Available online at www.sciencedirect.com
ScienceDirect
Food Chemistry 105 (2007) 771–783
Food Chemistry
www.elsevier.com/locate/foodchem

Analytical, Nutritional and Clinical Methods

A comprehensive study on the chemical composition and aromatic characteristics of lemon liquor

M.L. Crupi [a], R. Costa [a], P. Dugo [b], G. Dugo [a], L. Mondello [a,*]

[a] Dipartimento Farmaco-Chimico, Università di Messina, Viale Annunziata, 98168 Messina, Italy
[b] Dipartimento di Chimica Organica e Biologica, Università di Messina, salita Sperone 31, 98166 Messina, Italy

Received 18 July 2006; received in revised form 5 January 2007; accepted 21 January 2007

Abstract

Lemon liquor, commonly known as ''Limoncello'', obtained from maceration of lemon peels in ethanol, water and sugar, has become a product in great demand in the international market. Scientific literature reports just a few studies on Limoncello, however, in consideration of the data available, it can be assumed that in many cases the need of industry for standard products leads to the addition of essential oils and/or related to the alcoholic syrup. Aim of this study was therefore to investigate the volatile and non volatile fraction of lemon liquors of different commercial brands, using solid phase microextraction, gas chromatography-quadrupole mass spectrometry, chiral-gas chromatography and reversed phase HPLC.

Keywords: Limoncello; Lemon liquor; SPME; GC-MS; RP-HPLC; Chiral-GC

1. Introduction

Lemon liquor, commonly known as ''Limoncello'', has become a product in great demand in the international market. Its production is traditionally based on the alcoholic maceration of the external part (flavedo) of lemon peels. Water and sugar are the other two main ingredients of this liquor, that is technically better defined as ''rosolio''. It finds its origin in distant times, when housewives used to pick up flowers and fruits to make these natural liquors, whose preparation procedure well preserves the flavour and taste given by the vegetable part. The limoncello alcoholic grade hardly exceeds 30–32° and also for this reason the liquor has gained so much popularity. Other reasons are its digestibility, its sweetness and the incomparable lemon aroma and taste. Basically, the most suitable lemons for making limoncello are those free from pesticide residues. The best Citrus fruits utilized for limoncello are cultivated in the area around Salerno, Campania, Italy, where the variety ''Sfusato Amalfitano'' is now registered under Protected Geographical Indication (IGP) designation. This variety of lemon fruits, with light yellow and flavoured peels, is recorded in several historical documents as the one used for preparing limoncello due not only to its morphological properties but also to its eupeptic character. On the other hand, it cannot be underestimated the important role played by the Sicilian lemon fruits, from where probably the first Citrus fruits were introduced in Campania in the past. The Sicilian traditional recipe for making limoncello is based on the use of the variety *Femminello Santa Teresa*, in particular of the fruits produced in summertime after the third blossoming, which are called *Verdelli* (Cutuli et al., 1985; Dugo & Di Giacomo, 2002). It is a matter of fact that the evaluation of the organoleptic properties of limoncello is, although indirectly, connected to the analysis of the essential oil composition. The aroma of the liquor is actually one of the first consumer's perceptions that is crucial in establishing the preference among several products available in the market. For this reason, the analysis

* Corresponding author. Tel.: +39 090 6766536; fax: +39 090 6766532.
E-mail address: lmondello@pharma.unime.it (L. Mondello).

0308-8146/$ - see front matter © 2007 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2007.01.041

of the aromatic fraction of limoncello liquor seems to be an important item in assessing its genuineness and quality, besides "tracing" the various steps of the preparation procedure. Scientific literature reports just a few studies on Limoncello, however, in consideration of the data available it can be assumed that in many cases the need of industry for standard products leads to the addition of essential oils and/or related to the alcoholic syrup. The limoncello obtained with such a procedure is eventually an artificial product that does not have anything to deal with the original rosolio.

Apart from some attempts of investigation on lemon liquors reported in specific journals linked to the beverage industry (Moio, Piombino, Di Marzio, Incoronato, & Addeo, 2000; Bonomi, Lubian, Puleo, Tateo, & Fasan, 2001; Naviglio et al., 2001; Naviglio, Raia, Bergna, & Saggiomo, 2003; Romano, Schiavo, Iavarazzo, Battaglia, & Cassano, 2004; Naviglio, Pizzolongo, Mazza, Montuori, & Triassi, 2005), it can be stated that the increasing interest of the market toward limoncellos is not offset by the number of scientific papers.

One of the first papers that consistently faced up the analysis of limoncello liquor is by Dugo et al. (2000). The issues under investigation were in that case the physicochemical properties of different lemon liquors, ranging from the alcoholic grade, pH, etc., till the assessment of the volatile fraction chemical composition. The latter, in particular, was carried out by means of solvent extraction followed by the gas chromatographic analysis. In 2003, Versari et al. monitored various commercial limoncellos by means of both GC and HPLC techniques to establish quality markers, supporting their data with PCA analysis. Recently, Poiana, Attanasio, Albanese, and Di Matteo (2006) have investigated by means of GC-MS the volatile fraction of alcoholic extracts obtained from two different varieties of lemon fruits: *sfusato di Amalfi* and *limone di Sorrento*, with a particular attention to the seasonal variations. The need for a better comprehension of the chemical nature of limoncello is also dictated by the Council Regulation no. 1576/89 that lays down a definition and a description of spirit drinks. Besides giving a list of rules on alcoholic beverages, the regulation clearly states that even nature-identical flavouring substances and preparations shall not be authorized in liqueurs derived from Citrus fruits.

Aim of this study was therefore to investigate limoncello trying to gain a comprehensive knowledge about its qualitative and quantitative characteristics. In order to this, different analytical techniques have been applied: solid phase microextraction (SPME) (Pawlisyn, 1997), a well established sample preparation technique, was chosen to extract the volatiles of limoncello, whereas separation and identification of analytes were performed by gas chromatography-quadrupole mass spectrometry. Volatiles of lemon liquors of different commercial brands were then compared to the quali-quantitative composition of lab-made limoncellos. The volatile fraction also underwent chiral GC analysis, while the non volatile fraction was analyzed by RP-HPLC. A comparison between the alcohol content reported on the bottle labels and the one experimentally determined was made.

2. Materials and methods

2.1. Samples

Sixteen lemon liquors of commercial origin were purchased at the local grocery stores. Other two limoncello liquors were prepared in laboratory, each based on a different recipe. The first one, used for making a limoncello called *immersed*, has been carried out as follows: 12 lemon fruits (*Verdelli*), weighing about 900 g, were accurately peeled out and the skins, consisting of the *flavedo* parts, were put into 1 L of pure ethyl alcohol (95% vol.) and left to draw for 2 weeks. After this period, the lemon peels were taken out of the alcohol and a syrup made with 1 L of water plus 800 g of sugar was added to the ethanolic extract. The liquor obtained was let at rest for a couple of days before being analyzed. The other limoncello, called *exposed*, was obtained as follows: lemon skins, taken from 12 lemon fruits (*Verdelli*) weighing about 900 g in total, were put into a nylon net. The latter was suspended at the edge of a glass container, containing 1 L of pure ethanol (95% vol.), and the container sealed for 3 weeks in a dark and cool place. Indeed, the lemon oil extraction occurred without any direct contact of the solvent with the fruit peels.

All the limoncellos were kept in the refrigerator at about 7 °C. Furthermore, about 2 kg of lemons belonging to the same stock used for preparing the "lab-made" limoncello, were peeled out and the peels were manually squeezed to draw about 1.5 mL of essential oil from the utricles. The oil was diluted 1:10 in hexane prior to GC analysis.

2.2. SPME conditions

The SPME extractions were carried out in the headspace mode by means of an AOC-5000 autosampler (Shimadzu) hyphenated with the GC system. The experimental conditions were as follows: the fiber stationary phase, provided by Supelco, was a Polydimethylsiloxane (PDMS), 100 μm thick, 1 cm long. Samples were diluted 1:100 in HPLC grade water prior to the SPME extraction. An aliquot of 5 mL of each liquor solution was put into a 10 mL crimped vial spiked with 100 μL of a 100 ppm solution of nonane used as internal standard, and analyzed in triplicate. Samples were conditioned for 10 min at 40 °C, under agitation (clockwise, anticlockwise rotation at 500 rpm), and then underwent the extraction step for 30 min at 40 °C, still under agitation as previously indicated. Analytes were then desorbed for 5 min at 250 °C into the GC injector, that was kept in splitless mode during fiber desorption.

2.3. Calibration

Five compounds were chosen as key compounds representative of the main chemical classes determined in the aromatic fraction of lemon liquor. Based on theoretical considerations, compounds used for calibration should show the same behaviour of all the components of the same chemical class toward the PDMS fiber extraction process. In particular, limonene was chosen for monoterpenes, (E)-caryophyllene for sesquiterpenes, geranyl acetate for esters, neral for aldehydes and linalool for alcohols. In order to carry out accurate quantitative analysis, calibration graphs were made up on six points. Each measurement is the average of three independent analyses. The linearity of calibration graphs was tested over the expected concentration range of the chemical species as determined in the real liquors. Stock solutions of each standard were prepared in ethanol. Successive dilutions used for each calibration point were prepared using a mix of water/ethanol/sugar (1:1:0.8, v/v/w), in order to reduce the matrix effect.

After dilution, the standard compounds used for building up calibration curves were present in a concentration very close to the real one in limoncellos. Dilution was also necessary for decreasing the concentration of ethanol, that otherwise would have immediately saturated the SPME fiber. The resulting solutions were extracted by HS-SPME under the same conditions used for limoncello samples. In particular, each solution was diluted 1:100 with water. 5 mL of the final solution were put into an SPME vial and added with 100 μL of a 100 ppm standard solution of nonane (internal standard). The sample was then subjected to HS-SPME extraction and GC analysis, in triplicate. Data obtained for calibration were used to quantitate (as mg/L) groups of compounds in the real samples. Nonane was used as internal standard in order to correct absolute area shift of standard compounds when the fiber performance was lower because of the wear.

2.4. GC-FID analyses

For gas chromatographic separations, a Shimadzu GC-2010 system was used. The split/splitless injector was held at a temperature of 250 °C, and, after sampling time (5 min) in splitless mode, a split ratio of 1:50 was applied. Carrier gas was helium, at a constant linear velocity of 35.0 cm/s and a pressure of 120.4 kPa. All the analyses were carried out on a 30 m × 0.25 mm i.d. × 0.25 μm d_f SLB-5MS column (Supelco, Milan, Italy), temperature programmed as follows: 40 °C at 3 °C/min to 250 °C, held 2 min. The FID temperature was set at 260 °C (sampling rate 80 ms) and gas flows were 50 mL/min for hydrogen, 50 mL/min for makeup (N_2/Air) and 400 mL/min for air, respectively. Data were collected by using GCsolution software (Shimadzu).

2.5. GC-MS analyses

GC-MS analysis was performed on a Shimadzu QP2010, equipped with an SLB-5MS column (Supelco, Milan, Italy), 30 m × 0.25 mm i.d. × 0.25 μm d_f. Gas chromatographic parameters were the same as for GC-FID analyses, except for: carrier gas (He) linear velocity was 32.4 cm/s, split ratio was 1:10, sampling time was 1 min. The ion source temperature was set at 200 °C, the interface temperature was 250 °C, detector voltage was 0.94 kV. The acquisition took place in scan mode at a scan speed of 769 within a mass range of 40–400 amu. The software used was GCMS solution by Shimadzu.

2.6. Chiral GC

Chiral GC analyses were performed on a GC-2010 (Shimadzu) at the following experimental conditions: column used was a diethyltertbutylsilylbetaCDX 25 m × 0.25 mm i.d., 0.25 μm d_f (Mega, Milano, Italy). The injector temperature was set at 250 °C in split mode, with a split ratio equal to 30:1. Helium was chosen as carrier gas, at a linear velocity of 29.3 cm/s and a pressure of 96 kPa. The oven was temperature programmed from 45 °C, held 6 min, at 2 °C/min to 200 °C, held 5 min. FID temperature was set at 250 °C, with a sampling rate of 80 ms. Makeup gas (N_2) flow was 50 mL/min, H_2 flow was 50 mL/min, air flow was 400 mL/min.

2.7. RP-HPLC

Samples were filtered out through paper. One millilitre of each limoncello was added with 50 μL of internal standard solution (coumarin in acetonitrile 0.1% w/v). The HPLC system was equipped as follows: two SCL-10-AVP pumps, an SCL-10-AVP controller, a photodiode array detector (SPD-M10 Avp), a DGU-14A degasser (all the equipment was Shimadzu); the column used was a Discovery-HS C_{18} (Supelco, Milan, Italy), 250 × 4.6 mm i.d., 5 μm particle size. The mobile phase consisted of water (A) and acetonitrile (B), programmed as follows: 0–1 min, 30% B; 1–5 min, 60% B; 5–8 min, 60% B; 8–30 min, 100% B; 30–35 min, 30% B; 35–40 min, 30% B. All the solvents were HPLC grade (Merck, Germany). Volume injected was 20 μL at a flow rate of 1 mL/min. UV spectra were acquired from 190 to 370 nm, and the chromatogram was extracted at 315 nm. Sample frequency was 1.5625 Hz. Data were acquired by means of Class-VP software (Shimadzu).

2.8. Determination of alcohol content

The alcohol content of all the limoncellos was determined by means of a Malligand ebulliometer (Tecnolab, Belpasso, CT, Italy), diluting samples 1:2 with water prior to analysis.

3. Results and discussion

As reported in Section 1, the production of limoncello is not under a specific regulation of the EU. Based on what established by the Council Regulation No. 1576/89, the addition of Citrus essential oils and/or Citrus key compounds such as terpenes is not allowed and a valid method for assessing the correct procedure for making it becomes relevant in such a point of view. Furthermore, the unpleasant phenomenon known as "collarino" that happens to be in the bottles containing genuine limoncello and caused by the decrease of solubility of terpenes under refrigeration, pushes the producers to use terpeneless oils. It cannot be neglected that terpenes are unstable compounds, that very easily undergo degradation. The mechanism of their breakdown and reactivity in Citrus oils has been quite extensively studied (Schieberle & Grosch, 1989; Neumann & Garcia, 1992; Pokorny, Pudil, Volfova, & Valentova, 1998). Terpenes are also photosensitive: limonene and terpinenes, in particular, are involved in photodegradation, that is an irreversible process which degrades the oil and negatively affects the organoleptic properties of the beverage. For all these reasons, the terpene fraction and/or the presence of oxidized by-products can predict the origin and the quality of a limoncello.

3.1. SPME method development

All the SPME extraction steps were subjected to trials for method optimization. The choice of the fiber (PDMS) was dictated by the non-polar nature of the samples to be analyzed; 100 μm was chosen as the most proper film thickness in order to have the highest solute extraction of a poorly concentrated sample as can be the volatile fraction of a limoncello. The extraction time was tested at 10, 20, 30, 40 and 50 min, being the extraction yield more or less constant from 30 min on. For this reason, 30 min was chosen as the most reasonable fiber exposure time. Since it is well known that the SPME process, when carried out in the headspace (HS) mode, is definitely improved in the recovery yield if the fiber exposure starts in a chemically saturated environment, samples underwent previously an incubation period under agitation and warming up. Different incubation times (5, 10, 15 min) were tested and 10 min was chosen either for the headspace saturation or for time saving. Another trial was made on the desorption time of the fiber, by inserting the needle into the GC injection port for 1, 5 and 10 min, respectively. Five minutes was considered the best desorption time and this value was set as GC sampling time too.

3.2. GC-FID analysis

Table 1 reports quantitative data relative to the hand-squeezed lemon essential oil. With the exception of alpha-thujene (compound no. 2), its quali-quantitative composition falls within the ranges of a typical and genuine essential oil, as reported by Dugo et al. (1999): monoterpenes were 90.6%, sesquiterpenes were 1.0%, while oxygenated compounds were 8.4%. It has to be pointed out, though, that similar values of alpha-thujene, falling into the range of 0.27–0.43%, were found by other authors for laboratory extracted lemon oils (Ayedoun, Sossou, Mardarowicz, & Leclercq, 1996; Caccioni, Guizzardi, Biondi, Renda, & Ruberto, 1998) and for Mediterranean *Citrus limon* oils (Boelens & Jimenez, 1989).

Fig. 1 shows the GC chromatograms obtained for the two laboratory-made samples of limoncello. As can be seen, different recipes lead to different compositions of the aromatic fractions. In the case shown in Fig. 1, it is evident that the ethanolic extraction was less powerful in the limoncello *exposed*, where the oxygenated compounds (middle region of the chromatogram) are almost absent, as well as great part of sesquiterpenes, which are definitely less represented in this type of limoncello compared to the profile obtained for limoncello *immersed*. These observations can be confirmed with quantitative values reported in Table 2. This table reports the absolute amount (mg/L) of components identified in the HS-SPME extracts of all the analyzed samples. These values were calculated using calibration curves obtained from the HS-SPME extraction of key components, under the same experimental conditions used for the analysis of real samples, against *n*-nonane used as internal standard. Absolute amounts (mg/L) of monoterpene hydrocarbons in the two lab-prepared are comparable, while the amounts of oxygenated components and sesquiterpenes are lower in the *exposed* sample (see Table 2). The poorer aromatic profile of limoncello *exposed* could maybe due to the chemical process of extraction itself. Since no physical contact occurs between lemon peels and alcohol in the *exposed* limoncello, the only way for extracting the essential oil is by headspace. Perhaps, this process is less effective for heavier compounds, such as sesquiterpenes, or for more polar compounds such as oxygen containing molecules. In comparison to lemon oil, limoncello *immersed* had lower amounts of terpene hydrocarbons and higher amounts of oxygenated components. This is in accord with the procedure used to make limoncello. In fact, hydrocarbons have a lower solubility in alcohol than oxygenated compounds. Values obtained for limoncello *exposed*, on the contrary, presented a monoterpene hydrocarbons level higher than that of the oil obtained from the same lemon fruits, and lower values of sesquiterpene hydrocarbons and oxygenated components. These values are in accordance with the procedure followed for the preparation of this liqueur, where the components with the highest vapour pressure were more easily extracted.

The two lab-made limoncellos differ in their organoleptic properties as well: the colour of limoncello *immersed* is stronger and deeper (intense yellow with green shades), as well as its consistency, thicker and dense, and all these findings make it somehow lighter and finer to the taste.

Table 1
GC-FID analysis: composition of the volatile fraction of lab extracted lemon oil (mg/L) and of pseudo-limoncello (mg/L)

Compound	Oil (mg/L)	Liter range	Pseudolimoncello (mg/L)
Tricyclene	30	30–80	4.0
α-Thujene	2470	3700–5430	7.1
α-Pinene	14,510	14,960–24030	21.0
Camphene	340	460–810	4.8
Sabinene	17,570	11,280–27,940	211.3
β-Pinene	142,090	94,530–177,940	
Myrcene	11,900	10,530–18,600	18.8
Octanal + α-phellandrene	1210	210–1370	37.2
δ-3-Carene	40	10–100	4.4
α-Terpinene	1350	490–2510	6.1
p-Cymene	340	250–6750	4.5
Limonene	626,290	595,700–710,600	837.2
(Z)-β-ocimene	470	310–1490	5.1
(E)-β-ocimene	790	700–2040	5.4
γ-Terpinene	104,670	65,860–112,750	143.9
cis-Sabinene hydrate + Octanol	650	140–740	32.1
Terpinolene	3360	2050–4380	8.8
Linalool	1290	490–1790	85.1
Nonanal	1350	440–1940	44.4
cis-Limonene oxide	10	20–240	4.4
trans-Limonene oxide	10	20–190	4.4
(E)-myroxide	20		<0.1
Camphor	100	30–150	7.5
Citronellal	940	400–1660	30.8
Borneol	230	10–170	4.0
Terpinen-4-ol	200	100–800	11.6
p-Cymen-8-ol	70	–	<0.1
α-Terpineol	3190	580–2760	158.7
Dodecane	40	–	<0.1
Decanal	540	120–820	19.2
Octyl acetate	40	10–110	0.9
Citronellol	1160	60–1790	16.2
Neral	13,520	4550–13,330	390.0
Geraniol	270	40–590	3.2
Geranial	20,960	6020–22,520	581.6
Perillaldehyde	280	–	18.5
Perilla alcohol	20	–	3.9
Undecanal	290	20–460	12.0
Nonyl acetate	50	20–190	0.9
Citronellyl acetate	190	50–820	1.0
Neryl acetate	5480	2280–8830	4.8
Geranyl acetate	7190	1630–8090	5.9
Tetradecane	90	–	<0.1
α-*cis*-Bergamotene	110	–	<0.1
(E)-Caryophyllene	2170	1070–3340	0.8
α-*trans*-Bergamotene	2350	2110–5790	0.9
cis-β-Farnesene	40	–	<0.1
α-Humulene	300	70–340	0.1
(Z)-β-santalene	70	–	<0.1
Geranyl propionate	100	–	0.9
Germacrene D	100	30–190	<0.1
Valencene	980	10–880	0.3
Bicyclogermacrene	220	–	0.1
β-Bisabolene	3970	2950–9160	1.5
δ-Cadinene	30	–	<0.1
(E)-γ-bisabolene	80	–	<0.1
(E)-α-bisabolene	150	–	<0.1
Norbornanol	130	90–380	2.5
Campherenol	130	70–340	5.5
α-Bisabolol	20	90–300	<0.1
Nootkatone	60	10–100	5.8
Total	996,620		2779.7
Monoterpenes	926,250	921,830–966,440	1315.5

(*continued on next page*)

Table 1 (*continued*)

Compound	Oil (mg/L)	Liter range	Pseudolimoncello (mg/L)
Sesquiterpenes	10,760	8110–21,740	3.8
Total hydrocarbons	937,010	936,680–976,770	1319.3
Oxygenated compounds	59,600	42,790–115,980	1460.3

Values are means of three repetitions.

Table 2 reports the absolute amount (mg/L) relative to the peaks identified in the HS-SPME extracts also for all the other commercial samples analyzed. As can be seen, commercial limoncellos greatly differ in the amount of volatile components responsible for the aromatic profile. The analyzed samples presented values ranging from 155 mg/L (sample #3) to 2551 mg/L (sample #5). However, apart from sample #5, the volatile components of commercial limoncellos were always lower than 1050 mg/L with 10 samples vs. a total of 16 ranging from 400 to 1050 mg/L, and the remaining five samples ranging from 154 to 327 mg/L. Substantial quantitative differences can be seen for many of the components of the volatile fraction. However, all the samples presented a GC profile, from a qualitative point of view, similar to that of genuine cold-pressed lemon oil; in fact, the presence of peaks different from those encountered in genuine lemon oil has never been detected. If we observe the composition of the volatile fraction of some samples (#11, #14 and #16), an extremely high amount of neral and geranial (known as citral) is

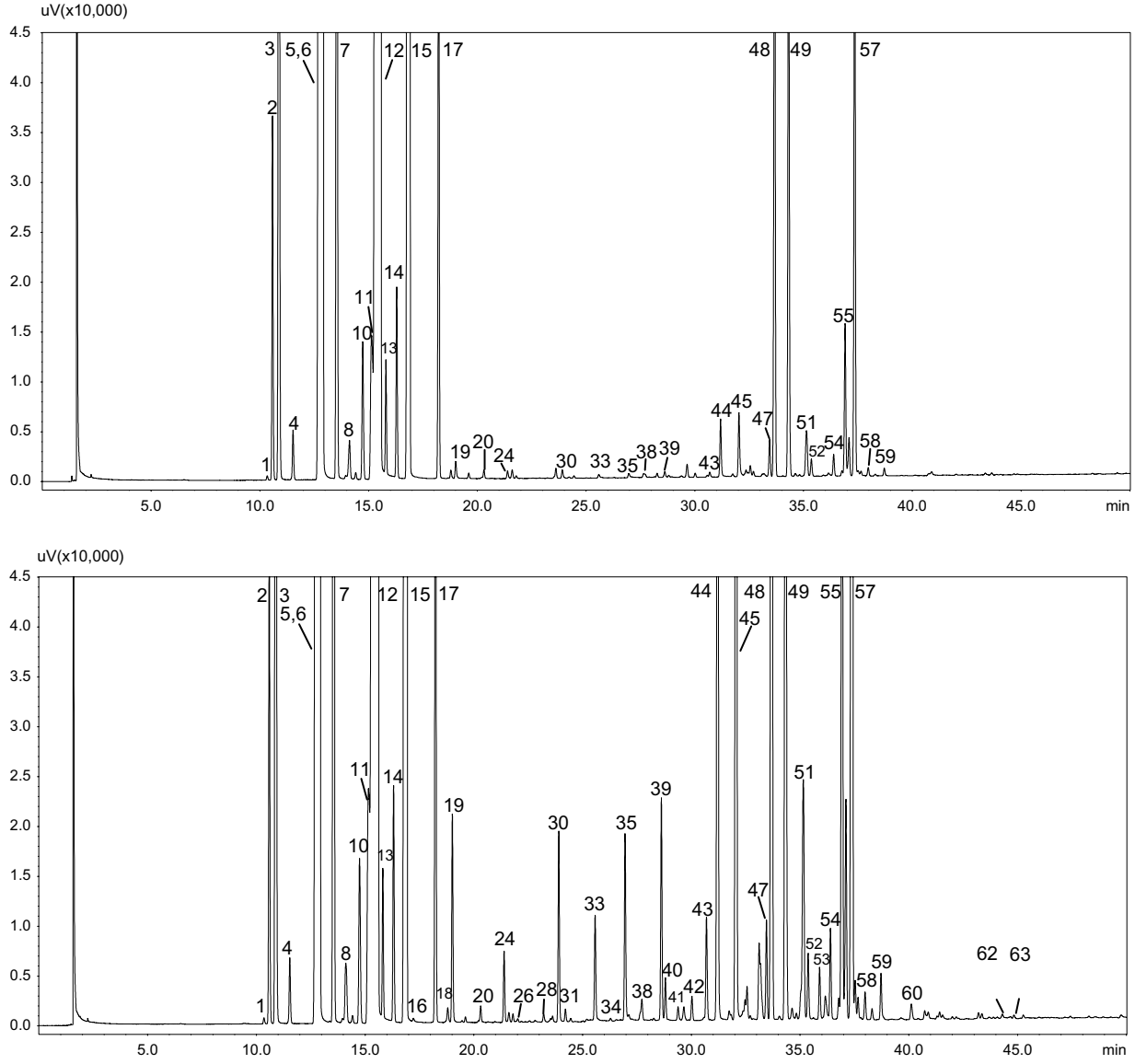

Fig. 1. HS-SPME-GC profiles of limoncello *exposed* (top) and *immersed* (bottom).

Table 2
SPME-GC-FID analysis: composition of the volatile fraction of limoncellos. Values are expressed as mg/L

No.	Compound	Limoncello																	
		Immersed	Exposed	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15	#16
1	Tricyclene	8.8	8.8	–	–	–	–	8.8	–	–	–	–	–	–	–	–	–	–	–
2	α-Thujene	11.8	11.9	–	9.4	–	8.9	13.0	9.1	–	8.9	8.9	8.7	8.8	–	9.6	8.8	8.7	–
3	α-Pinene	29.4	29.9	8.8	13.4	8.9	12.6	36.5	11.6	10.6	12.5	9.9	8.9	9.7	9.7	14.6	9.2	9.0	8.9
4	Camphene	9.2	9.2	–	8.8	–	8.9	9.4	8.8	8.9	8.9	8.8	–	8.8	8.9	8.9	8.7	–	–
5	Sabinene	35.1	37.8	–	14.4	9.0	–	20.8	8.8	–	–	9.7	9.0	–	–	14.9	–	–	9.0
6	β-Pinene	228.6	247.3	9.5	66.8	11.6	37.1	247.9	42.4	12.2	44.5	23.4	11.2	18.4	9.3	78.5	13.6	11.0	11.6
7	Myrcene	28.2	29.3	8.9	16.1	9.0	13.0	35.7	12.1	11.0	11.0	9.2	9.1	10.5	10.2	15.0	9.5	9.3	9.1
8	Octanal + α-phellandrene	19.4	19.0	18.6	18.4	–	18.6	19.2	8.8	21.1	26.3	15.3	21.4	22.0	21.3	23.2	10.8	10.1	10.5
9	δ-3-Carene	8.8	8.8	–	–	–	–	8.8	8.8	–	–	–	–	8.8	–	–	–	–	–
10	α-Terpinene	10.1	10.2	8.8	–	–	9.4	13.4	9.1	8.9	8.7	8.8	8.8	8.9	9.0	9.4	8.8	8.8	8.9
11	*p*-Cymene	12.6	11.3	9.2	84.9	13.3	17.4	11.8	17.2	17.6	28.7	12.7	10.9	14.5	15.3	58.5	13.2	9.5	11.3
12	Limonene	1214.5	1263.9	26.5	634.6	43.6	353.3	1671.0	272.1	212.1	288.78	42.6	45.3	179.3	141.4	475.7	50.2	60.6	57.9
13	(Z)-β-ocimene	9.8	9.8	–	–	–	–	10.2	–	–	–	8.9	8.7	–	–	9.0	–	–	–
14	(E)-β-ocimene	10.4	10.5	–	8.8	–	9.1	11.2	8.9	8.9	8.9	8.8	8.8	8.8	8.8	9.0	–	–	8.8
15	γ-Terpinene	228.6	233.5	9.5	11.9	10.3	47.8	281.9	31.5	30.2	34.0	12.3	10.1	13.8	21.6	25.1	8.9	10.9	15.9
16	*cis*-Sabinene hydrate + octanol	2.7	–	–	1.7	–	–	1.7	–	–	–	2.0	–	–	–	–	–	–	–
17	Terpinolene	15.4	15.2	8.8	8.9	8.7	12.0	20.1	9.7	11.1	9.0	8.9	8.9	9.2	12.1	10.3	8.9	8.9	9.0
18	Linalool	7.5	4.1	1.7	5.9	2.1	9.2	5.0	2.2	5.2	5.6	4.3	3.0	2.1	5.8	4.7	5.2	–	2.3
19	Nonanal	49.4	13.6	10.6	14.1	12.5	–	–	–	11.5	10.3	–	16.7	–	9.7	–	20.3	10.5	13.3
20	*cis*-Limonene oxide	8.9	8.8	–	9.0	–	–	9.4	–	–	–	8.9	8.7	–	–	9.0	–	–	8.8
24	Citronellal	24.4	11.1	11.0	10.7	–	–	9.0	–	–	–	–	9.6	13.9	19.6	–	12.7	17.0	13.4
26	Terpinen-4-ol	2.3	–	1.6	–	0.6	5.6	0.6	0.5	–	3.6	–	–	–	–	–	3.3	–	2.9
28	α-Terpineol	4.3	–	1.6	3.1	1.7	6.1	3.0	2.3	26.9	13.9	3.2	5.0	1.8	17.0	3.0	7.6	–	6.2
30	Decanal	47.7	11.5	13.2	13.5	11.1	10.1	10.9	–	12.3	16.2	–	20.8	9.8	11.6	–	16.4	15.0	15.5
31	Octyl acetate	1.8	–	–	1.7	–	–	1.7	–	1.8	1.8	–	–	–	1.7	–	1.8	–	–
33	Neral	33.5	10.0	30.4	10.8	–	23.6	11.7	13.5	10.2	11.0	10.1	24.8	64.0	26.5	9.9	44.6	26.3	85.0
34	Geraniol	1.8	–	3.3	4.9	1.9	64.7	10.9	10.9	296.1	106.2	4.6	2.5	6.2	177.5	4.6	21.8	2.8	3.6
35	Geranial	50.8	10.3	40.2	10.1	–	24.5	17.9	–	9.7	13.7	10.2	32.1	94.0	19.4	11.3	65.2	40.4	133.5
36	Perillaldehyde	10.4	–	–	–	–	–	–	–	–	16.6	9.6	9.8	–	–	–	–	–	–
37	Isobornyl acetate	–	–	2.6	1.8	–	–	–	–	–	–	–	–	–	–	–	–	–	–
38	Perilla alcohol	12.1	2.6	–	–	3.6	13.3	10.4	10.7	22.3	25.2	6.3	4.8	4.2	13.7	6.8	–	5.2	9.5
39	Undecanal	53.2	10.4	10.4	13.2	–	–	–	–	10.3	9.9	–	13.5	–	9.0	–	12.0	10.4	13.5
40	Nonyl acetate	1.9	–	–	1.7	–	–	–	–	1.7	–	–	–	–	–	–	1.7	–	1.7
41	Methyl geranoate	–	–	–	1.7	–	–	1.7	–	–	1.7	2.0	–	–	–	–	1.7	–	–
42	Bicycloelemene	<0.1	–	–	–	–	–	<0.1	–	–	–	–	–	–	–	–	–	–	
43	Citronellyl acetate	2.3	1.7	1.8	2.2	1.7	1.9	1.8	–	2.0	2.0	1.9	1.8	1.9	2.6	1.9	3.4	1.8	2.1
44	Neryl acetate	10.9	2.1	2.3	4.8	2.0	2.7	3.8	2.2	4.6	5.1	4.1	2.0	2.5	7.2	4.2	11.0	2.3	3.7
45	Geranyl acetate	14.7	2.2	2.1	4.4	2.0	2.6	2.3	2.0	5.0	5.3	2.8	1.7	2.2	3.2	2.6	12.4	2.1	3.3
47	α-*cis*-Bergamotene	0.2	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
48	(E)-caryophyllene	8.0	1.8	0.5	4.2	0.3	2.8	4.5	0.6	1.2	3.4	0.5	0.2	2.2	0.9	2.8	1.1	0.8	1.3
49	α-*trans*-Bergamotene	5.9	1.7	0.3	7.7	0.3	2.5	7.9	0.8	1.7	4.3	2.2	0.1	0.8	0.5	5.8	1.8	0.3	1.2
51	α-Humulene	0.9	0.1	0.1	0.7	<0.1	0.6	0.7	0.1	0.3	0.3	0.1	<0.1	0.4	0.1	0.5	0.1	0.2	0.2
52	(Z)-β-santalene	0.2	<0.1	–	0.2	–	<0.1	0.2	<0.1	0.1	0.1	<0.1	–	<0.1	<0.1	0.1	<0.1	–	<0.1
53	Geranyl propionate	0.1	–	<0.1		–	–	–	–	<0.1	<0.1	–	–	0.3	<0.1	–	<0.1	0.2	<0.1
54	Germacrene D	0.3	<0.1	–	0.3	–	0.1	0.3	<0.1	<0.1	0.2	<0.1	–	<0.1	–	0.3	<0.1	–	–
55	Valencene	3.0	0.3	<0.1	0.7	<0.1	0.2	1.3	<0.1	0.4	0.7	0.2	<0.1	0.4	<0.1	1.2	0.4	0.2	<0.1

(*continued on next page*)

Table 2 (*continued*)

No.	Compound	Limoncello															
		Immersed	Exposed	#1	#2	#3	#4	#5	#6	#7	#8	#9	#10	#11	#12	#13	#14	#15	#16
56	Bicyclogermacrene	0.7	<0.1	<0.1	0.3	<0.1	0.3	0.6	<0.1	0.3	0.4	0.2	–	–	–	0.4	<0.1	–	0.2
57	β-Bisabolene	11.9	1.6	0.7	12.9	0.4	3.9	10.1	0.8	4.1	8.0	3.5	0.1	1.4	0.7	6.7	3.2	0.6	2.8
58	δ-Cadinene	0.1	–	–	–	–	–	<0.1	–	–	–	–	–	–	–	–	–	–	–
59	(E)-γ-bisabolene	0.1	–	–	<0.1	–	<0.1	0.1	–	<0.1	<0.1	<0.1	–	–	–	<0.1	–	–	<0.1
60	(E)-α-bisabolene	<0.1	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
62	Campherenol	2.4	–	–	2.6	–	–	1.7	1.7	3.1	2.1	–	–	–	–	–	1.7	–	–
63	α-Bisabolol	2.3	–	–	2.9	–	–	2.1	2.1	3.2	3.4	3.0	–	–	–	2.3	1.6	–	–
Total identified fraction		2256.8	2053.4	245.1	1044.2	154.6	722.9	2551.0	509.4	786.3	760.9	267.9	327.1	529.3	594.2	839.7	408.8	283.0	493.7
Monoterpene hydrocarbons		1879.4	1955.3	98.6	895.7	114.3	538.3	2419.2	458.9	340.1	472.6	181.7	165.9	308.0	255.0	756.4	139.7	136.6	167.9
Sesquiterpene hydrocarbons		31.3	5.5	1.8	27.0	1.1	10.5	25.7	2.4	8.1	17.4	6.8	0.5	5.6	2.3	17.8	6.7	2.3	5.8
Total hydrocarbons		1910.7	1960.9	100.4	922.7	115.3	548.8	2444.8	461.3	348.2	490.1	188.5	166.4	313.6	257.3	774.1	146.4	139.0	173.7
Oxygenated compounds		346.1	92.5	144.7	121.5	39.3	174.2	106.1	48.1	438.1	270.9	79.4	160.7	215.8	336.9	65.6	262.4	144.0	320.0

present. Citral is known to be the responsible of lemon aroma. Probably in the above mentioned samples, citral has been added in order to increase the flavour of the liqueur. Another point, for these samples with very high amount of oxygenated components, is the possibility, in their preparation, of the use of terpeneless oils. One important issue is also the content of *p*-cymene, that is considered a sort of storage time marker in a lemon oil: some limoncellos (brands #2, 8 and 13) presented an extremely high content of this hydrocarbon, up to values of ~85 mg/L.

In order to confirm data obtained from calibration procedures, a pseudo-limoncello was prepared by adding 25 μL of the lab extracted lemon oil to 10 mL of the alcoholic syrup (water/ethanol/sugar, 1:1:0.8, v/v/w). Once prepared, the pseudo-limoncello underwent HS-SPME extraction and, afterwards, GC-FID analysis, at the same experimental conditions as for limoncellos. Data relative to this analysis are reported in Table 1, from where it can be seen that the absolute amount of the total identified fraction is very near to the true value (~2500 mg/L) with a relative error of $E_r = 11.2\%$.

3.3. GC-MS analysis

Peak assignment was substantially performed on the basis of the extensive knowledge of Citrus essential oils and on the several papers of the research group to which authors refer (Cotroneo et al., 1986; Verzera et al., 1987; Verzera et al., 1999; Dugo et al., 2002). However, GC-MS analysis was greatly supported by the use of a laboratory constructed library, named *FFNSC ver. 1.2* (*Flavour and Fragrance Natural and Synthetic Compounds*) (Shimadzu, Milan, Italy). This GC-MS database contains spectra derived from pure chemicals, essential oils and commercial fragrances. Unlike common GC-MS libraries, the FFNSC, provided with a very versatile software (GCMS solution), works as a dual-filter GC-MS library, basically by means of the Linear Retention Indices calculated and registered for each compound. In fact, if different molecules, after ionization, can give rise to almost identical spectra, it is less likely that they have the same LRI. The latter is used to filter the library search results, since spectra with an LRI very dissimilar from the one of the unknown compound, are automatically rejected. This innovative tool gives often an easy solution to the GC-MS user in trouble with the identification of molecules having similar structure, a quite common occurrence when dealing with flavours and fragrances (Kovats, 1958; van den Dool & Kratz, 1963; Mondello, Dugo, Basile, Dugo, & Bartle, 1995).

3.4. e-GC analysis

There is an extensive knowledge on the importance of the enantiomeric distribution of the components in essential oils (Mondello, Dugo, & Dugo, 2002). The assessment of the enantiomeric ratios is considered one of the most significant parameters linked to the genuineness and authenticity

of an oil. Essential oils inevitably contain molecules with one or more asymmetric carbon atoms, that give rise to different enantiomers. The distribution of the enantiomer ratios represents a powerful tool to detect essential oil adulteration by means of nature-identical synthetics. In fact, the addition of these synthetic compounds to the natural oil in most cases distorts the enantiomeric ratios of the naturally occurring compounds. This type of adulteration is definitely well investigated by means of gas chromatography with chiral stationary phases (modified cyclodextrins are the most popular and effective). Quite often, due to the different olfactive impact of one enantiomer compared to another one, this adulteration can change the effective organoleptic properties of an oil (e.g. (−)-carvone is minty, (+)-carvone is caraway-like).

Fig. 2 reports two e-GC chromatograms, the upper one relative to limoncello *immersed*, the lower one to a commercial limoncello (brand #6). Table 3 reports the enantiomeric distribution of β-pinene, sabinene and limonene in all the limoncello samples. The enantiomeric distribution of lab-prepared limoncellos for β-pinene, sabinene and limonene has been found to be very similar to that presented by the lemon essential oil obtained in laboratory from the same fruits used for the preparation of limoncello. These data are in perfect agreement with those reported in literature for cold-pressed genuine lemon oils (Mondello et al., 1999). This result demonstrates that the preparation of limoncello following the traditional recipe does not alter the enantiomeric distribution of monoterpene hydrocarbons. If we observe the results obtained for commercial limoncellos, some data are out of the ranges presented by genuine lemon oils. The enantiomeric ratios determined for sabinene in some limoncellos (brands #3, #6, #10 and #16) were approximately racemic, as well as for β-pinene in brand #12. Also, the enantiomeric ratio of (+)/(−)-limonene was anomalous in brands #7, #10, #12 and #16, and according to the data above discussed, it is likely that these limoncellos were made following different procedures, i.e. adding reconstituted oils containing citrus oils or citrus terpenes different from lemon and/or synthetic

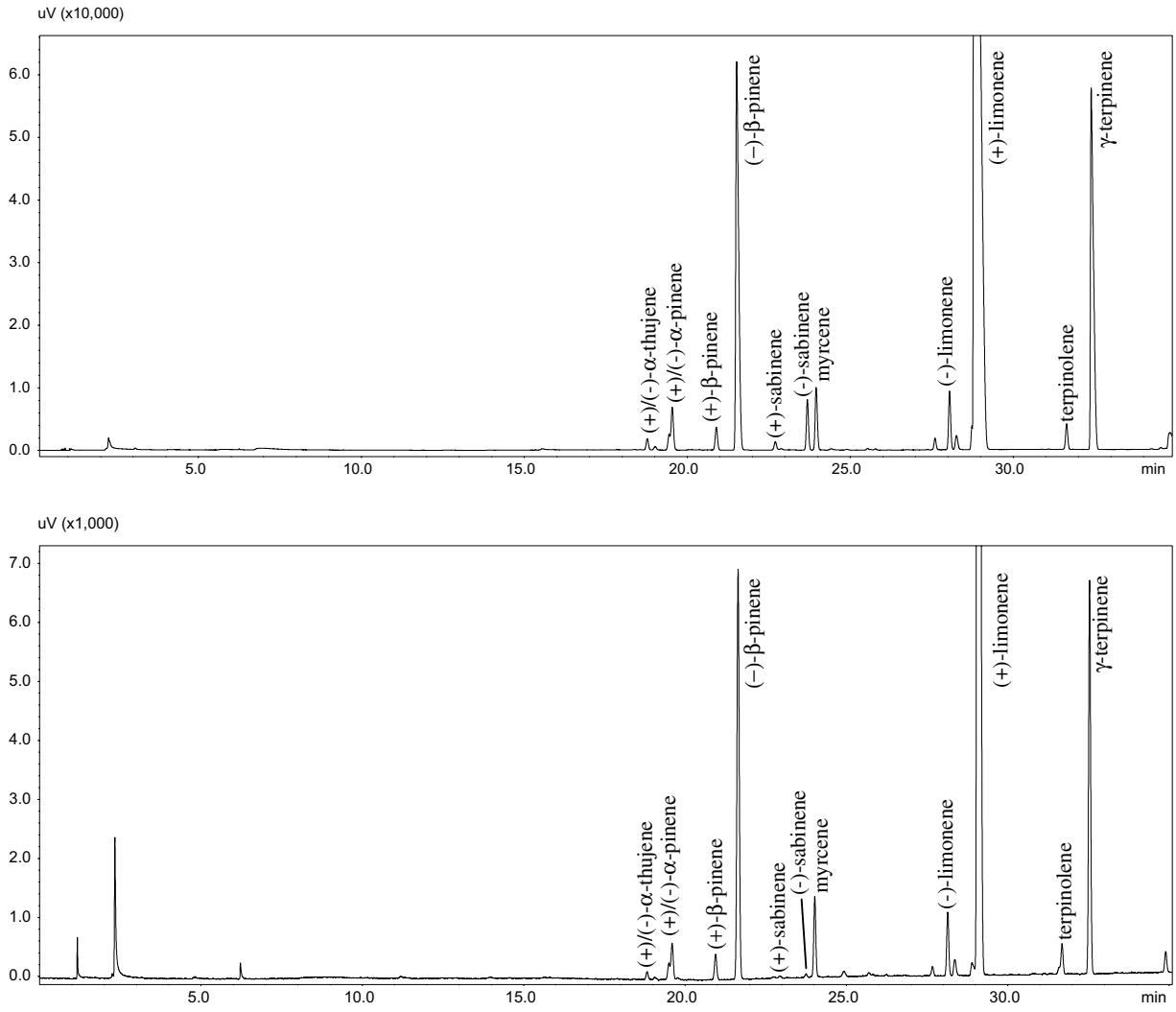

Fig. 2. Enantiomeric GC profile of the HS-SPME extracts of limoncello *immersed* (top) and brand #6 limoncello (bottom).

Table 3
Enantiomeric composition of lemon oil, commercial and lab-made limoncellos

Limoncello	1R,5R-(+)-β-Pinene	1S,5S-(−)-β-pinene	1R,5R-(+)-sabinene	1S,5S-(−)-sabinene	4S-(−)-limonene	4R-(+)-limonene
Literature[a]	4.2–7.0	95.8–93.0	12.5–15.3	87.5–84.7	1.5–2.0	98.5–98.0
Lemon oil	4.5	95.5	13.1	86.9	1.8	98.2
Immersed	4.6	95.4	16.3	83.7	2.1	97.9
Exposed	4.5	95.5	13.2	86.8	1.8	98.2
Brand #1	6.3	93.7	–	–	1.3	98.7
Brand #2	6.3	93.7	16.0	84.0	1.5	98.5
Brand #3	6.2	93.8	46.1	53.9	1.6	98.4
Brand #4	6.2	93.8	–	–	1.9	98.1
Brand #5	6.0	94.0	15.6	84.4	1.7	98.3
Brand #6	6.0	94.0	31.3	68.7	1.7	98.3
Brand #7	5.4	94.6	–	–	2.5	97.5
Brand #8	5.7	94.3	–	–	1.6	98.4
Brand #9	4.5	95.5	14.0	86.0	2.1	97.9
Brand #10	5.5	94.5	44.2	55.8	14.2	85.8
Brand #11	5.7	94.3	–	–	1.3	98.7
Brand #12	43.3	56.7	–	–	3.8	96.2
Brand #13	5.7	94.3	14.7	85.3	1.7	98.3
Brand #14	4.9	90.1	–	–	1.9	98.1
Brand #15	5.0	95.0	–	–	1.3	98.7
Brand #16	6.9	93.1	54.1	45.9	2.2	97.8

[a] Average ranges reported by Mondello et al. (1999).

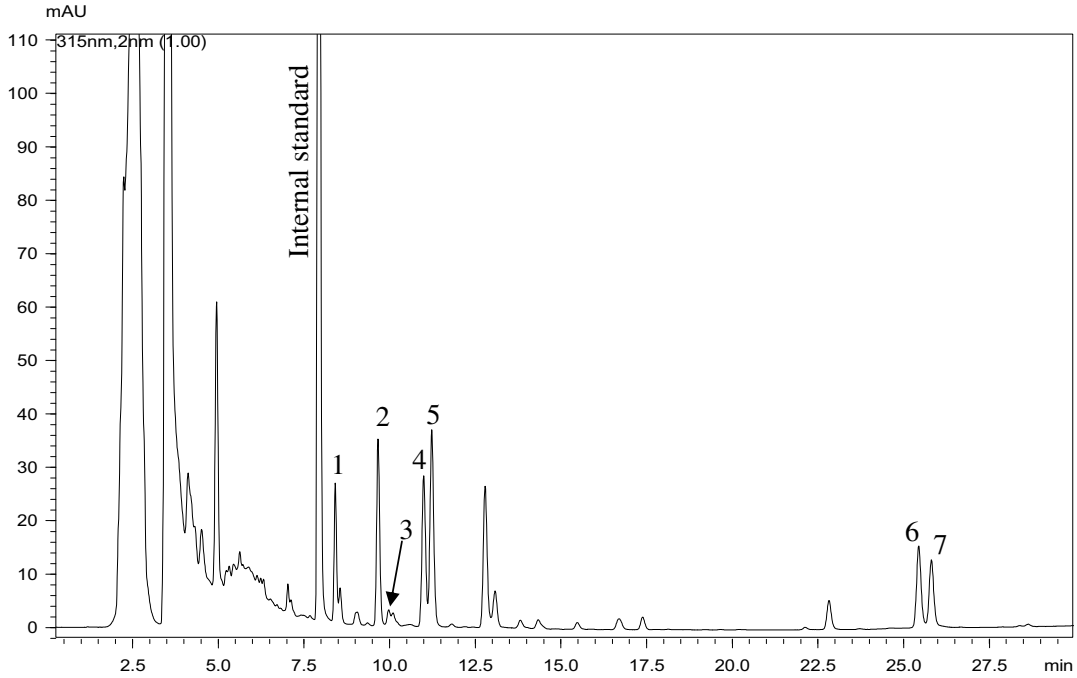

Fig. 3. HPLC chromatogram of limoncello *immersed* (see Table 4 for peak identification).

components. As an example, some brands (in particular #10 and #12) showed an altered ratio of (+)/(−)-limonene: it is a common adulteration to add (−)-limonene in order to adjust the value of the optical rotation (Dugo, Stagno d'Alcontres, Donato, & Dugo, 1993; Dugo, Verzera, Trozzi, & Cotroneo, 1994; Mondello, Catalfamo, Dugo, Proteggente, & Dugo, 1997; Dugo, Mondello, Cotroneo, Bonaccorsi, & Lamonica, 2001). This becomes somehow necessary to oil traders in the preparation of reconstituted oils, that is, made from nature identical synthetics, trying to achieve an analytical level as close as possible to the one of the genuine essential oil of interest.

3.5. HPLC analysis

Fig. 3 shows the HPLC chromatogram of coumarins and psoralens in a sample of limoncello *immersed*. These components are constituents of the non volatile fraction

Table 4
Content of coumarins and psoralens expressed as mg/L

Limoncello	1	2	3	4	5	6	7	Total
	Herniarin	Citropten	Bergapten	Byakangelicol	Oxypeucedanin	Bergamottin	5-Geranyloxy-7-methoxycoumarin	
Literature*	–	520–1420	–	660–1230	890–1570	1600–2910	1800–2500	–
Lemon oil	715.6	1712.3	280.9	334.3	4591.1	3291.1	2393.8	16,330.2
Immersed	1.2	2.6	0.3	4.9	6.7	3.1	2.4	21.2
Exposed	0.1	0.2	<0.1	0.3	0.5	0.4	0.3	1.9
Brand #1	<0.1	0.4	<0.1	<0.1	0.1	0.3	0.2	1.1
Brand #2	–	2.7	–	0.7	1.0	2.0	1.6	7.9
Brand #3	0.6	1.5	0.4	–	–	0.7	0.8	4.1
Brand #4	–	0.4	–	<0.1	–	0.5	0.4	1.4
Brand #5	–	2.0	–	0.5	0.7	2.5	1.9	7.6
Brand #6	<0.1	0.9	–	0.2	0.7	0.5	0.4	2.7
Brand #7	1.1	3.0	0.3	–	0.5	1.1	0.9	7.1
Brand #8	<0.1	2.3	–	0.4	–	1.1	0.7	4.5
Brand #9	0.5	1.5	0.1	0.3	0.4	1.1	0.6	4.5
Brand #10	0.1	0.7	<0.1	0.1	0.1	0.3	0.3	1.6
Brand #11	–	0.4	<0.1	0.2	0.2	0.1	0.1	1.0
Brand #12	<0.1	0.8	–	–	0.1	0.4	0.3	1.5
Brand #13	0.1	2.1	–	0.3	0.5	1.3	1.0	5.3
Brand #14	–	0.3	<0.1	<0.1	0.1	0.2	0.1	0.8
Brand #15	–	0.3	–	0.4	0.1	0.2	0.2	1.2
Brand #16	<0.1	0.7	<0.1	0.1	0.3	0.9	0.7	2.7

For the HPLC analytical parameters, s.

of cold-pressed lemon oil. Table 4 reports quantitative results obtained for all the analyzed samples, compared to those of the literature for cold-pressed lemon oils and to those obtained for the lab-made lemon oil using *Verdelli* fruits.

As can be seen, limoncello *immersed* presents a higher amount of coumarins and psoralens in comparison to the other analyzed samples, showing more than 20 mg/L. Limoncello *exposed*, prepared with the same *Verdelli* lemons as the immersed sample, shows a ten times lower amount of all the non-volatile components if compared to limoncello *immersed*. This behaviour might be due, as previously reported, to the peculiar process of extraction based on an ethanolic saturated headspace. Coumarins and psoralens are oxygen heterocyclic molecules, non volatile, that show the same behaviour toward the headspace ethanolic extraction as the oxygenated compounds in the GC-FID analysis. Values found for commercial limoncellos ranged from a minimum of 0.8 mg/L to values higher than 7 mg/L in samples #2, #5 and #7.

Some commercial samples did not contain herniarin and bergapten. This finding can be in accordance with literature data, that report herniarin and bergapten as present only in particular varieties of lemons (Dugo, Mondello, Cogliandro, Cavazza, & Dugo, 1998). Byakangelicol and oxypeucedanin are epoxypsoralens, that can hydrolyse to their corresponding diols. For this reason, they resulted absent in some samples.

However, the different amount of coumarins and psoralens can be related to the different procedures used to prepare limoncello, and, in some cases, to the use of terpeneless oils, obtained by distillation procedure, and of oils free from non volatile components.

3.6. Alcohol content

Some disagreement was found in between the values reported on the label and those experimentally determined for certain limoncellos (brands #2, #3, #4, #9, #10, #12, #13, #14). In these cases, values labelled on the bottles were 1–2 units lower than the real alcohol content. It seems proper to remember that council regulation no. 92/109 on foodstuff labelling lays down that a tolerance of ±0.3% towards the difference of the real and the labelled alcoholic contents is allowed. The highest alcohol content was found in lab-made limoncellos, suggesting that the industrial procedure of preparation is based on the use of a higher amount of water, whereas for lab-made limoncellos the proportion alcohol:water was 1:1.

4. Conclusion

As previously stated throughout the text, a deep knowledge of all the chemical aspects of a limoncello could greatly help with assessing its authenticity and genuineness. An issue of interest for manufacturers is undoubtedly the possibility of overcoming all the drawbacks arising from the use of Citrus material, which means terpenes. Obviously, the interest of industry in limoncello production is also economic: having a final product that looks and tastes like the same through the whole year, is definitely cheaper, more attractive to the consumer and easier to be produced on an industrial basis. In consideration of the results obtained in this research, it seems very likely that several of the limoncellos analyzed were produced not only by the addition of essential oil, instead of using the real fruits, but also by using terpeneless oils and sometimes synthetic

products of reconstituted oils. Among the commercial limoncellos analyzed in this study, brand #5 shows to be of the highest quality, due to its high similarity to lab prepared samples. Also sample #2 can be considered of high quality, but the high value of *p*-cymene can indicate a long-time stored sample. The availability of analytical methods capable of revealing the origin, the authenticity and the quality of a limoncello may encourage the producers to prepare high quality products, appreciated by the consumer not because of the massive advertisement, but for the characteristics of their composition. The analytical method developed in this research for volatiles extraction proved to be innovative and versatile: it seems fair to point out that the absolute amount of about 50 components (extracted by SPME), present in different ranges of concentration and belonging to various chemical groups, was successfully determined. The reliability of the HS-SPME method was also demonstrated by the values of CV% that were, on average, 4% ranging from 0.05% to 9.8%. In some cases (i.e. oxygenated compounds) the CV% was much higher, but this result might be due to the nature of the SPME fiber (PDMS), that was chosen basically for the predominant non polar properties of the analytes (terpene hydrocarbons). Moreover, for the first time, chiral GC analysis was applied to the analysis of lemon liquors, highlighting once again the possibility of evaluating the enantiomeric ratios as genuineness markers, not only in limoncellos, but also in other types of beverages.

References

Ayedoun, A. M., Sossou, P. V., Mardarowicz, M., & Leclercq, P. A. (1996). Volatile constituents of the peel and leaf oils of Citrus limon L. Burm. F. from Benin. *Journal of Essential Oil Research, 8*, 441–444.

Boelens, M. H., & Jimenez, R. (1989). The chemical composition of some Mediterranean citrus oils. *Journal of Essential Oil Research, 1*, 151–159.

Bonomi, M., Lubian, E., Puleo, L., Tateo, F., & Fasan, S. (2001). Criterio di caratterizzazione PCA di liquori "limoncello". *Industrie delle Bevande, 30*(174), 371–374.

Caccioni, D. R. L., Guizzardi, M., Biondi, D. M., Renda, A., & Ruberto, G. (1998). Relationship between volatile components of citrus fruit essential oils and antimicrobical action on Penicillum digitatum and Penicillum Italicum. *International Journal of Food Microbiology, 43*, 73–79.

Cotroneo, A., Dugo, G., Licandro, G., Ragonese, C., & Di Giacomo, G. (1986). On the genuineness of Citrus essential oils. Part XII. Characteristics of Sicilian lemon essential oil produced with the FMC extractor. *Flavour and Fragrance Journal, 1*, 125–134.

Cotroneo, A., Verzera, A., Lamonica, G., Dugo, G., & Licandro, G. (1986). On the genuineness of Citrus essential oils. Part X. Research on the composition of essential oils produced from Sicilian lemons using "Pelatrice" and "Sfumatrice" extractors during the entire 1983/84 production season. *Flavour and Fragrance Journal, 1*, 69–86.

Cutuli, G., Di Martino, E., Lo Giudice, V., Pennini, L., Raciti, G., Russo, F., et al. (1985). *Trattato di Agrumicoltura*. Bologna, Italy: Ed agricole.

Dugo, G., & Di Giacomo, A. (2002). *Citrus*. London, NY: Taylor & Francis.

Dugo, G., Bartle, K. D., Bonaccorsi, I., Catalfamo, M., Cotroneo, A., Dugo, P., et al. (1999). Advanced analytical techniques for the analysis of Citrus essential oils. Part. 1. Volatile fraction: HRGC/MS analysis. *Essenze E Derivati Agrumari, 69*, 79–111.

Dugo, G., Cotroneo, A., Verzera, A., & Bonaccorsi, I. (2002). Composition of the volatile fraction of cold-pressed citrus peel oils. In G. Dugo & A. Di Giacomo (Eds.), *Citrus* (pp. 201–317). London, NY: Taylor & Francis.

Dugo, P., Mondello, L., Cogliandro, E., Cavazza, A., & Dugo, G. (1998). On the genuineness of citrus oils. Part LIII. Determination of the composition of the oxygen heterocyclic fraction of lemon essential oils (Citrus limon (L.) Burm, f.) by normal-phase high performance liquid chromatography. *Flavour and Fragrance Journal, 13*, 329–334.

Dugo, G., Mondello, L., Cotroneo, A., Bonaccorsi, I., & Lamonica, G. (2001). Enantiomeric distribution of volatile components of Citrus oils by MDGC. *Perfumer and Flavorist, 26*, 20–35.

Dugo, P., Russo, M., Mondello, L., Dugo, G., Postorino, S., & Carnicini, A. (2000). Chemical and physicochemical parameters and composition of the aromatic fraction of limoncello. *Italian Journal of Food Science, 12*, 343–351.

Dugo, G., Stagno d'Alcontres, I., Donato, M. G., & Dugo, P. (1993). On the genuineness of Citrus essential oils. Part XXXVI. Detection of reconstituted lemon oil in genuine cold-pressed lemon essential oil by high resolution gas chromatography with chiral capillary columns. *Journal of Essential Oil Research, 5*, 21–26.

Dugo, G., Verzera, A., Trozzi, A., & Cotroneo, A. (1994). Automated HPLC-HRGC: a powerful method for essential oil analysis. Part I. Investigation on enantiomeric distribution of monoterpene alcohols of lemon and mandarin essential oils. *Essenze E Derivati Agrumari, 1*, 35–44.

Kovats, E. (1958). Gas-chromatographische Charakterisierung organischer Verbindungen. Teil I: Retentions Indices aliphatischer Halogenide, Alkohole, Aldehyde und Ketone. *Helvetica Chimica Acta, 41*, 1915–1932.

Moio, L., Piombino, P., Di Marzio, L., Incoronato, C., & Addeo, F. (2000). L'aroma del liquore di limoni. *Industrie delle Bevande, 29*, 499–506.

Mondello, L., Catalfamo, M., Cotroneo, A., Dugo Giacomo Dugo, et al. (1999). Multidimensional capillary GC–GC for the analysis of real complex samples. Part IV. Enantiomeric distribution of monoterpene hydrocarbons and monoterpene alcohols of lemon oils. *Journal of High Resolution Chromatography & Chromatography Communications, 22*, 350–356.

Mondello, L., Catalfamo, M., Dugo, P., Proteggente, A. R., & Dugo, G. (1997). La gascromatografia multidimensionale per l'analisi di miscele complesse. Nota preliminare. Determinazione della distribuzione enantiomerica di componenti degli olii essenziali agrumari. *Essenze E Derivati Agrumari, 67*, 62–85.

Mondello, L., Dugo, P., Basile, A., Dugo, G., & Bartle, K. (1995). Interactive use of linear retention indices, on polar and apolar columns, with a MS-Library for reliable identification of complex mixtures. *Journal of Microcolumn Separations, 7*(Sep), 581–591.

Mondello, L., Dugo, P., & Dugo, G. (2002). The chiral compounds of citrus essential oils. In G. Dugo & A. DiGiacomo (Eds.), *Citrus* (pp. 461–495). London, NY: Taylor & Francis.

Naviglio, D., Ferrara, L., Montesano, D., Mele, G., Naviglio, B., Tomaselli, M., et al. (2001). New solid–liquid extraction technology for the production of lemon liquor. *Industrie delle Bevande, 30*, 362–370.

Naviglio, D., Pizzolongo, F., Mazza, A., Montuori, P., & Triassi, M. (2005). Chemical–physical study of alcoholic extract of flavedo lemon and essential lemon oil to determine microbic charge responsible of limoncello turbidity. *Industrie delle Bevande, 34*, 425–431.

Naviglio, D., Raia, C., Bergna, M., & Saggiomo, S. (2003). Stability control and genuineness evaluation of lemon liquor: use of dynamic headspace. *Industrie delle Bevande, 32*, 121–126.

Neumann, M., & Garcia, N. A. (1992). Kinetics and mechanisms of the light-induced deterioration of lemon. *Journal of Agricultural and Food Chemistry, 40*, 957–960.

Pawlisyn, J. (1997). *Solid-phase microextraction: theory and practice*. New York: Wiley-VCH.

Poiana, M., Attanasio, G., Albanese, D., & Di Matteo, M. (2006). Alcoholic extracts composition from lemon fruits of the Amalfi-Sorrento Peninsula. *Journal of Essential Oil Research, 18*, 432–437.

Pokorny, J., Pudil, F., Volfova, J., & Valentova, H. (1998). Changes in the flavor of monoterpenes during their autoxidation under storage conditions. *Developments in Food Science, 40*, 667–677.

Romano, R., Schiavo, L., Iavarazzo, E., Battaglia, A., & Cassano, A. (2004). Qualitative and quantitative characteristics of lemon juice concentrate obtained by osmotic distillation. *Industrie delle Bevande, 33*, 529–532.

Schieberle, P., & Grosch, W. (1989). Potent odorants resulting from the peroxidation of lemon oil. *Zeitschrift fur Lebensmittel-Untersuchung und-Forschung, 189*, 26–31.

van den Dool, J., & Kratz, P. D. (1963). A generalization of the retention index system including linear programmed gas–liquid partition chromatography. *Journal of Chromatography, 11*, 463–471.

Versari, A., Natali, N., Russo, M. T., & Andrea, A. (2003). Analysis of some Italian lemon liquors (Limoncello). *Journal of Agricultural and Food Chemistry, 51*, 4978–4983.

Verzera, A., Cotroneo, A., Dugo, G., & Salvo, F. (1987). On the genuineness of Citrus essential oils. Part XV. Detection of added orange oil terpenes in lemon essential oils. *Flavour and Fragrance Journal, 2*, 13–16.

Verzera, A., Dugo, P., Mondello, L., Trozzi, A., & Cotroneo, A. (1999). Extraction technology and lemon oil composition. *Italian Journal of Food Science, 11*, 361–370.