

# Analytical Methods and Phytochemistry of the Typical Italian Liquor “Limoncello”: A Review

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## 1. Introduction

The analyses of food or food-derived products that naturally occur in plants are essential especially related to food safety, food composition, adulterations, and food quality for Protected Geographical Indication (IGP), Controlled Origin Denomination (DOC) or Controlled and Guaranteed Origin Denomination (DOCG) designation.

For these reasons in this field of research a multidisciplinary approach between analytical chemistry, phytochemistry, organic synthetic chemistry, and biochemistry is strongly recommended.

In this contest, the Italian liqueur “Limoncello”, obtained by maceration of lemon skin in a hydroalcoholic solution of saccharose, is an explicative example.

Limoncello is produced in Southern Italy, mainly in Campania region (Gulf of Naples, the Sorrentine Peninsula, the coast of Amalfi, and islands of Procida, Ischia, and Capri) but also in Sicily and Sardinia.

Following an ancient tradition, limoncello is a homemade liqueur obtained from peels of Sorrento lemons. However nowadays, due to the fact that its production on industrial scale is widespread and the demand on the international market is increasing, virtually all kinds of lemons are used to make this liqueur. Its main distinctive feature is a bright yellow colour. Limoncello is served especially after-dinner as a digestive after having been stored in a refrigerator at 4 °C. It is very popular and commonly consumed in all Italy and many areas of the Mediterranean region but it is also becoming famous in other countries like United States, Canada, Australia, New Zealand, and northern Europe.

Being so popular, limoncello is also an ingredient for the preparation of several cocktails due to its strong lemon flavour without the sourness or bitterness of lemon juice.

A typical procedure for the preparation of limoncello include as ingredients 8 Sorrento lemons, 1 litre of water, 1 litre of absolute ethanol and 800 g of sugar. Lemons are then peeled in such a way to get only the flavedo (the very external part of the skin coloured in yellow); the resulting raw vegetable material is then macerated in the dark for 15 days. The

alcoholic solution from maceration is then added to the solution of sugar in water and the resulting mixture is filtered. The liqueur so obtained is kept in the dark for 40 days after which period is stored in the refrigerator and consumed as described above.

Resulting from an ethnically ancient tradition and being used as a digestive in several part of the world, it is interesting to investigate the chemical composition and medical properties of Limoncello.

The Council Regulation no. 1576/89 lays (Council Regulation, 1989) down a definition and a description of spirit drinks and also dictates the need for a better comprehension of the chemical nature of limoncello. 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.

Apart from some investigations on lemon liquors reported in journals specifically linked to beverage industry (Bonomi et al., 2001; Moio et al., 2000; Naviglio et al., 2001, 2003, 2005a, 2005b; Romano et al., 2004), it can be stated that the increasing interest of the market toward limoncellos is not offset by the number of scientific papers.

The effective problem connected to investigation about phytochemicals is the correct and real identification and quantification in natural sources by means of very sensitive, selective, and validated analytical methodologies.

In 1986, the first study describing the chemical composition of the essential oil derived from Sicilian lemons was reported in the literature by Cotroneo and coworkers (Cotroneo et al., 1986). The same group got further insights on the same topic with a series of manuscript published from the beginning of 90s until 2001 (Dugo G. et al., 1993, 1999, 2001; Dugo P. et al., 1998, 2000; Mondello et al., 1999). These Authors used very sensitive and selective analytical methodologies like high-resolution GC coupled with chiral capillary columns-mass spectrometry (HRGC-MS) and bi-dimensional capillary GC (2D-GC). In 2002 they finally summarized in a review article all the works they made on *Citrus* species (Dugo et al., 2002).

Examining the papers by Dugo and coworkers, the need of using advanced instrumental techniques for the analyses of raw sample extracted from Citrus fruits, mainly due to their chemical complexity, can be pointed out.

In the next paragraph, we'll make a survey of the current reported literature data about extraction procedures and instrumental configuration used for the analyses of this Italian liqueur obtained by maceration of lemon skin.

## 2. Analytical methods

In the last twenty years a huge progress was made concerning instrumental configuration, sensitivity and selectivity improvement (Locatelli et al., 2011) for analytical methods applied in many fields, especially for food safety.

The main problem consisted in the great complexity of extracted samples and the large number of components. For these reasons the qualitative and quantitative analyses must be very specific and robust enough to isolate, qualificate, and to quantificate the target compound (s).

In this field the best practices and improvements concerns purification and analytes extraction techniques and especially analytical instrument configuration applied to this kind of analyses.

## 2.1 Extraction procedures

Sample preparation steps are often sensitive to the matrix and, generally, contributed with approx. at 75% to the final error. This is particular true when multiple steps are involved into the procedure, where the final uncertainty is compounded.

The sample preparation has a straight impact on method performances, in particular trueness, precision, limits of quantitation, linearity, reproducibility, and is often the rate-limiting step for several analytical assays.

Sample processing can broadly range in complexity from simple dissolution in an appropriate solvent to a complicated extraction procedure, followed by derivatization and further extraction.

There are three general extraction methods, solvent extraction (or liquid-liquid extraction, LLE), solid phase extraction (SPE), and solid phase micro-extraction (SPME).

The use of these techniques, however, often entails the use of precipitation procedures, crude separation processes, and subsequent concentration methods.

Traditional liquid-liquid extraction procedures employ in a serial of extraction of an aqueous sample with an immiscible organic solvent resulting in a large solvent volume that must be dried and then concentrated prior to analysis, bring to an expensive, and in some cases non-reproducible procedure.

For this reason, further extraction methodologies were developed and validated. In particular, in SPE procedures, a solid sorbent material is packed into a cartridge or imbedded in a disk and performs essentially the same function as the organic solvent in liquid-liquid extraction, with a smaller organic solvent volume consumption.

SPE can be applied in several fields, from bio-analytical to environmental analyses, but it requires a sample volume adequately to extract targets analytes and the possibility to replicate the analysis because SPE is a destructive methodology.

In the early 1990s, the development of a new sampling method, non-destructive, sensitive, reproducible, relatively inexpensive and in particular solventless, allowed the trapping of volatile organic compounds (VOCs) on a silica optical fibre coated with a polymer thin layer followed by their identification by GC.

For the analyses of Limoncello the first extraction procedure used to determine the product volatile components was Liquid-Liquid Extraction (LLE).

In this methodology, extraction organic solvent generally used for this purpose is a hexane/ethyl acetate mixture (Starrantino et al., 1997, Dugo et al., 2000, Versari et al., 2003).

In particular the analyses of lemon-derived products were achieved with a preliminary dilution (1:10)-extraction step with hexane and ethyl acetate (75:25, v/v) (Starrantino et al., 1997) or pure hexane (Mondello et al., 2003) followed by direct analyses of extracts.

Solid-phase micro extraction (SPME) derives principally from SPE technologies and improves the concept of reducing solvent consumption, economic characteristic, and involved through two different extraction methods: headspace SPME (HS-SPME) and contact SPME.

The headspace SPME is based on the absorption of the analytes on a fibre coating placed in the sample's headspace volume and on the partition of the target analytes between the sampling matrix and the fibre. After exposure the fibre bearing the concentrated analytes was retracted, removed from the sample vial and VOCs were thermally desorbed by insertion of the fibre into the injector port of the chromatograph.

Recently in the preparative sample scenario appeared several automated and fully independent instrumentation that allow to process high sample number (high-throughput), and especially with an improvement of efficiency and process reproducibility.

When gas-chromatographic assays were used to quantify diluted Limoncello extracts it was also necessary to decrease the concentration of ethanol that tended to immediately saturate the SPME fibres. The best election, and generally, used methodology for the analysis of volatile components was headspace SPME (HS-SPME) on polydimethylsiloxane (PDMS) as thin layer (TL) due to non-polar characteristic of samples compounds.

This technique can be easily automated (Crupi et al., 2007) to improve analyses number (high throughput assay) and to obtain a better control on overall analysis steps, which bring to higher methodology reproducibility.

## 2.2 Instrumental analysis

Mono-dimensional chromatographic processes (1D) are widely applied in the analysis of food and food-derived products.

Although such methods often provide satisfying analytical results, the complexity of several matrices exceeds the resolution capacity of a single dimension separation system.

In the past years several efforts have been dedicated to the combination of independent techniques with the aim of reinforcement resolving power, until the use of multi-dimensional chromatography (MDC).

A typical comprehensive bi-dimensional chromatographic separation (2D) is achieved, generally, on two distinct columns connected in series with a special transfer system located between them. The type of interface used is connected to the specific methodology. The function of the interface is to cut and then release continuous fractions of the primary column effluent onto a fast separation column. In order to achieve comprehensive analysis and to preserve the 1D separation, the bands injected onto the secondary column must undergo elution before the following re-injection. Secondary retention times must be, at the most, equal or less than the duration of a single modulation period.

Coupled to these chromatographic techniques (both gas chromatography and high-performance liquid chromatography), several detectors are used for a clear and univocal identification and quantification of target analyte(s).

The most used are Flame Ionisation Detector (FID) coupled with GC, Mass Spectrometry Detector (MS) coupled to GC and HPLC, and Tandem Mass Spectrometry (MS/MS) interfaced with HPLC.

Due to the complex chemical composition of Limoncello extracts (comprising several classes of volatile compounds, 85-98%), the election chromatographic techniques are certainly gas-chromatographic (GC) ones.

In mono-dimensional GC the stationary phase is bonded and highly cross-linked; silphenylene polymer (polarity similar to 5% phenyl polymethylsiloxane) in programmable thermal analyses, split/splitless injector in splitless mode. This capillary column is used with both FID and MS detector, with the unique difference in helium flow rate (minor in the MS interfacing).

With this system is possible the analyses of all volatile components fraction derived from SPME extraction, while for the analyses of enantiomeric compounds chiral capillary column characterized by diethyltertbutylsilyl- $\beta$ -cyclodextrins as stationary phases are generally used.

These analyses are carried out separately to obtain a complete chemical composition of volatile components. Recently Mondello and coworkers (Mondello et al., 2006) coupled these two gas-chromatographic separations in an innovative multi-dimensional GC (MDGC) system, to obtain the complete chemical composition on volatile components including enantiomeric resolution in a single analysis.

The main goal of this configuration is especially due to the possibility of evaluating the enantiomeric ratios as genuineness markers, especially in complex matrices as Limoncellos.

To detect analytes of interest flame ionisation detector (FID) and mass spectrometric detector (MS) are generally chosen.

With the first, that is a "universal" gas-chromatographic detector is possible to obtain, in a single run a complete chemical fingerprint of volatile fraction, with identification of various components by retention index ( $R_i$ ). This configuration is very useful if are at disposition data bank with  $R_i$  or chemical standards. If no pure chemical standards are available, the best choice is gas-chromatography-mass spectrometry interfacing (GC-MS), because is possible to obtain  $R_i$  of several volatile components and, in addition, the mass-to-charge ratio for the correct identification of targeted compounds.

Another trend in the last years is the use of MDGC, coupled with mass spectrometry. Gas-chromatographic determination has the disadvantage that by this technique it is difficult to analyse non-volatile components of extracted samples.

High performance liquid chromatography (HPLC) is generally used for the non-volatile components. Is a well-defined technique, robust and reproducible that generally is coupled with "universal" detector as Diode Array Detector (DAD).

In the literature several paper dealing with the analysis of flavones, coumarins, and furanocoumarins composition of lemon-derived products by HPLC using different chromatographic column were reported.

In particular for the analyses of coumarins and furanocoumarins in Limoncellos samples the most widely used column are silica based (generally 300 x 4 mm, 10  $\mu$ m particle size) to obtain a complete resolution in normal phase mode (Starrantino et al., 1997, Versari et al., 2003), while for the analyses of phenolic compounds Octadecylsilane (ODS) column in reversed phase mode was used (Versari et al., 2003).

Ultraviolet-visible (UV/Vis) detection was carried out at 315-330 nm for coumarins and furanocoumarins and at 280 nm for phenolic compounds.

The analyses, due to the samples matrices complexity, are carried out in gradient elution mode. In particular for the analyses of coumarins and furanocoumarins was used a mobile Phase constituted by hexane: ethyl acetate (92:8 or 88:12, v/v) and hexane: ethyl alcohol (90:10, v/v).

Only recently Crupi and coworkers (Crupi et al., 2007) developed a unique reversed phase method for simultaneous determination at 315 nm of coumarins and furanocoumarins using water and acetonitrile as mobile phase in gradient elution mode.

### 3. Limoncello chemical composition

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 are crucial in establishing the preference among several products available in the market. For this reason, the analysis 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.

The current chemical composition of this Italian liqueur is mainly related to the aromatic fraction that was reported by Crupi and coworkers (Crupi et al., 2007) using previously mentioned analytical techniques.

In their comprehensive studies, after SPME extraction of volatile components and chromatographic analyses, both GC and HPLC, they detected several monoterpenes, sesquiterpenes, and oxygenated compounds, as reported in the Table 1.

Versari and coworkers reported similar analyses of several commercial Limoncello samples and chemometric elaboration with Principal Components Analysis (PCA) technique to obtain two main group: the first that showed a composition similar to lemon essential oils (high content of  $\beta$ -pinene, myrcene, *trans*- $\alpha$ -bergamotene, and  $\beta$ -bisabolene, and a low content in neral and geranial) (Versari et al., 2003). The composition of the second group suggested the presence of oxidative phenomena and (or) addition of flavours, in particular the presence of compounds as ethyl acetate, acetaldehyde, 2-methyl-1-propanol and glycerol indicate that fermentation process probably occurred in the sugar syrup during Limoncello dilution step after the extraction process.

The best recurrent molecules of the oil and of Limoncello (both homemade and commercial ones) belonging the volatile compounds family are  $\alpha$ -pinene, sabinene,  $\beta$ -pinene, myrcene, *p*-cymene, limonene,  $\gamma$ -terpinene, neral, geraniol, and geranial, and as reported in the Table 1. As results of their investigation, Crupi and coworkers (Crupi et al., 2007) underline that most of analysed Limoncello samples were effectively obtained directly from fruits, but also using terpeneless oils and in some cases synthetic products of reconstituted oils.

From data reported in the Tables 1 and 2 is possible to indicate that the commercial #05 and #02 are of high quality, even if commercial #02, due to high level of *p*-cymene can indicate a long storage time.

Compounds	Oil	Home-made Limoncello	Commercial Limoncello															
			#01	#02	#03	#04	#05	#06	#07	#08	#09	#10	#11	#12	#13	#14	#15	#16
$\alpha$ -Pinene	14.510	21.0	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
Sabinene	17.570	211.3	-	14.4	9.0	-	20.8	8.8	-	-	9.7	9.0	-	-	14.9	-	-	9.0
$\beta$ -Pinene	142.090	-	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
Myrcene	11.900	18.8	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
<i>p</i> -Cymene	340	4.5	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
Limonene	626.290	837.2	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
$\gamma$ -Terpinene	104.670	143.9	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
Neral	13.520	390.0	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
Geraniol	270	3.2	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
Geranial	20.960	581.6	40.2	10.1	-	24.5	17.9	-	9.7	13.7	10.2	32.1	94.0	19.4	11.3	65.5	40.4	133.5

Table 1. Amount (mg/L) of major representative volatile components in several lemon samples-derivatives in oil, homemade Limoncello, and commercial Limoncello products (Crupi et al., 2007).

Compound	Commercial Limoncello												
	#01	#02	#03	#04	#05	#06	#07	#08	#09	#10	#11	#12	
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	
5-geranyloxy-7-methoxycoumarin	0.7	1.6	0.8	0.5	0.5	1.3	0.8	7.0	1.2	0.4	0.2	1.2	
Citroptene derivate	-	-	0.1	0.1	-	-	-	0.3	-	-	-	-	
5-isopentenyl-7-methoxycoumarin	-	-	0.4	0.2	-	-	-	1.0	-	-	-	-	
Citroptene	2.0	-	1.5	1.0	1.3	0.3	1.8	4.0	0.4	1.1	0.4	1.0	
Imperatorin	4.4	0.8	-	0.6	-	-	-	1.6	-	-	-	-	
Eriocitrin	16.9	-	27.8	21.7	65.9	-	-	71.2	-	29.1	-	22.3	
Sinapinic acid	0.4	-	0.5	-	1.0	-	-	Trace	-	1.5	-	0.5	
Narirutin	5.2	14.5	1.8	0.8	2.2	-	-	2.2	-	-	-	5.9	
Naringin	-	1.5	-	1.2	Trace	-	-	Trace	Trace	Trace	1.4	1.3	
Hesperidin	16.7	-	25.3	12.0	32.1	-	-	42.3	-	6.7	-	7.6	
Neohesperidin	0.6	-	Trace	Trace	Trace	-	-	1.2	-	Trace	-	0.4	
Diosmin	1.9	13.3	7.6	Trace	7.9	-	5.3	-	0.8	4.0	Trace	8.6	

Table 2. Identified and quantified coumarins, psoralen, and phenolics composition (mg/L) of several commercial Limoncello samples (Versari et al., 2003).

Terpenes are photosensitive compounds; in particular limonene and terpinenes are involved in this irreversible process that negatively affects the organoleptic properties of the beverage. The terpenes fraction and/or the presence of oxidized by-products can predict the origin and the quality of a Limoncello.

Versari and coworkers (Versari et al., 2003) reported that other mainly present compounds, analysed by HPLC-DAD, were phenolics, coumarins, and psoralen derivatives. These compounds are mainly flavones, coumarins, and furanocoumarins, as represented in Figure 1.

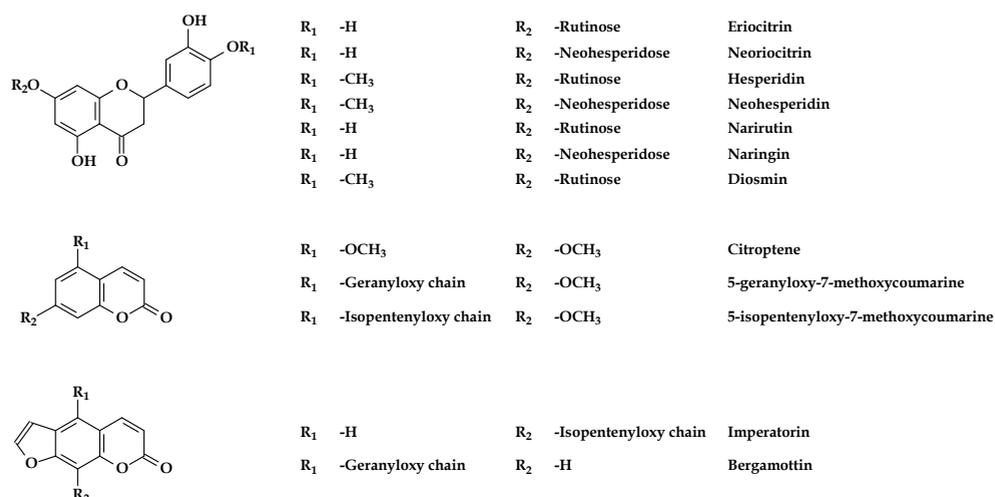


Fig. 1. Chemical structures of flavones, coumarins, and furanocoumarins identified and quantified in Limoncello by Versari and co-worker (Versari et al., 2003).

Problems connected to storage time were reported also by Da Costa and Theodore (Da Costa and Theodore, 2010) in a paper that report a complete study of over two-hundred volatile components in Limoncello and their changes that occurred with aging.

Another well-recognized question investigated by Poiana and coworkers (Poiana et al., 2006) regarded the differentiation from sample-to-sample about chemical and physical-chemical parameters of alcoholic extracts of lemons deriving from the Amalfi and Sorrento areas. These discrepancies are probably correlated with the different cultivars and growing conditions.

In particular this research underlined the evolution during the season for some classes of components. The carbonyl-to-oxygenated, alcohols-to-oxygenated and esters-to-oxygenated compounds ratios are indices of flavouring quality. These showed similar trends for both lemon types. In comparison to other lemon productions a higher amount of minor classes of components were also recorded.

#### 4. Citrus essential oil heavy metal composition – food quality

Citrus essential oil is a very complex mixture of several classes of volatile (85-98%) and non-volatile (2-15%) compounds, as reported in the previous paragraphs. In this classification,

the main present compounds are terpenes, hydrocarbons, esters, aldehydes, and ketones. The key difference between essential oils from different raw plant varieties is especially related to the composition of the volatile fraction (Steuer et al., 2001). *Citrus* essential oil is generally used as aromatising agent and additive for food and food-derived products, beverages, cosmetics and in some pharmaceuticals. In this sample extract is possible to incur into the presence of organic and inorganic contaminants, as well documented by the presence of pesticide residues (Saitta et al., 2000, Dugo et al., 1997; Verzera et al., 2004) and plasticizers (Di Bella et al., 1999, 2000, 2001; Saitta et al., 1997); however, there is a needs of available data regarding the presence of heavy metals in this products. Some reports concerning the microelements composition of *Citrus* peel extracts were published (Gorinstein et al., 2001; Simpkins et al., 2000). Metals, such as iron, copper, zinc, and cobalt, are non-toxic at modest concentrations, while cadmium, lead, mercury, and arsenic are toxic even in very low concentration level and constitute a significant health hazard (Rojas et al., 1999).

Metals levels in *citrus* essential oils mainly depend on the type of soil and treatment, but are also affected by the extraction procedures to manufacture food-derived products, such as scraping or pressing because the fruits inevitably come in contact with metallic surfaces.

In this field the best instrumental analysis concerns especially the use of electrochemical techniques, such as derivative potentiometric stripping analysis (dPSA) and Atomic Absorption Spectroscopic methods (AAS) in Graphite Furnace Atomic Absorption Spectroscopy Analysis (GFAAS) configuration to determine trace metals concentrations in a variety of matrices, such as alloys, food, biological materials, and environmental samples.

Recently La Pera and coworkers (La Pera et al., 2003) published an interesting work inherent the use of these two techniques for the simultaneous determination of cadmium, copper, lead, and zinc in *Citrus* essential oils with high recoveries values from real samples extracts.

In the Table 3 were reported the mean concentration determined by dPSA and AAS both on acid and methanol extracts.

Sample	Treatment	Cd		Cu	
		dPSA	AAS	dPSA	AAS
Lemon	Acid extracts	1.57±0.03	1.43±0.03	16.94±0.21	16.00±0.20
Lemon	Methanol extracts	1.63±0.03	1.55±0.05	21.65±1.59	18.10±2.65
Sample		Pb		Zn	
		dPSA	AAS	dPSA	AAS
Lemon	Acid extracts	111.24±0.81	103.60±1.10	802.55±2.48	799.60±3.00
Lemon	Methanol extracts	113.24±0.72	103.30±7.15	809.62±2.26	788.99±9.10

Table 3. Heavy metal mean concentration (ng/g; n=3) and standard deviation (n=3) detected by dPSA and AAS in Lemon samples (La Pera et al., 2003).

These results indicate that *Citrus* lemon essential oils contain several heavy metals and that a deep control on food-derived products is required to obviate any significant health hazard.

In particular, Verzera and coworkers (Verzera et al., 2004) showed that there are great differences between lemon biological oils and traditional ones inherent in the organophosphorus pesticide content.

In particular, these authors report that Parathion methyl, Parathion ethyl, Quinalphos, Methidathion, Clorpyrifos methyl, and Azinphos ethyl are the most representative organophosphorus pesticides found in lemon oil samples, both in biological and traditional agricultural methods.

Traditional oils were found to contain from 3.52 to 3.85 ppm of previously cited compounds, while biological ones were found to contain from 0.17 to 0.74 ppm.

Only in lemon oils obtained from fruits deriving from traditional agricultural methods were found Dicofol as organochlorine pesticides at 1.0 ppm level.

Previously reported values inherent in organophosphorus pesticides were obtained by gas chromatography coupled with Flame Photometric Detector (FPD) that is similar to FID in that the sample exits the analytical column into a hydrogen diffusion flame. Where the FID measures ions produced by organic compounds during combustion, the FPD analyzes the spectrum of light emitted by the compounds as they luminesce in the flame.

Organochlorine pesticides were also obtained by gas chromatography coupled with Electron Capture Detector (ECD). The ECD measures electron-capturing compounds (frequently halogenated compounds) by creating an electrical field in which molecules exiting a GC column can be detected by the drop in current in the field.

## 5. Conclusion

A deep knowledge of all the chemical aspects of a Limoncello, in this context, could greatly help with assessing its authenticity and genuineness. The analysis of food products may be directed to the assessment of food quality and authenticity, the control of a technological and production process, the determination of nutritional values, and the detection of molecules and secondary metabolites eventually present in food-derived products with a possible advantageous effect on human health and safety.

Consequently, a main aim in food chemistry regards especially the continuous improvement, development, and in particular, validation of increasingly sensitive and selective analytical techniques.

The availability of hyphenated analytical methods and 2D chromatographic 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.

Further investigations must be devoted especially to the improvement of the instrumental performance and high-throughput analyses, to the implementation of supplementary

options such as cryo-trapping and to the enhancement of MS and MS/MS detection in both conventional gas and high performance liquid chromatography, and multi-dimensional gas-chromatography (MDGC) applications.

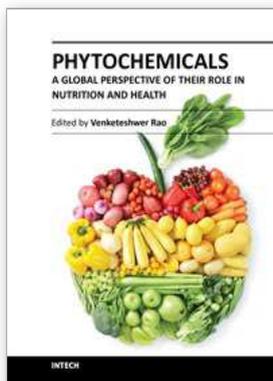
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