹⁷ The predominance of limonene reported for grapefruit oil is even more striking (86.3%), followed by myrcene (6.28%).¹⁸

Table 2. Sources of EOs, Extraction Experimental Details, and Yields

source	power (W)	time (min)	fresh weight (g)	volume essential oil (mL)	essential oil yield (fresh weight, %)	essential oil yield (dry weight, %)
orange outer skin	600 until 100 °C, then 500	75	445	1.8	0.4	1.48
orange peel	600 until 100 °C, then 500	60	500	0.6	0.12	0.43
orange waste	600 until 100 °C, then 500	80	500	1.4	0.28	1.63
lemon outer skin	600 until 100 °C, then 500	60	500	1.3	0.26	1.34
lemon peel	700 until 100 °C, then 500	80	500	0.4	0.08	0.50
lemon waste	600 until 100 °C, then 500	70	500	0.6	0.12	0.80
grapefruit peel	600 until 100 °C, then 500	70	500	0.35	0.07	0.33

The 2D structures of some of these constituents are included in Table 1.

The vibrational spectra of the prevalent constituents of EOs such as limonene,^{19,20} β -pinene,²¹ γ -terpinene,²² β -myrcene,^{23,24} linalyl acetate,^{25,26} linalool,²⁷ citral,²⁸ polymethoxyflavones,²⁹ and furanocoumarins³⁰ have been analyzed in the literature. In this work, the EOs extracted from different fruits (orange, lemon, and grapefruit) and fruit parts (outer skin, peel, and waste) are comprehensively characterized by infrared spectroscopy in a straightforward, nondestructive procedure. The aim is to obtain the distribution of the main constituents by the different fruits and fruit parts, rather than obtaining a mere quantitative analysis or a complete analysis of all the 50–100 components of the EOs, highlighting the relevance of the differences found on the possible applications of the newly extracted EOs.

METHODS

Seven different citrus peel samples were subjected to microwave-assisted extraction. Orange and lemon outer skin, peel, and waste were used as EO sources, whereas in the case of grapefruit only the peel was used. In detail, orange and lemon matrices consisted of the outer skin cut with a knife, the peel obtained by hand skinning, and the waste obtained after industrial squeezing using a new cholea at industrial premises (Ortogel, Caltagirone, Italy). The experimental details and yields are summarized in Table 2.

The extraction and separation were carried out using a commercially available extractor (Ethos X, Milestone). No organic solvent was employed. In a typical extraction on laboratory scale, 500 g of lemon peel and 400 mL of distilled water were added to a glass vessel. Power was set at 600 W until the temperature reached 100 °C, and then lowered to 500 W. The extraction lasted for a variable time, depending on the matrix, between 60 min for orange peel and 80 min for orange waste. The EO was collected through the distillation module, while the pectin was extracted in the water phase. Displaying the simplicity of the experimental setup, Figure 1 shows a sample of lemon peels prior and after extraction.

The molecular structure of the extracted oils was assessed by Fourier transform infrared spectroscopy in diffuse reflectance mode (DRIFT), using a Mattson FTIR spectrometer with a Specac Selector, in the range of 4000–400 cm^{-1} (wide-band MCT detector), at 2 cm^{-1} resolution. The sample preparation consisted in depositing a drop of each EO on a bed of ground KBr (Aldrich, FTIR grade). The spectra were the result of ratioing 1000 added scans for each sample against the same number of scans for the background (ground KBr).

RESULTS AND DISCUSSION

The DRIFT spectra of the EOs from different citrus (orange, lemon, and grapefruit) and fruit regions (outer skin, peel, and waste) are compared in Figures 2 and 3. The spectra of EOs extracted from different orange regions are similar in terms of band positions, and the same is true for lemon (Figure 2). However, the relative intensities of the bands are influenced by the fruit region in lemon EOs, whereas for orange only those



Figure 1. Lemon peels prior to and after microwave-assisted hydrodistillation.

from the outer skin show significant differences. More appreciable changes are observed only when comparing the EOs from equivalent regions of different fruits (Figure 3).

The proposed band assignments, as well as compatible oil constituents, are summarized in Table 3. Some comments seem pertinent at this stage. In all the spectra, the strongest bands (between 2800 and 3100 cm^{-1}) are characteristic of CH stretching vibrations of both sp^2 and sp^3 hybridized carbon atoms. The band at $\sim 3080 \text{ cm}^{-1}$ is indicative of $=\text{CH}_2$ groups (stretching modes). In addition, a sharp C=C stretching mode can be observed at 1678 cm^{-1} , along with a $=\text{CH}_2$ out-of-plane bending mode of vinylidene groups at 887 cm^{-1} and a =CH out-of-plane mode of a trisubstituted alkene at 798 cm^{-1} .³¹ All the EO samples show a medium/strong band at 1643 cm^{-1} , assigned to the stretching mode of ring C=C bonds.¹⁹ The very strong doublets at $\sim 2964/2920 \text{ cm}^{-1}$ and 2857/2840 cm^{-1} are assigned to stretching modes of CH_3/CH_2 groups, antisymmetric and symmetric, respectively. The corresponding deformations of CH_3 groups are also clear at 1439 and 1377 cm^{-1} , and their rocking mode at 914 cm^{-1} . Additionally, all the spectra present medium/weak bands at 1051 cm^{-1} , assigned to C–C stretching modes. This pattern, common to the spectra of all the EOs, is very similar to that of limonene (Figure S1), suggesting its predominance, although not excluding the presence of other monoterpenes.

Also observed in all the spectra are the bands at 1782, 1255, and 1155 cm^{-1} , assigned to C=O and antisymmetric and symmetric C–O–C stretching modes, respectively, indicating the presence of an ester such as linalyl acetate. The in-plane deformations of CH_2 , at 1016 and 990 cm^{-1} , are considered fingerprints of linalyl acetate, as well as the characteristic intensity ratio between the $\nu\text{C}=\text{O}$ and $\nu_{\text{as}}\text{C}-\text{O}-\text{C}$ bands.^{25,26}

The broad νOH band centered at $\sim 3480 \text{ cm}^{-1}$ appears in the EOs from lemon, orange outer skin, and grapefruit peel,

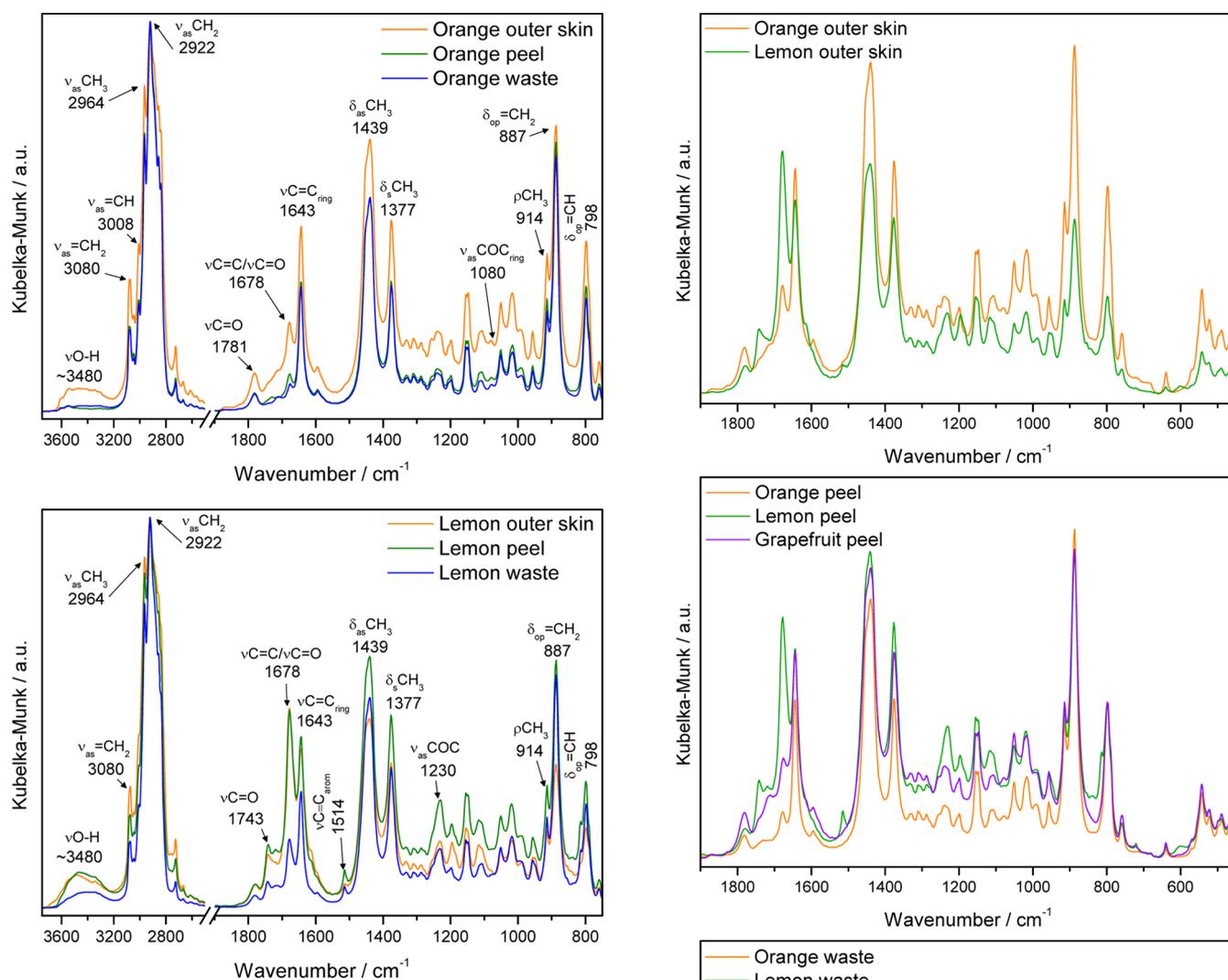


Figure 2. DRIFT spectra of the EOs extracted from different regions of the same fruit, as indicated, normalized to the wavenumber of maximum absorption ($\nu_{\text{as}}\text{CH}_2$).

suggesting that a constituent with one or more alcohol groups is present in these EOs, especially in those from grapefruit peel, where the band is stronger. Probable candidates are linalool (the most common hydrolysis product of linalyl acetate), borneol, or limocitrol (trihydroxylated polymethoxyflavone). The presence of such constituent could justify the distinct relative band intensities observed in the spectrum of EO from orange outer skin in comparison to that of other orange EOs.

The weak bands at 1514, 1232, 1117, 840, and 721 cm^{-1} in the EOs from lemon are consistent with the presence of at least one constituent with aromatic rings and methoxy substituents, such as PMFs or partially hydroxylated PMFs. In contrast, the medium/weak bands at 1240 and 1147 cm^{-1} ($\nu_{\text{as}}\text{C}-\text{O}-\text{C}$ and $\nu_{\text{s}}\text{C}-\text{O}-\text{C}$) suggest the presence of a constituent with an ether group different from a PMF, especially in orange and grapefruit EOs; the weak band at 1078 cm^{-1} ($\nu_{\text{as}}\text{C}-\text{O}-\text{C}$ in a 5-member rings) points to a furanocoumarin.

Only in orange and grapefruit EOs is the band at 1595 cm^{-1} observed ($\nu_{\text{C}}\cdots\text{C}$), indicating that a monoterpene with nonaromatic conjugated $\text{C}=\text{C}$ double bonds, such as β -myrcene, is present. The assignment of the band at 1718 cm^{-1} to the $\nu_{\text{C}}\cdots\text{O}$ mode of a carbonyl group in resonance with a

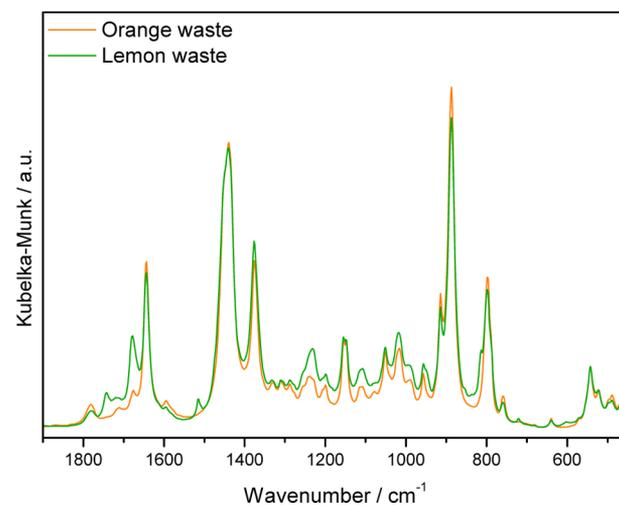


Figure 3. DRIFT spectra (1900–550 cm^{-1} range) of the EOs extracted from equivalent regions of different fruits, as indicated, normalized to the wavenumber of maximum absorption.

nonaromatic chain opens the possibility of a different oxygenated terpene, such as *trans*-citral (geranial) or *cis*-citral (neral), present only in the EOs from orange and lemon waste and from lemon and grapefruit peel.

In summary, the direct observation of the spectra shows that most extracted EOs are predominantly limonene and that they contain other monoterpenes (e.g., γ -terpinene and β -pinene)

Table 3. Observed Bands and Proposed Assignments of the DRIFT Spectra of EOs Extracted from Different Orange and Lemon Regions and Grapefruit Peel

orange		lemon		grapefruit peel	assignment ^b	possible oil constituents ^{b,2-30,c}
outer skin	peel	outer skin	peel			
~3470 br	~3480 m	~3480 br	~3470 br	~3480 br,m	ν O-H	LOH, BOH, HPMFs
~3080 m	~3080 m	3075 m	3075 m	3080 vw	ν_{as} =CH ₂	L _s , β P, β M, LOH, LAc
3047 w	3047 w	3045 m	3045 w	3046 vw	ν_s =CH ₂	L _s , β P, β M, LAc, LOH
3008 s	3008 m	3008 sh	3008 m	3010 w	ν =CH	L _s , γ T, β M, PMFs, HPMFs
2964 VS	2964 S	2964 VS	2964 VS	2965 S	ν_{as} CH ₃	L _s , β P, γ T, β M, LOH, LAc, Ci, Ilm
2922 VS	2922 VS	2926 VS	2922 VS	2919 VS	ν_{as} CH ₂	L _s , β P, γ T, β M, LOH, LAc, Ci
2858 VS	2856 VS	2862 VS,sh	2858 VS	2856 S	ν_s CH ₃	L _s , β P, γ T, β M, LOH, LAc, Ci, Ilm
2841 VS	2839 S	2837 S,sh	2837 S,sh	2841 m	ν_s CH ₂ (ring)	L _s , β P, γ T
2727 w	2725 w	2727 w	2727 w	2727 w	ν_s CH ₂	β M, LOH, Ilm
1782 w	1782 w	1780 w	1780 w	1782 w	ν C=O	LAc
		1741 w	1741 w	1743 w	ν C...O	Ilm
		1718 w	1718 w	1712 w	ν C...O	Ci
1678 m	1676 w	1678 S	1678 S	1676 m	ν C=C/ ν C...O	L _s , β P, β M, LOH, LAc/Ci, PMFs, HPMFs
1643 S	1643 m	1643 S	1643 S	1643 S	ν C=C _(ring)	L _s , γ T
1595 w	1595 w	1514 w	1514 w	1595 w	ν C...C	β M
		1452 sh	1452 w		ν C...C _(anom-ring)	PMFs
1439 VS	1439 S	1441 S	1441 VS	1439 VS	δ CH	L _s , γ T
1377 S	1377 m	1377 m	1377 S	1377 S	δ_{as} CH ₃	L _s , β P, γ T, β M, LOH, LAc, Ci, BOH, Ilm
1331 w	1331 w	1333 w	1333 w	1331 w	δ_s CH ₃	BOH, Ilm
1309 w	1311 w	1309 w	1309 w	1309 w	δ CH ₂	β M, LOH, Ilm
1288 w	1288 w	1288 w	1286 w	1288 w	δ CH ₂ (ring)	L _s , γ T
1255 w	1254 w	1255 sh	1255 sh	1257 sh	ν_{as} C-O-C	PMFs, HPMFs
1240 m	1240 w	1232 w	1232 m	1240 w	ν_{as} C-O-C _(ester)	LAc
		1196 w	1198 m	1200 w	ν_{as} C-O-C	Ilm
1200 w	1155 w	1155 m	1155 m	1155 m	ν_{as} C-O-C	PMFs
1155 m	1147 w	1149 m	1149 m	1147 m	ν_{as} C-O-C	PMFs, HPMFs, Ilm/LOH, Ci
1147 m	1147 w	1117 w	1117 m	1147 m	ν_{as} C-O-C	LAc
		1109 w	1109 w	1107 w	ν_{as} C-O-C _(6 ring)	PMFs, HPMFs
1080 w	1078 w	1078 w	1078 w	1078 w	ν_{as} C-O-C _{(6 ring)/νC-O(H)}	BOH, Ilm
1051 m	1051 w	1051 w	1051 m	1051 m	ν_{as} C-O-C _(5 ring)	Ilm
1016 m	1016 w	1018 w	1018 m	1018 m	ν C-C	L _s , β P, γ T, β M, LOH, LAc, Ci, BOH, Ilm
993 m	991 w	989 w	993 w	993 w	ν C-C	LAc
957 m	957 w	957 w	957 w	957 w	δ_{ip} =CH/ δ_{op} =CH ₂	LAc/ β M
914 S	914 m	914 w	914 m	914 m	δ CH _(ring)	L _s , γ T, PMFs, Ilm
887 VS	887 VS	887 m	887 VS	887 VS	ρ CH ₃	L _s , β P, γ T, β M, LOH, LAc, Ci
		845 w	837 w	841 w	δ_{op} =CH ₂ / ρ CH ₃	L _s , β M, β P/LOH, LAc, Ilm
		798 m	798 m	798 m	$\rho(O)CH_3$	PMFs, HPMFs
		798 m	798 m	798 m	δ_{op} =CH	L _s , γ T, β M, LAc, LOH

Table 3. continued

orange			lemon			grapefruit peel	assignment ^b	possible oil constituents ^{1,2,30,c}
outer skin	peel	waste	outer skin	peel	waste			
760 w	760 w	760 w	760 w	760 w	760 w	760 w	$\rho\text{CH}_2(\text{ring})$	L, βP , γT
640 vw	640 w	640 w	721 w	721 w	721 w	640 w	$\delta_{\text{op}}=\text{CH}_{(\text{anom. ring})}$	PMFs, HPMFs
569 w			640 w	640 w	640 w	571 sh	$\delta_{\text{op}}=\text{CH}_{(\text{anom. ring})}$	Ilm
			571 sh	571 w	571 w	542 w	$\delta_{\text{ip}}\text{C}-\text{O}-\text{C}$	PMFs, HPMFs, Ilm
			542 w	542 w	542 w		$\delta_{\text{ip}}\text{C}-\text{O}-\text{C}$	PMFs, HPMFs, Ilm

^aVs, very strong; S, strong; m, medium; w, weak; vw, very weak; br, broad; sh, shoulder. ^bip, in-plane; op, out-of-plane. ^cL, limonene; βP , β -pinene; γT , γ -terpinene; βM , β -myrcene; LOH, linalool; LAc, linalyl acetate; Ci, citral; BOH, borneol; PMFs, polymethoxyflavones; HPMFs, hydroxylated polymethoxyflavones; Ilm, isoperatorin.

and linalyl acetate. Linalool, borneol, or hydroxylated PMFs are present mostly in the EO from grapefruit peel and in lemon EOs. Orange-derived EOs are free of alcohols, which appear to be present only in the outer skin. Furanocoumarins (e.g., isoperatorin) were detected in the EOs from orange and grapefruit, whereas polymethoxyflavones were identified in the EOs from lemon.

This spectral observation is straightforward but has a weakness, due to the extensive band overlapping of different constituents. In an attempt to quantify the analysis, a spectral deconvolution into a sum of Gaussian and Lorentzian components was performed by a nonlinear least-squares fitting method. Three regions were considered (3700–2600, 1900–1550, and 1550–650 cm^{-1}), and the corresponding deconvolutions are shown in Figures S2–S4. Some band components identified by deconvolution are unequivocally associated with specific constituents, as indicated in Tables S1–S3. This is the case of the component recovered at 888 cm^{-1} , the exact wavenumber of the strongest limonene band in this region, assigned to the out of plane $=\text{CH}_2$ mode. Other monoterpenes exhibit the same strong mode but shifted to higher wavenumbers, namely, β -myrcene, where the $=\text{CH}_2$ group is resonant with another chain vinylidene group, and β -pinene, where the $=\text{CH}_2$ group is a substituent of a saturated six-member ring. Therefore, the component retrieved at 888 cm^{-1} was assigned exclusively to limonene and was used to determine the factor f that compensates for limonene's contribution to this wavenumber. The ratio between the spectral integrated areas of pure limonene (multiplied by f) and EO is an estimate of limonene content in the EO. The results in terms of percentage are summarized in Figure 4.

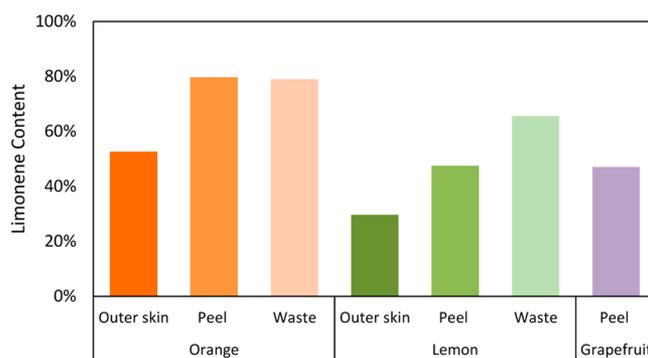


Figure 4. Limonene percentage in the different EOs, estimated from the DRIFT spectral deconvolution.

In fact, except for the EO from lemon outer skin, limonene is the predominant constituent (above 50%) of all the extracted EOs. Those from orange peel and waste are the richest (~80%), followed by lemon waste (66%). For the minor constituents, only a relative contribution to each EO was obtained from the ratio of the corresponding components' areas to the total area of the spectrum. These relative intensities were converted to an arbitrary scale from 1 to 7 (minimum and maximum values, respectively, of the % area obtained for a given constituent in the 7 EO samples). In the absence of a band component associated with a constituent, its content was considered zero. This was the case of γ -terpinene in the EOs of orange and grapefruit: three characteristic infrared bands ($\nu\text{C}=\text{C}$, δCH (ring), and ρCH_3 , at 1613, 953, and 816 cm^{-1} , respectively) were recovered only in the spectra of lemon

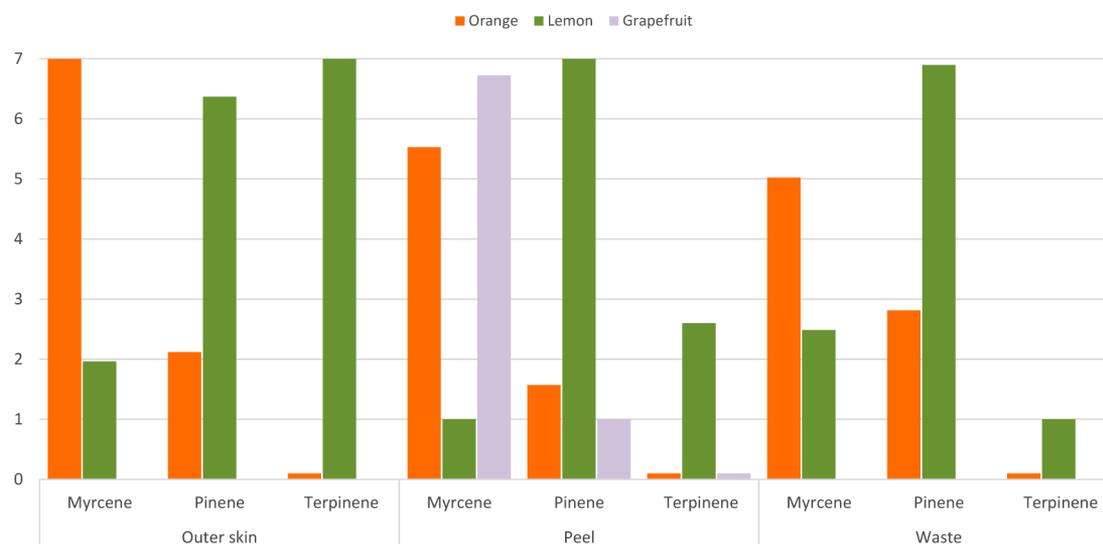


Figure 5. Summary, on a scale from 1 to 7, of the relative proportions of minor monoterpene constituents of the EOs extracted from different regions of orange, lemon, and grapefruit.

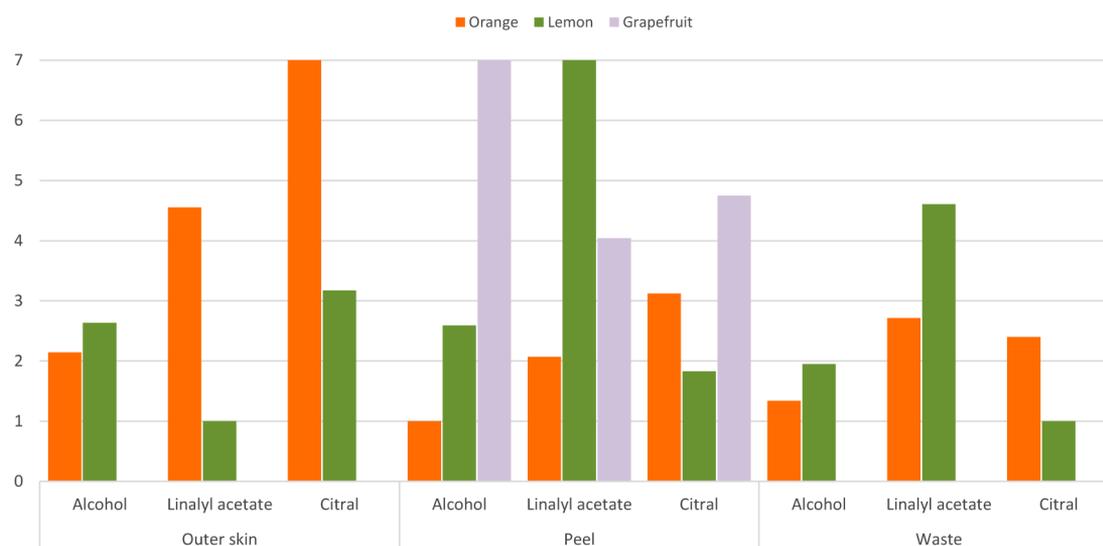


Figure 6. Summary, on a scale from 1 to 7, of the relative proportions of minor constituents (oxygenated monoterpenes) of the EOs extracted from different regions of orange, lemon, and grapefruit.

EOs. A summary of the results thus obtained is shown in Figure 5 (for monoterpene hydrocarbons), Figure 6 (for oxygenated monoterpenes or terpenoids), and Figure 7 (for polymethoxyflavones and furanocoumarins).

The EOs from lemon are the richest in some monoterpenes other than limonene, especially γ -terpinene (outer skin and peel) and β -pinene (peel, waste, and outer skin), responsible for the characteristic “green peely” odor.³² β -Myrcene was found mostly in orange outer skin and peel and in grapefruit peel.

The EO from grapefruit peel is the richest in alcohols, followed by lemon outer skin and peel and by orange outer skin, whereas orange peel is the poorest in such constituents. Contributions from linalool, borneol and hydroxylated polymethoxyflavones must be considered, since the calculation took into account all the OH stretching bands. Given the anti-inflammatory activity of linalool,³³ the effect of *R*-(2)linalool in decreasing the heart rate and in producing calm mood states,³⁴ and the chemopreventive effects of hydroxylated PMFs,

including anticancer, anti-inflammation, antiatherosclerosis, and neuroprotection,³⁵ EOs from grapefruit peel should be preferred if these applications are intended. However, maximum content in another anti-inflammatory agent, linalyl acetate,³³ was found in lemon peel oil, followed by lemon waste and orange outer skin oils. Orange EOs, especially from the outer skin, are relatively richer in citral, which has a significant ability to suppress oxidative stress, possibly through induction of an endogenous antioxidant protein that reacts rapidly and specifically with the active isomer, geranial.³⁴

It is widely recognized that the monoterpene fraction of EOs oxidizes readily in air, with the result that the EOs develop unpleasant odors and flavors. The EOs richer in oxygenated compounds are more stable on storage, besides being more soluble in water and common polar solvents. Thus, the concentrations of aliphatic and monoterpene aldehydes (especially citral isomers), as well as those of esters and alcohols in EOs are critical information in the perceived quality

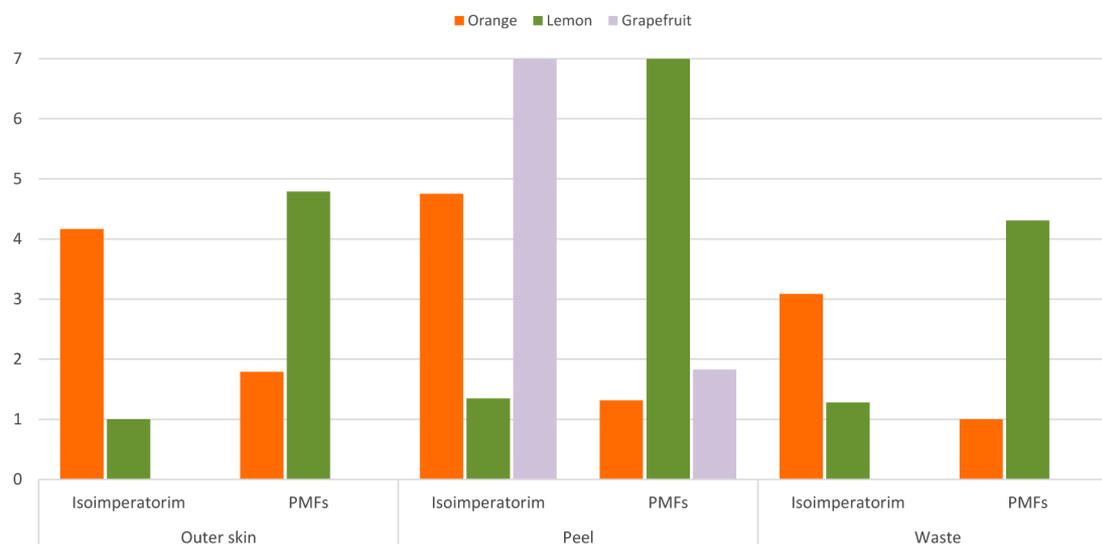


Figure 7. Summary, in a scale from 1 to 7, of the relative proportions of minor constituents (PMFs and furanocoumarins) of the EOs extracted from different regions of orange, lemon and grapefruit.

of an oil.³² From this point of view, all the analyzed EOs from the Sicilian citrus are high-quality.

The beneficial effects of flavonoids are well-documented in the literature: their cholesterol- and triacylglycerol-lowering potential³⁶ and strong anti-inflammatory effects³⁷ are responsible for increased metabolic stability and membrane transport in the intestine and liver, improving their oral bioavailability,³⁸ thus the great potential of PMFs as oral cavity cancer chemopreventive agents.³⁹ The anticancer activity of citrus peel flavonoids has been evaluated on several animal models, including skin, colon, prostate, lung, and liver, among others.⁴⁰

Contrary to the results of other authors,¹⁷ the present study shows that the EOs from Sicilian lemon, especially from the peel, are richer in PMFs than those from orange and grapefruit. The opposite was found for furanocoumarins, such as isoimperatorin, which are predominant in grapefruit peel and orange oils. This may be an advantage for these EOs, given the potential effect of isoimperatorin in protecting cells from the hepatotoxic and hepatocarcinogenic effects of Aflatoxin B1 (AFB1) that may form in improperly stored foods.⁴¹

CONCLUSIONS

In this study we provide spectroscopic evidence that all the extracted oils from lemon, orange, and grapefruit harvested in Sicily during winter and obtained using solvent-free microwave extraction are rich in *d*-limonene, particularly those from orange. This is compensated for by lemon EOs being richer in other monoterpenes, namely, γ -terpinene. All the EOs are high-quality, due to the presence of oxygenated constituents (alcohols, aldehydes, esters, oxygenated PMFs, and/or furanocoumarins), with those from the wastes being poorer in these constituents. It is also recognized that mature fruits generally contain less EOs than unripe ones, but their quality increases with maturation.¹² The new microwave-based procedure is highly compatible with the actual industrial processes of juice production, since it simply requires the installation of industrial-scale microwave extractors⁴² downstream from the orange, lemon, or grapefruit juice extraction units.

The demand for high quality citrus EOs is rapidly growing, with growing consumer awareness regarding benefits of these

oils, driving increasing demand in the developing and developed regions.⁴³ Increasing demand for orange oil faced with the constant or diminishing availability of orange crops in Florida and Brazil currently translates into a shortage of supply and higher prices,⁴⁴ whereas relevant innovative and ecologically friendly new methods of orange oil usage continue to emerge, such as in the case of its use as basic ingredient for an advanced pesticide of broad scope.⁴⁵ Essential oils have a wide range of classical applications, such as perfumes, creams, soaps, flavor additives for food, fragrances for household cleaning products, and industrial solvents. Moreover, their beneficial biological effects are today well-established.⁶ *In vitro* physicochemical assays characterize most of them as antioxidants, and recent work has shown that they can act as prooxidants at cellular level, affecting inner cell membranes and organelles such as mitochondria. Depending on the type and concentration, EOs exhibit cytotoxic effects on living cells but are usually nongenotoxic.

The current industrial process of the citrus EO production, namely, a cold-pressed procedure, works at room temperature and is among the cleanest industrial processes in the food industry. The hydrodistillation process in the microwave-assisted mode proposed herein, carried out at 100 °C, has a significantly higher energy input, but it affords high-quality EOs in higher yields, partly fulfilling the approach to the “dry” biorefinery proposed by Chemat and co-workers.⁴⁶ Buyers in the nutraceutical, cosmetic, and pharmaceutical industries require pure EOs devoid of pesticide residues that not only would make the product unsuitable for their needs but whose use in cultivation of citrus crops is known to lower the quality of citrus EOs, diminishing the content of valued aldehyde compounds.⁴⁷

Every season Sicily produces a significant amount of citrus fruits, mainly, orange, lemon, grapefruit, and mandarin. (In the 2015/2016 season 1.62 million tons of citrus were produced.)⁴⁸ The outcomes of this study suggest that the newly developed microwave-assisted extraction of EO (and pectin) from different citrus fruits and from different parts of biologically grown citrus crops is likely to become shortly an important asset of the emerging bioeconomy in this region and in other countries where citrus trees are grown on large areas of land.

■ ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acssuschemeng.7b01046.

DRIFT spectrum of *d*-limonene and deconvolution of the sample spectra, along with assignments of the spectral band components recovered by deconvolution (PDF)

■ AUTHOR INFORMATION

Corresponding Authors

*E-mail: lilharco@tecnico.ulisboa.pt.

*E-mail: mario.pagliaro@cnr.it.

ORCID 

Mario Pagliaro: 0000-0002-5096-329X

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

This work is dedicated to the memory of Davide Tamburino (1961–2017), gentleman and passionate citrus entrepreneur. We thank his company, Ortogel (Caltagirone, Italy), for providing most citrus fruits and derivatives used in this work.

■ REFERENCES

- (1) Fidalgo, A.; Ciriminna, R.; Carnaroglio, D.; Tamburino, A.; Cravotto, G.; Grillo, G.; Ilharco, L. M.; Pagliaro, M. Eco-Friendly Extraction of Pectin and Essential Oils from Orange and Lemon Peels. *ACS Sustainable Chem. Eng.* **2016**, *4*, 2243–2251.
- (2) Ciriminna, R.; Chavarría-Hernández, N.; Inés Rodríguez Hernández, A.; Pagliaro, M. Pectin: A new perspective from the biorefinery standpoint. *Biofuels, Bioprod. Biorefin.* **2015**, *9*, 368–377.
- (3) Ciriminna, R.; Lomeli-Rodríguez, M.; Demma Carà, P.; Lopez-Sanchez, J.; Pagliaro, M. Limonene: a versatile chemical of the bioeconomy. *Chem. Commun.* **2014**, *50*, 15288–15296.
- (4) Ferhat, M. A.; Meklati, B. Y.; Chemat, F. Comparison of different isolation methods of essential oil from Citrus fruits: cold pressing, hydrodistillation and microwave 'dry' distillation. *Flavour Fragrance J.* **2007**, *22*, 494–504.
- (5) *Essential Oils in Food Preservation, Flavor and Safety*; Preedy, V. R., Ed.; Academic Press: New York, 2015.
- (6) Bakkali, F.; Averbeck, S.; Averbeck, D.; Idaomar, M. Biological effects of essential oils - A review. *Food Chem. Toxicol.* **2008**, *46*, 446–475.
- (7) Kamaliroosta, L.; Zolfaghari, M.; Shafiee, S.; Larijani, K.; Zojaji, M. Chemical Identifications of Citrus Peels Essential Oils. *J. Food Biosci. Technol.* **2016**, *6*, 69–76.
- (8) Hamdan, D.; El-Readi, M. Z.; Nibret, E.; Sporer, F.; Farrag, N.; El-Shazly, A.; Wink, M. Chemical composition of the essential oils of two Citrus species and their biological activities. *Pharmazie* **2010**, *65*, 141–147.
- (9) Lota, M.-L.; de Rocca Serra, D.; Tomi, F.; Jacquemond, C.; Casanova, J. Volatile Components of Peel and Leaf Oils of Lemon and Lime Species. *J. Agric. Food Chem.* **2002**, *50*, 796–805.
- (10) Mc Hale, D.; Sheridan, J. B. The oxygen heterocyclic compounds of citrus peel oils. *J. Essent. Oil Res.* **1989**, *1*, 139–149.
- (11) Nasser AL-Jabri, N.; Hossain, M. A. Comparative chemical composition and antimicrobial activity study of essential oils from two imported lemon fruits samples against pathogenic bacteria. *Beni-Suef Univ. J. Appl. Sci.* **2014**, *3*, 247–253.
- (12) Argyropoulou, C.; Daferera, D.; Tarantilis, P. A.; Fasseas, C.; Polissiou, M. Chemical composition of the essential oil from leaves of *Lippia citriodora* H.B.K. (Verbenaceae) at two developmental stages. *Biochem. Syst. Ecol.* **2007**, *35*, 831–837.
- (13) López Mahía, P. L.; Gándara, J. S.; Losada, P. P. Infrared spectrophotometric determination of citral corrected for limonene interference in lemon and orange essential oils. *Food Chem.* **1993**, *46*, 193–197.
- (14) Kamal, G. M.; Anwar, F.; Hussain, A. I.; Sarri, N.; Ashraf, M. Y. Yield and chemical composition of Citrus essential oils as affected by drying pretreatment of peels. *Int. Food Res. Journal* **2011**, *18*, 1275–1282.
- (15) Lawrence, B. M. Progress in Essential Oils, Lemon Oil. *Perfum. Flavor.* **1996**, *21*, 41–45.
- (16) Macheix, J.-J.; Fleuriet, A.; Billot, J. *Fruit Phenolics*; CRC Press: Boca Raton, FL, 1990.
- (17) Manthey, J. A. Fourier transform infrared spectroscopic analysis of the polymethoxylated flavone content of orange oil residues. *J. Agric. Food Chem.* **2006**, *54*, 3215–3218.
- (18) Ahmad, M. M.; Rehman, S.-U.; Iqbal, Z.; Anjum, F. M.; Sultan, J. I. Genetic variability to essential oil composition in four citrus fruit species. *Pak. J. Bot.* **2006**, *38*, 319–324.
- (19) Partal Ureña, F.; Moreno, J. R. A.; López González, J. J. Conformational study of (R)-(+)-limonene in the liquid phase using vibrational spectroscopy (IR, Raman, and VCD) and DFT calculations. *Tetrahedron: Asymmetry* **2009**, *20*, 89–97.
- (20) Zapata, R. B.; Villa, A. L.; Montes de Correa, C.; Williams, C. T. In situ Fourier transform infrared spectroscopic studies of limonene epoxidation over PW-Amberlite. *Appl. Catal., A* **2009**, *365*, 42–47.
- (21) Schulz, H.; Özkan, G.; Baranska, M.; Krüger, H.; Özcan, M. Characterisation of essential oil plants from Turkey by IR and Raman spectroscopy. *Vib. Spectrosc.* **2005**, *39*, 249–256.
- (22) Baranska, M.; Schulz, H.; Krüger, H.; Quilitzsch, R. Chemotaxonomy of aromatic plants of the genus *Origanum* via vibrational spectroscopy. *Anal. Bioanal. Chem.* **2005**, *381*, 1241–1247.
- (23) Svatos, A.; Attygalle, A. B. Characterization of Vinyl-Substituted, Carbon-Carbon Double Bonds by GC/FT-IR Analysis. *Anal. Chem.* **1997**, *69*, 1827–1836.
- (24) Yang, X.; Li, S.; Xia, J.; Song, J.; Huang, K.; Li, M. Renewable myrcene-based UV-curable monomer and its copolymers with acrylated epoxidized soybean oil: design, preparation, and characterization. *BioResources* **2015**, *10*, 2130–2142.
- (25) Lalfal, S.; Vanloot, P.; Bombarda, I.; Kister, J.; Dupuy, N. Identification of metabolomic markers of lavender and lavandin essential oils using mid-infrared spectroscopy. *Vib. Spectrosc.* **2016**, *85*, 79–90.
- (26) *Citrus Essential Oils: Flavor and Fragrance*; Sawamura, M., Ed.; Wiley-Blackwell: Hoboken, NJ, 2011.
- (27) Rummens, F. H. A. A study in monoterpene chemistry by physical methods. Doctoral dissertation, Technische Hogeschool Eindhoven, Eindhoven, 1963.
- (28) Shahzadi, P.; Muhammad, A.; Mehmood, F.; Chaudhry, M. Y. Synthesis of 3, 7-Dimethyl-2, 6-cctadienal acetals from citral extracted from lemon grass, cymbopogon citrates. *J. Antivirals Antiretrovirals* **2014**, *6*, 28–31.
- (29) Jasim, A. R.; Hussein, A. A.; Nasser, A. M. A. G. Phytochemical investigation of flavonoids in different fruit parts of Iraqi sour orange. *World J. Pharm. Pharm. Sci.* **2016**, *5*, 109–128.
- (30) Lee, K.-H.; Soine, T. O. Coumarins X: Spectral studies on some linear furanocoumarins. *J. Pharm. Sci.* **1969**, *58*, 681–683.
- (31) Socrates, G. *Infrared and Raman Characteristic Group Frequencies: Tables and Charts*, 3rd ed.; John Wiley & Sons, Ltd.: Chichester, U.K., 2004.
- (32) Rousef, R.; Perez-Cacho, P. R. Citrus flavour. In *Flavours and fragrances chemistry, bioprocessing and sustainability*; Burger, R. G., Ed.; Springer: Berlin, 2007; pp 117–134.
- (33) Peana, A. T.; D'Aquila, P. S.; Panin, F.; Serra, G.; Pippia, P.; Moretti, M. D. L. Anti-inflammatory activity of linalool and linalyl acetate constituents of essential oils. *Phytomedicine* **2002**, *9*, 721–726.
- (34) Fukumoto, S.; Sawasaki, E.; Okuyama, S.; Miyake, Y.; Yokogoshi, H. Flavor components of monoterpenes in citrus essential oils enhance the release of monoamines from rat brain slices. *Nutr. Neurosci.* **2006**, *9*, 73–80.

(35) Lai, C. S.; Wu, J. C.; Ho, C. T.; Pan, M. H. Disease chemopreventive effects and molecular mechanisms of hydroxylated polymethoxyflavones. *Biofactors* **2015**, *41*, 301–313.

(36) Kurowska, E. M.; Manthey, J. A. Hypolipidemic effects and absorption of citrus polymethoxylated flavones in hamsters with diet-induced hypercholesterolemia. *J. Agric. Food Chem.* **2004**, *52*, 2879–2886.

(37) Gossiau, A.; Chen, K. Y.; Ho, C.-T.; Li, S. Anti-inflammatory effects of characterized orange peel extracts enriched with bioactive polymethoxyflavones. *Food Sci. Hum. Wellness* **2014**, *3*, 26–35.

(38) Walle, U. K.; Walle, T. Bioavailable flavonoids: cytochrome P450-mediated metabolism of methoxyflavones. *Drug Metab. Dispos.* **2007**, *35*, 1985–1989.

(39) Walle, T. Methoxylated flavones, a superior cancer chemopreventive flavonoid subclass? *Semin. Cancer Biol.* **2007**, *17*, 354–362.

(40) Rawson, N. E.; Ho, C.-T.; Li, S. Efficacious anti-cancer property of flavonoids from citrus peels. *Food Sci. Hum. Wellness* **2014**, *3*, 104–109.

(41) Pokharel, Y. R.; Han, E. H.; Kim, J. Y.; Oh, S. J.; Kim, S. K.; Woo, E.-R.; Jeong, H. G.; Kang, K. W. Potent protective effect of isoimperatorin against aflatoxin B1-inducible cytotoxicity in H4IIE cells: bifunctional effects on glutathione S-transferase and CYP1A. *Carcinogenesis* **2006**, *27*, 2483–2490.

(42) Ciriminna, R.; Carnaroglio, D.; Delisi, R.; Arvati, S.; Tamburino, A.; Pagliaro, M. Industrial Feasibility of Natural Products Extraction with Microwave Technology. *ChemistrySelect* **2016**, *1*, 549–555.

(43) *Citrus Oils Market: Global Industry Analysis and Opportunity Assessment, 2016–2026*; Future Markets Insights: Pune, India, 2017.

(44) “The market is suffering a significant lack of supply needed to keep up with the consistent and, in some cases, increasing demand for orange oil and its derivatives.” *Market Intelligence, February 2017*; Treatt: Suffolk, U.K., 2017.

(45) Ciriminna, R.; Meneguzzo, F.; Pagliaro, M. Orange oil. In *Green Pesticides Handbook: Essential Oils for Pest Control*; Nollet, L. M. L., Rathore, H. S., Eds.; CRC Press: Boca Raton, FL, 2017; Chapter 15.

(46) Jacotet-Navarro, M.; Rombaut, N.; Deslis, S.; Fabiano-Tixier, A.-S.; Pierre, F.-X.; Bily, A.; Chemat, F. Towards a “dry” bio-refinery without solvents or added water using microwaves and ultrasound for total valorization of fruit and vegetable by-products. *Green Chem.* **2016**, *18*, 3106–3115.

(47) Verzera, A.; Trozzi, A.; Dugo, G.; Di Bella, G.; Cotroneo, A. Biological lemon and sweet orange essential oil composition. *Flavour Fragrance J.* **2004**, *19*, 544–548.

(48) *Economie regionali - L'economia della Sicilia*; Banca d'Italia: Rome, 2016; p 50.