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Analysis of Some Italian Lemon Liquors (Limoncello)

VERSARI ANDREA,† NATALI NADIA,† RUSSO MARIA TERESA,‡ AND ANTONELLI ANDREA*,§

Dipartimento di Scienze degli Alimenti, Università di Bologna, via Ravennate 1020, Cesena (FC) 47023, Italy, Dipartimento di Scienze e Tecnologie Agroforestali ed Ambientali, Università di Reggio Calabria, P.zza San Francesco di Sales 7, Gallina 89061, Reggio Calabria, Italy, and Dipartimento Interdisciplinare di Scienze Agrarie, Università di Reggio Emilia, via Kennedy 17, Reggio Emilia, 42100, Italy

The chemical composition of several commercial Italian Limoncellos, lemon-peel-based alcoholic beverages, was studied by chromatographic techniques. These methods allowed a rapid monitoring of Limoncello, giving information on quality markers and possible adulteration of the product. Quantitative data for more than 60 compounds are reported. Limoncellos were characterized by the presence of selected volatile (terpenes, aldehydes, alcohols) and nonvolatile compounds (psoralens, coumarins, phenolics, carbohydrates and acids). On the basis of their composition, the samples were grouped by PCA analysis in two sets; the first group showed a composition similar to lemon essential oils, with a high content of b-pinene, myrcene, trans-a-bergamottene, and b-bisabolene, and a low content in neral and geranial. The composition of the second group suggested the occurrence of oxidative phenomena and/or the addition of flavors. The presence of ethyl acetate, acetaldehyde, 2-methyl-1-propanol and glycerol showed that a fermentation probably occurred in the sugar syrup used to dilute the Limoncello after the extraction process.

KEYWORDS: Lemon liquor; composition; flavonoids; coumarins; psoralens; essential oils; chromatography

INTRODUCTION

Citrus peel (i.e., flavedo or hepicarp), a tissue rich in secondary plant metabolites and characterized by pleasant flavors, is commonly used for preparation of marmalades, candied peels, and essential oil (EO). In Italy, lemon peels are also used to produce a high-value product called Limoncello.

Limoncello is a typical Italian lemon liquor obtained from the alcoholic extraction of essential oils from lemon peel (*Citrus limon* (L.) Burm. f.). This beverage is becoming increasingly popular in Italy and abroad due to its natural aroma and taste, which recall the fresh lemon, as well as for its digestive properties. A total production of 15 million liters of Limoncello per year is estimated (*1*). The production process is simple and includes the following main phases: (i) Washing and peeling the fruits. (ii) Infusion of the lemon peels in 95% ethyl alcohol for 2−7 days. During this phase, the extraction of essential oils and other peel constituents occurs. (iii) Dilution with a sugar syrup to obtain a final product with ca. 32% ethyl alcohol. (iv) Bottling the Limoncello. In the European Community, addition of natural or synthetic flavor/essential oil to the Limoncello is regulated by the Council Regulation No 1576 (*2*).

Besides ethanol and water, Limoncello contains several volatile and nonvolatile minor compounds (ca. 2%), which are fundamental for its sensory characteristic. The former are terpenic compounds, which form the EO, and the latter include several classes of nonvolatile compounds (**Figure 1**) with potential health-related properties, such as flavonoids, coumarins, and psoralens (*3*−*6*). In addition to their remarkable olfactory and taste properties, these compounds may play an important role as markers for chemotaxonomic studies and for the evaluation of quality and genuineness of citrus juice (*7*, *8*), fruit (*9*, *10*), and EO (*11*). For example, coumarins and psoralens are helpful in detecting the adulteration of cold-pressed EO by the addition of steam-distilled oil, which lacks these volatile components.

Literature mainly concerns the volatile fraction composition of Limoncello (*12*−*15*). Further data on the analytical characterization of Italian Limoncello are needed to protect the consumers and to draw guidelines for the quality control and the regulation of this industry.

In this preliminary study, twelve samples were analyzed for their flavors, sugars, acids, polyalcohols, coumarines, psolarenes, and phenolics content to find out the best classes of compounds for further investigations on quality control as well as regulatory assessments.

MATERIALS AND METHODS

Chemicals. Standards of volatile compounds, coumarins, phenolics, carbohydrates, polyalcohols, and acids were purchased from commercial

* To whom correspondence should be addressed: Tel.: +39-0522-522023. Fax: +39-0522-522059. E-mail: andrea.antonelli@unimo.it.
† Università di Bologna.
‡ Università di Reggio Calabria.
§ Università di Reggio Emilia.

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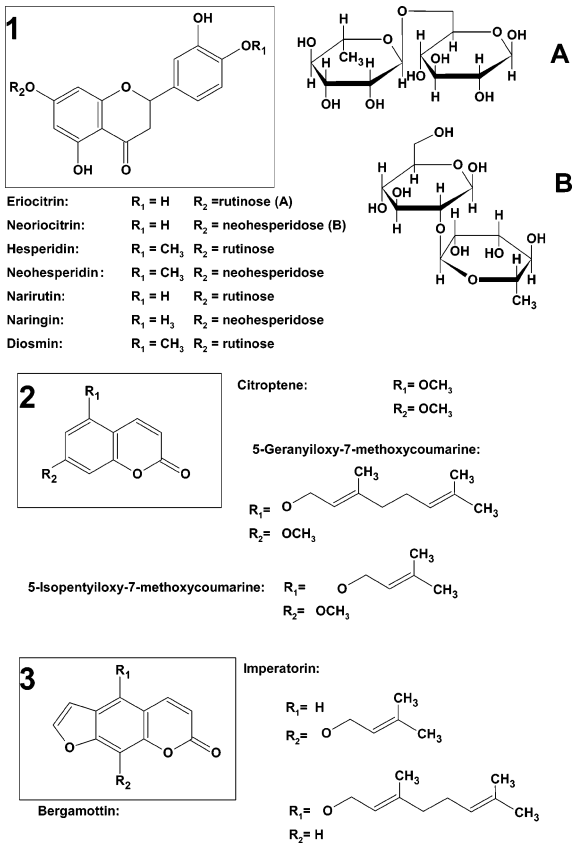

Figure 1. Chemical structure of flavanones (1), coumarins (2), and furanocoumarins or psoralens (3) identified in Limoncello.

sources reported as follows in **Tables 1**−**6**. Standards of volatile compounds and naringin were dissolved in ethanol, whereas (i) was 5-geranyloxy-7-methoxycoumarin in a mixture ethanol/ethyl acetate (50:50, v/v); (ii) was citropten and imperatorin in chloroform; (iii) was narirutin and neohesperidin in a water mixture (*N*,*N*-dimethylformamide (50:50, v/v)); and (iv) was sinapic acid diosmin, eriocitrin, hesperidin, and neoeriocitrin in *N*,*N*-dimethylformamide.

Acetic acid, water HPLC grade, chloroform, ethanol, hexane, ethyl acetate, *N*,*N*-dimethylformamide, hexamethyldisilazane, pyridine, and trimethylchlorosilane of analytical grade purity were purchased by different suppliers.

Samples. Twelve Limoncello liqueurs were provided from commercial sources and protected from light at −18 °C until analyzed in duplicate. Volatile compounds, carbohydrates, and acids were analyzed by GC, whereas coumarins, psoralens, and phenolic compounds were analyzed by HPLC.

Analytical Determination. *Acids, Carbohydrates, and Polyalcohols Analyses.* An aliquot of sample was added with β-phenylglucopyranoside as internal standard, then dried, silylated, and submitted to GC analysis onto a SE 52 capillary column as previously described (*16*). The extraction procedure reported in (*16*) was ommitted to prevent trimethylsilyl ester hydrolisis.

Higher Alcohol Analysis. A 50-mL sample of Limoncello was analyzed according to AOAC procedure (*17*).

GC Analysis. *Volatile extraction.* Stock ethanol solutions (10 g/L) were prepared for each standard. An aliquot of each solution (100 μL) was introduced into a 10-mL volumetric flask to have a standard mixture (MIX). A model Limoncello was obtained with 500 μL of MIX and taken to the final volume of 10 mL into a volumetric flask with a solution of 30% ethanol and 20% sucrose. Volatile compounds were liquid−liquid extracted from 10 mL of Limoncello with hexane in accordance with the literature (*12*).

Analysis of Oil Composition by GC and GC-MS. Gas chromatographic analyses were performed by injecting 1 μL of the extract using splitless mode (30 s splitless-time, split ratio 1:45) into an HRGC 5160 system (Carlo Erba Strumentazione, Milano, Italy) equipped with a 25-m, 0.25-mm id, 0.25-μm film thickness SE 52 capillary column (Mega, Legnano, Italy). Hydrogen was used as carrier gas (80 Kpa; linear velocity 69 cm/sec). The oven was set at 50 °C and then programmed at 4 °C/min up to 70 °C, which was maintained for 8 min. Then the temperature was programmed at 5 °C/min up to 200 °C, which was maintained for 6 min. Finally, oven temperature was raised ballistically up to 300 °C. The injector was kept at 250 °C, and the flame ionization detector was set at 300 °C. Chromatograms were displayed and integrated by a Chrom-Card data handling system (Fisons Instruments, Milano, Italy).

A QMD 1000 instrument (Fisons Instruments) was used for GC-MS, with the same column as described previously. It was operated with helium as carrier gas (80 Kpa; linear velocity 55 cm/sec). Mass spectra were recorded from 33 to 350 *m*/*e* at 70 eV.

Chromatographic peaks were identified by t_R and mass spectra of authentic compounds when available and by comparing literature data.

HPLC Analyses. Coumarins, psoralens, and phenolic compounds were analyzed by direct injection after sample filtration on 0.2-μm cellulose acetate membrane (Orange Scientific, Braine l'Alleud, Belgium). An HPLC 980 system (Jasco, Tokyo, Japan) was equipped with an MD-1510 diode-array detector, data were acquired in the range of 200−450 nm and processed using a Borwin-PDA version 1.50 software (JMBS Developments, Grenoble, France). Samples were injected with a 20-μL loop using a 7125 valve (Rheodyne, Cotati, CA), and separation occurred at 30 °C using a column oven (Jones Chromatography, Mid Glamorgan, UK).

Coumarins and psoralens were analyzed onto a μ-porasil silica column (300 × 3.9-mm; 10-μm i.d.; Waters, Milford, MT) and monitored at 330 nm. The flow rate was 1 mL/min using hexane/ethyl acetate (88:12, v/v) as eluent A and hexane/ethanol (90:10, v/v) as eluent B. Coumarins and psoralens were separated with the following linear gradient elution conditions (min/A%): 0/100, 10/100, and 30/0, followed by reconditioning of the column.

Phenolics were analyzed onto a Luna Explore RP−C18 column (150 × 4.6-mm; 3-μm i.d.; Phenomenex, Torrance, CA) and monitored at 280 nm. The flow rate was 0.5 mL/min using water/acetic acid (98:2, v/v) as eluent A, and water/acetic acid/methanol (34:1:65, v/v/v) as eluent B. Phenolics were separated with the following linear gradient elution conditions (min/A%): 0/60, 10/60, 25/50, and 30/0, followed by column reconditioning.

Peak Identification and Quantification. The retention time (t_R), the UV−vis spectra and the mass spectra provided structural information without the need of isolating the individual compounds. Peaks were identified by comparing t_R of pure standards, by spiking technique and comparison with data from literature. (*8, 11*). In particular, MS and UV−vis spectra were used for volatile and nonvolatile compounds, respectively. Quantification was performed using calibration curves fitted by linear regression analysis with the correlation coefficient ($r > 0.99$) (Statistica 5.1, StatSoft, Tulsa, OK).

Multivariate Analysis. Principal component analysis (PCA), a pure display method, was used to reduce the number variables in the data matrix and to select the most discriminating parameters. Data were standardized (1/SD) and mean centered before PCA (*18*).

RESULTS AND DISCUSSION

Acids, Carbohydrates, and Polyalcohols. Quantitative data of acids (**Table 1**), carbohydrates, and polyalcohols (**Table 2**) are provided. The samples were characterized by a high content of citric acid, followed by oxalic, lactic, and ascorbic acids. The high citric acid content found in samples 1 and 8 could be due to lemon juice addition.

Carbohydrates such as fructose and glucose are naturally present in lemon peel, whereas sucrose is added. The total sugar content ranged from 18 to 28%, the highest value (277 g/L) being associated with the highest alcohol content (40%) in sample 8. These data are in good agreement with the Limoncello production practice, which recommends 20−28% of sugar concentration. The high sugar percentage is commonly used to balance product with a high alcoholic strength.

Table 1. Acid Content (mg/L) of Limoncello Samples

	Limoncello											
compound L	1	2	3	4	5	6	7	8	9	10	11	12
lactic acid[a]	9	25	8	24	23	10	27	7	3	21	39	86
oxalic acid[a]	79	25	116	87	86	77	81	78	68	75	85	53
malonic acid[a]	4	6	5	7	1	3	5	5	2	27		5
phosphoric acid[a]	94	5	14	2	2	3	3	3	6	2	3	1
succinic acid[a]	29	23		2	4		4	10		13		14
malic acid[a]	20	16	28	6	4	8	4	7	18	14	20	8
citric acid	2044	438	190	209	475	152	83	1059	172	395	846	301
ascorbic acid	26	13	10	10	23	11	17	10	44	6	16	9

[a] Standards from Sigma-Aldrich, Milano, Italy.

Table 2. Polyalcohols and Sugars Content of Limoncello Samples

		Limoncello											
compound		1	2	3	4	5	6	7	8	9	10	11	12
glycerol[a]	mg/L	2	3				2	1					
meso-erithrytol[a]	mg/L						4	4	3	3			
sorbitol[a]	mg/L	2									1		
mio-inositol[a]	mg/L	32	29	27	37	62	4			11	26		17
total	mg/L	36	32	27	37	62	10	5	3	14	27	0	17
L-arabinose[a]	mg/L	13	8			17	10	15	41	20	26		6
rhamnose[a]	mg/L	56						3	6		3	6	
D–α-arabinose	mg/L	54	80	55	60	72	48	70	22	42	69	46	55
D–β-arabinose	mg/L	15	12	8	12	22	13	14	31	8	19	15	20
fructose[a]	g/L	0.4	1.2	0.3	0.3	3.5	0.2	0.1	1.1	0.2	0.1	1.3	0.4
glucose[a]	g/L	1.9	3.5	0.8	0.7	2.2	4.4	0.9	5.6	4.6	2.5	5.7	1.3
saccharose[a]	g/L	180	181	185	184	196	257	259	270	260	220	257	197
total	g/L	182	186	186	185	202	262	260	277	265	223	264	199

[a] Standards from Sigma-Aldrich, Milano, Italy.

Table 3. Content of Ethanol (% v/v) and Higher Alcohols (mg/L) of Limoncello Samples

	Limoncello											
compound[a]	1	2	3	4	5	6	7	8	9	10	11	12
ethanol	32	31	31	31	30	30	29	40	30	27	29	28
acetaldhyde	16	7	13	13	15	14	24	18	21	23	17	16
ethyl acetate	1	1	1			2	4	8	1	1	1	
metahnol	3	4		1				8	1			
propanol	36	8	33	12	19	4	9	25		43		12
i-butanol	2		2	1	2			2		4		3

[a] Standards from Prolabo, Paris, France.

Small amounts of glycerol were detected in some samples (nos. 1, 2, 6, 7, and 8). This compound partially overlapped with phosphoric acid. However, the MS spectra and the spiking technique confirmed the occurrence of H_3PO_4 in Limoncello.

Alcohols. Ethanol content was in the range of 27−40% (**Table 3**). A value of 31−32% of ethanol is considered as optimal for Limoncello; lower strength may affect the beverage stability (EO separation), whereas higher values might not fit the consumer's preference. In fact, Limoncello is considered a beverage, not a spirit. Ethanol content was in good agreement with the value reported on the label, the only exception being sample no. 8 (ethanol = 35% declared vs 40% found).

Methanol showed values below the legal limit, whereas acetaldehyde content was above the regulatory limit for neutral ethanol, which is set at 0.5 g/hL (5 mg/L) of anhydrous alcohol (**Table 3**). Similar results were found for the 2-methyl-1-propanol. Sample 8 was the only one with an ethyl acetate content (2.0 g/hL = 20 mg/L of anhydrous alcohol) exceeding the legal limit for neutral ethanol (*2*).Despite the fact that these limits concern only the ethanol used as ingredients and are not applicable to the Limoncello as a liquor, these findings require a clarification. Two hypothesis were formulated: (i) the addition of low-quality ethyl alcohol for the extraction process of lemon peel and (ii) the occurrence of fermentation process of the sugar solution used to dilute the lemon peel extract. The high acetaldehyde level and the occurrence of glycerol suggested the latter hypothesis as more probable. Moreover, the low methanol content, as a consequence of the use of high quality ethanol, would confirm it.

Volatile Compounds. Some samples (nos. 2, 3, 6, 7, 8, 9, 11, and 12) did not reach about 1 g/L of total volatiles, whereas the others (nos. 1, 4, 5, and 10) reached or exceeded this reference level, estimated as shown in **Table 4**. The value of 0.26−0.59% as extraction yield of EO from lemon peels has been reported (*20*). However, these data refer the extraction from the whole lemon peel, whereas the extraction process for Limoncello production uses only the flavedo, which is about 25−50% of the total peel weight. For this reason, we consider about 1 g/L of EO as a reasonable estimate extraction yield for Limoncello.

The sum of total volatiles resembled the composition of EO with only few exceptions (**Figure 2**). The first group of samples (nos. 2, 3, 6, 7, 8, 9, 11, and 12) showed an anomalous level of oxygenated compounds. The extraction condition enhances more polar moieties, whereas hydrocarbons are less soluble in ethanol. Moreover, the same samples showed an unusually high level of citral, up to 10 times the regular EO content. It is reasonable to argue that these Limoncellos were added of deterpenated EO

Table 4. Volatile Composition (mg/L) of the Limoncello Samples. Identifications Were Carried out by GC-MS (MS), by Comparison of Literature Data (L) (*19*), and by Pure Standards (S)

compound[a]	MS	L	S	1	2	3	4	5	6	7	8	9	10	11	12
α-thujene	X	X		10.0	0.9	6.9	5.3	7.9	0.7	0.2	6.2	4.0	3.8	1.2	0.9
α-pinene	X	X	X	32.0	6.0	37.0	33.0	29.0	6.3	2.1	38.0	13.0	20.0	5.8	7.0
camphene	X	X	X		0.1	0.2		0.4		0.2	0.1		0.4		0.8
β-pinene	X	X	X	239.0	52.0	205.0	226.0	181.0	46.0	16.0	222.0	30.0	94.0	27.0	33.0
myrcene	X	X	X	16.0	3.6	23.0	14.0	12.0	10.0	7.1	31.0	3.0	13.0	5.5	8.7
octanal	X	X	X	3.1	1.4	1.5	2.2	2.8	3.0	9.1	6.0	4.0	1.5	0.4	2.9
p-cimene	X	X	X	17.0	22.0				19.0	12.0		18.0		2.7	3.7
limonene	X	X	X	694.0	164.0	1.1	747.0	553.0	460.0	60.0	1.3	179.0	650.0	276.0	436.0
(Z)- β-ocimene	X	X	X	0.7	0.1	0.2	0.6	0.6			1.0		0.3	0.1	0.2
(E)- β-ocimene	X	X	X	1.5	0.2	0.3	1.4	1.5	0.1		2.0	0.5	0.3	0.4	0.6
γ-terpinene	X	X	X	102.0	14.0	14.0	98.0	110.0	13.0	0.9	144.0	0.5	20.0	34.0	29.0
trans-sabinene hydrate	X	X		1.4	2.3	1.8	0.4	1.8	0.6	2.3	0.3		1.4	0.1	0.3
terpinolene	X	X	X	5.6	1.4	2.8	5.0	5.1	1.0		8.0		1.9	2.1	1.5
linalool	X	X	X	3.3	6.3	3.7	1.9	3.0	9.9	10.0	7.0	10.0	4.6	2.9	5.2
nonanal	X	X	X	9.1	9.3	4.7	3.0	4.7	2.7	13.0	1.0	1.0	3.1	3.7	2.4
cis-limonene oxide	X	X	X	1.6	1.6	2.3	0.8	1.8		0.6	2.0	0.5			
trans-limonene oxide	X	X	X	0.5	0.8	0.4		0.6	1.3	0.5	1.0				
borneol	X	X	X	0.9	2.5	3.2	0.6	1.7	1.3	2.2	6.0	10.0	1.6	0.5	1.0
terpinen-4-ol	X	X	X	1.0	5.1	6.3	1.0	4.4	6.1	5.1	25.0	7.0	12.0	7.6	8.8
α-terpineol	X	X	X	7.3	13.0	11.0	8.7	13.0	11.0	27.0	26.0	13.0	15.0	15.0	13.0
nerol and citronellol	X	X	X	6.8	14.0	9.0	12.0	13.0	11.0	18.0	15.0	12.0	19.0	2.4	6.8
neral	X	X	X	3.3	5.8	0.7	4.8	8.7	29.0	5.0	11.0	39.0	2.3	22.0	21.0
geraniol	X	X	X	6.4	12.0	8.1	10.0	9.3	20.0	9.6	33.0	41.0	20.0	1.4	9.0
geranial	X	X	X	15.0	19.0	4.2	18.0	40.0	115.0	18.0	51.0	234.0	11.0	95.0	82.0
undecanal	X	X	X	0.6	0.6	2.2	0.5	0.6		0.4	1.0		1.2	0.2	
citronellyl acetate	X	X	X	0.6	0.5	0.6	0.6	0.7	0.6		1.0		0.6	0.7	0.2
neryl acetate	X	X	X	7.7	4.0	4.8	5.8	9.3	1.5	0.5	11.0	2.0	6.2	8.2	27.0
geranyl acetate	X	X	X	6.2	4.5	5.7	5.8	7.2	1.4	0.6	13.0	2.0	7.5	7.1	3.4
β-caryophyllene	X	X	X	2.1	0.9	3.4	2.4	1.9	2.5	2.0	4.0	1.0	1.5	1.8	0.9
trans-α-bergamottene	X	X		2.5	1.0	4.9	3.2	3.4	1.7		4.0	1.0	2.5	2.2	0.7
β-bisabolene	X	X		4.0	2.0	7.6	7.8	5.4	2.8		8.0	1.0	4.1	3.1	1.1
Total				1201.2	370.9	376.6	1219.8	1033.8	777.5	222.4	679.9	626.5	918.8	529.1	707.1

[a] Standards from Sigma-Aldrich, Milano, Italy.

Table 5. Coumarins and Psolarens Composition (mg/L) of Limoncello Samples

	Limoncello												
compound	1	2	3	4	5	6	7	8	9	10	11	12	**EO**[a]
bergamottin	1.7	4.7	21.5	1.1	0.9	3.8	2.3	19.6	3.7	1.2	1.0	3.6	2200
5-geranyloxy-7-methoxycoumarin[b]	0.7	1.6	0.8	0.5	0.5	1.3	0.8	7.0	1.2	0.4	0.2	1.2	1600
citropten derivate			0.1	0.1				0.3					
5-isopentyloxy-7-methoxycoumarine			0.4	0.2				1.0					80
citropten[b]	2.0		1.5	1.0	1.3	0.3	1.8	4.0	0.4	1.1	0.4	1.0	650
imperatorin[b]	4.4	0.8		0.6				1.6					60
total	8.7	7.1	24.3	3.5	3.9	5.5	4.9	35.2	5.5	2.8	1.6	5.8	4590

[a] Essential oil composition from literature (*19*). [b] Standards from Extrasynthese, Genay, France.

(that is the oxygenated fraction of EO) or simply with citral. This latter deduction seems to be very likely for sample 9, in which citral was 45% of the total volatile amount.

In any case, limonene was the main compound, followed by β-pinene (**Table 4**). The content of *p*-cymene is usually very low in fresh EO, but it can increase as a consequence of γ-terpinene oxidation (*21*), citral degradation (*22*), and under mild acid condition starting from various monoterpenes (*21*, *23*). As a matter of fact, a very low γ-terpinene content was found in several samples (nos. 2, 3, 6, 7, 9, and 10), even if *p*-cimene, its major oxidation product, was not always present. Moreover, Limoncellos 3 and 10 were also low in citral. Limonene oxides are reliable oxidation markers; they are not present in fresh essential oil, but they appear after air exposition. From a chemical point of view, it is well known that limonene oxidation yields its oxides as primary byproducts (*24*, *25*). However, the chemistry of these compounds is complex, and a lack of correlation between limonene content and its oxide occurred in Limoncellos (**Table 4**).

Almost all samples showed a high content of terpinen-4-ol. According to Moio et coll. (*13*), this compound indicates the addition of aroma. However, its origin can also be related to acid-catalyzed phenomena (*21*, *23*).

One sample (no. 8) showed a high concentration of geranyl acetate, which was probably due to seasonal variability or technological process. More difficult to explain was the amount of 27 mg/L of neryl acetate (sample 12), which content was 1 order of magnitude more concentrated than that of the geranyl isomer.

Coumarins and Psoralens. Four major compounds, bergamottin, 5-geranyloxy-7-methoxycoumarin, citropten, and imperatorin, were detected in all samples. Additional minor compounds were present, including a citropten derivative and the 5-isopentenoxy-7-metoxycumarin. The qualitative composition of Limoncello mainly resembled that one of lemon EO reported in the literature (*11*), (**Table 5**). Taking into account that Limoncello contains about 1 g/L of EO, and that essential oil contains about 5000 mg/L of coumarins and psoralens

Table 6. Phenolic Composition (mg/L) of Limoncello Samples

	Limoncello											
compound	1	2	3	4	5	6	7	8	9	10	11	12
eriocitrin[a]	16.9		27.8	21.7	65.9			71.2		29.1		22.3
sinapic acid	0.4		0.5		1.0			tr		1.5		0.5
peak no. 3[c]						1.7	5.4		64.7		9.7	
narirutin[a]	5.2	14.5	1.8	0.8	2.2			2.2				5.9
naringin[a]		1.5		1.2	tr			tr	tr	tr	1.4	1.3
hesperidin[b]	16.7		25.3	12.0	32.1			42.3		6.7		7.6
neohesperidin[a]	0.6		tr	tr	tr			1.2		tr		0.4
diosmin[a]	1.9	13.3	7.6	tr	7.9		5.3		0.8	4.0	tr	8.6
peak no. 9	5.0	6.2	0.6	3.0	tr	tr	0.8		tr	3.7	tr	2.1
peak no 10	tr[d]	5.5	1.4	tr	tr	tr	0.8		tr	3.6	tr	2.4
peak no 11	tr		tr		1.0	2.4	0.7		3.2		5.5	3.7
total phenolics	46.7	41.0	65.0	38.7	110.1	4.1	13.0	116.9	68.7	48.6	16.6	54.8

[a] Standards from Extrasynthese, Genay, France. [b] Standards from Sigma-Aldrich, Milano, Italy. [c] **Figure 3**. [d] Trace.

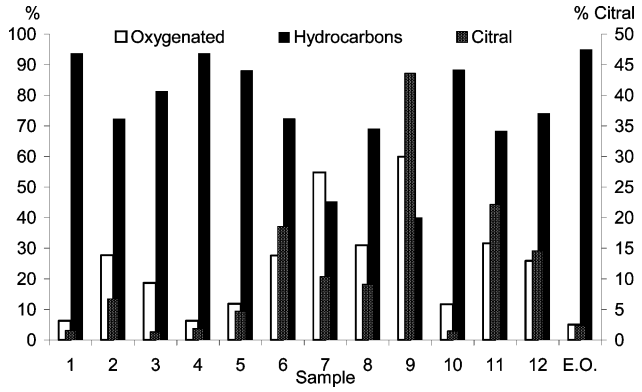

Figure 2. Content (%) of volatile compounds in Limoncello. (EO): Essential oil composition from the literature (19).

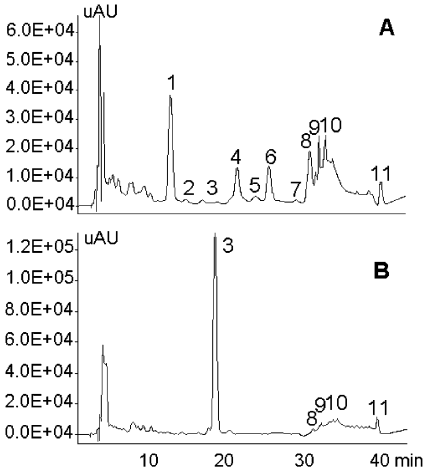

Figure 3. HPLC chromatogram of phenolics in Limoncello (280 nm). Peak legend: (1) eriocitrin, (2) sinapic acid, (3) unknown, (4) narirutin, (5) naringin, (6) hesperidin, (7) neohesperidin, (8) diosmin, (9) unknown, (10) unknown, (11) unknown. (A) Sample no. 12, (B) sample no. 9.

(*11*, *19*), a cumulative amount of at least 5 mg/L for these compounds is expected. Most of the samples reached this theoretical level, whereas samples 10 and 11 were lower (2.8 and 1.6 g/L, respectively). On the other hand, samples 3 and 8 showed a concentration about 5 and 7 times higher (24 and 35 mg/L, respectively).

In the EO, bergamottin, 5-geranyloxy-7-methyoxycoumarin and citroptene are the main constituents. In our samples, this general pattern was confirmed, but compositional details were less straightforward. In fact, even if bergamottin was almost always the main compound (except for sample 1), its relative concentration varied from 88% (sample 3) to 31% (sample 3). In sample 1, its concentration was as low as 20%, and it was not the main compound.

Moreover in EO, 5-geranyloxy-7-methyoxycoumarin is about one-third of the whole coumarin and psolaren content. In Limoncellos, this figure was never reached, whereas citropten in some cases (samples 1, 4, 5, 7, 10, and 11) greatly exceed EO concentration. Samples 2, 3, 6, and 9, on the contrary, showed low amounts of citroptene, whereas samples 8 and 12 seemed the most similar to EO composition. Due to the limited number of samples analyzed the meaning of this behavior is difficult to explain.

Phenolics. The HPLC profile of the Limoncello showed up to eleven peaks, which eluted within 45 min. According to the HPLC profile, the samples were divided in two groups: Limoncello 1, 3, 4, 5, 8, 10, and 12 (**Figure 3A**) and 2, 6, 7, 9, and 11 (**Figure 3B**). The first group was characterized by a high content of eriocitrin (17−71 mg/L), hesperidin (7−42 mg/L), and diosmin (2−13 mg/L), and a low content of sinapic acid, narirutin, neohesperidin, and naringin. The second group showed a simple HPLC profile with a major unknown peak eluting at t_R 18.7 min [λ_{max} = 263 nm] along with others minor unidentified peaks (**Table 6**). It is interesting to note that, with the only exception of sample 12, the HPLC of phenolics confirmed the results obtained with the GC of volatile compounds. In fact, the Limoncellos were grouped in the same two sets.

PCA Analysis. PCA analysis allowed the selection of the most important X-variables and the reduction of the dimensionality of the data. The first two PCs explained 92% of the X-variables using eight parameters, including a polyalcohol (mio-inositol), a sugar (saccharose), five flavors (β-pinene, myrcene, neral, geranial, and β-bisabolene), and one phenolic compound (esperidin) (**Figure 4**). Limonene, a typical lemon compound, was not included in the model because its addition reduced the X-explained variance. Samples no. 1, 3, 4, 5, and 8 grouped on the right side the plot, whereas samples no. 2, 6, 7, 9, 10, 11, and 12 were spread on the left side of the plot. The former group of Limoncellos was characterized by a high content of β-pinene, myrcene, β-bisabolene, and esperidine, and a low content in neral and geranial. On the basis of their composition the latter group included samples probably added with essential oil and low quality oxidized product. Sample no. 9, a possible outlier, was very high in neral and geranial (i.e., citral).

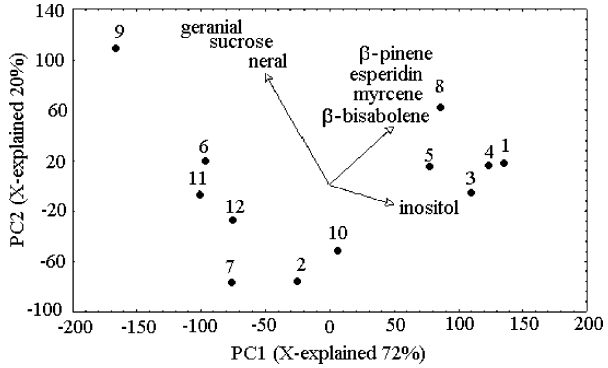

Figure 4. PCA plot of the 12 Limoncellos.

In conclusion, several of the compounds herein studied provide useful information for quality control. The flavor analysis is particularly useful to verify the addition of essential oils (deterpenated oil or citral) to the Limoncello to enhance lemon scent. In this case, an increase of oxygenated compounds and a loss of hydrocarbons can be detected. Esperidin as well as terpene hydrocarbons are natural markers to monitor the occurrence of lemon peel maceration. At present, the lack of extraction kinetic studies limits the full appreciation of these findings. Citric acid content indicates the addition of lemon juice. Ethyl acetate, acetaldehyde, 2-methyl-1-propanol, and glycerol are most probably related to the occurrence of microbiological activity in the sugar syrup used in the Limoncello formulation.

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