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# Multivariate analysis discrimination of various cold-pressed lemon oils from different geographical regions

**Sanja Kostadinović [1], Marina Stefova [1], Diana Nikolova [2], Daniela Nedelcheva [2], Natalia Martinez [3], Daniel Lorenzo [3], Eduardo Dellacassa [3] and Hamed Mirhosseini [4]***

*[1] Institute of Chemistry, Faculty of Science, Sts. Cyril and Methodius University, Skopje, Macedonia. [2] Institute of Organic Chemistry, Centre of Phytochemistry, Bulgarian Academy of Sciences, Sofia, Bulgaria. [3] Cátedra de Farmacognosia y Productos Naturales, Departamento de Química Orgánica, Facultad de Química, Universidad de la República. Montevideo, Uruguay, 20 Gral. Flores 2124. 11800-Montevideo, Uruguay. [4] Department of Food Technology, Faculty of Food Science and Techology, University Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia. *e-mail: hamedmi@food.upm.edu.my*

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**Abstract**

The main objective of present study was to investigate the influence of geographical region on the flavour compounds of various cold-pressed lemon oils, i.e. Argentinean, Ivory Cost, Italian, Spanish and Uruguayan. Among the flavor compounds, the major volatile flavour compounds from different chemical classes namely monoterpene hydrocarbons (i.e. limonene, sabinene plus β-pinene, γ-terpinene, myrcene, α-pinene and α-terpinolene), esters (i.e. neryl acetate and geranyl acetate), alcohol (i.e. α-terpineol) and aldehyde compounds (i.e. geranial and neral) were composed of > 90% of total volatile flavor compounds of target cold pressed lemon oils. Uruguayan essential oil had the least concentration level of all major classes of flavor compounds except for alcohol compounds; while the highest content of all chemical classes of flavor compounds identified in this study. The principle component analysis (PCA) allowed us to discriminate various cold-pressed lemon oil based on their origin resource, thereby classify their sensorial attributes.

***Key words:*** Cold-pressed lemon oil, volatile flavour compounds, monoterpene hydrocarbons, sesquiterpenes hydrocarbons, aldehydes, esters, alcohols, GC-MS, GC-FID, alkanes, principle component analysis.

## Introduction

Citrus and lemony flavours represent a very large portion of the world market for flavour ingredients. An expanding market for lemon flavoured drinks uses the oils of lemon, orange, mandarin, grapefruit and bitter orange [1]. Citrus oils are extremely important for the flavouring of food, beverages, confectionary, cookies and dessert is growing steadily at more then three per cent per year. Lemon oil is one of the most popular citrus flavors used to induce refreshing topnote to almost any fragrances. The oil expressed from the lemon rind has a characteristic lemon aroma-sweet, fresh and sharp. Lemon flavor has many applications in food, drug and cosmetic industries especially as flavouring agent, blender or modifier in fruit juice and beverages, bakery products and confections. Lemon scented oils are also in great demand for use in aromatherapy. The main properties attributed to their application are analgesic, antidepressant, aphrodisiac, antiseptic, antispasmodic, carminative, cordial, deodorant, digestive, expectorant, sedative and tonic [2-4]. Cold-pressed lemon oils are a mixture of several compounds that can be classified into three classes: terpenes, which form more than 90% of the oil, oxygenated compounds and sesquiterpenes. Cold-pressed citrus oil is a mixture of volatiles, such as terpenes and oxygenated compounds, and nonvolatiles, such as waxes and pigments. Terpene content should be minimal in order to insure product stability in the perfume industry, whereas limonene, a principal constituent of terpenes has recently been praised as a superior solvent for polystyrol in chemical recycling processes.

Terpenes are unstable to heat and light and they degrade to produce undesirable off-flavor compounds. Terpenes do not contribute much to the flavour or fragrance of the oil, even at higher concentrations. Furthermore, nonvolatiles such as waxes and pigments must be removed because they are prone to highly viscous and phototoxic activity, then produce the turbid in the oil. The characteristic flavor of citrus oil is provided by the oxygenated compounds (oxygenated terpenes), which mainly consist of alcohols, aldehydes, and ester such as linalool, citral, and linalyl acetate [5]. The volatile flavor composition of lemon aroma has been studied by previous researchers [6-13]. However, most of these studies concerned oils for neither commercial interest nor the production origin were specified.

Citrus essential oil is present in oil sacs or oil glands located at different depths in the peel and the cuticles of the fruit. Peel and cuticle oils are removed mechanically by cold pressing and, since cold pressing yields a watery emulsion, this emulsion is then subjected to centrifugation to separate out the essential oil. Distillation is also used in some countries as an economical way to recover *Citrus* essential oils from *Citrus* by products. The processing of *Citrus* fruits to obtain juices produces a series of by-products, one of which is the peel, from which a variety of products, including essential oil, can be extracted. During distillation, *Citrus* peels exposed to boiling water or steam release their essential oils through evaporation. Recovery of the essential oil is facilitated by distillation of two immiscible liquids, viz. water and essential oil, based on the principle that, at the boiling temperature, the combined vapour pressure equals the ambient pressure [14]. Thus, the variety of *Citrus* fruits and extraction technique are considered as two important factors affecting the key volatile composition extracted from *Citrus* fruits.

It is necessary to have quantitative information about the most characteristic aromatic components in lemon flavour oil. This information allows the manufacture for the modifications and/or enhancement of the aroma freshness. The present study was conducted to obtain more information about the chemical variability of lemon oils with respect to the geographic area of production. In this study, the flavour composition of 6 types of commercial lemon oils produced with the same extraction technology obtained from the main citrus producing regions in the world was evaluated. For creation of lemon flavor in beverage industry or any other area of food industry the base of the lemon flavor is cold pressed lemon essential oil. Usually 90% of the flavors for beverage soft drinks are consist from the cold pressed lemon essential oils. The main objectives of present study were to (i) investigate the effect of geographical region on the flavour composition of various cold pressed lemon oils obtained from different local resources in Argentina, Ivory Cost, Italy, Spain and Uruguay and subsequently, (ii) differentiate various cold pressed lemon oils based on their chemical classes by using principle component analysis (PCA). This study allows studying the composition and geographical variation of the volatile constituents present in commercial lemon oils obtained from major producing regions of the lemon oil in the world.

## Materials and Methods

***Materials:*** The pure standard of lemon oil composition namely α-thujene (98.5%), α-pinene (99.5%), camphene (98%), sabinene (99%), β-pinene (98.5%), myrcene (95%), α-phellandrene (99%), α-terpinene (95%), p-cymene (98%), limonene (99%), camphor (99%), γ-terpinene (98.5%), α-terpinolene (99%), linalool (95%), nonanal (95%), citronellal (99%), terpinen-4-ol (98.5%), α-terpineol (98%), decanal (99%), nerol (98%), citral (95%),undecanal (99%), tetradecanal (98%), citronellyl acetate (98%), neryl acetate (98.5%), geranyl acetate (95%) and valencene (95%) were supplied by Fluka (Buch, Switzerland) and Sigma-Aldrich (St. Louis, MO, USA). Different varieties (i.e. Argentina, Brazil, Ivory Coast, Spain, Italy and Uruguay) of cold pressed lemon oils were provided by different flavour companies, Danisco (Cultor, Aarhus, Denmark), Givaudan (Dubendorf, Switzerland) and Symrise (Nördingen, Germany).

***Qualitative and quantitative analyses of lemon oil compounds:*** The flavour compounds of cold-pressed lemon oils were analyzed using a Hewlett-Packard 6890 GC equipped with a Mass Spectrometer (MS) and an HP-5 cross-linked fused-silica capillary column (i.d. = 0.25 mm, length = 30 m, film thickness = 0.25 μm) supplied by Agilent Inc. (PA, USA). In qualitative and quantitative analyses, 0.2 μl of cold pressed lemon oil was injected in the split mode (1:100). Oven temperature was programmed at 40°C isothermally for 5 min, then ramped to 180°C at 4°C/min and then increased to 260°C at 20°C/min and held for 10 min at the final temperature. Helium was used as the carrier gas with a flow-rate of 5.3 (ml/min). Injector and detector temperatures were set at 270 and 300°C, respectively. The volatile flavour compounds were initially detected and confirmed using a Hewlett-Packard 6890N GC system (Wilmington, DE) and the NIST library version 2.0. The components of the essential oil were identified by comparison of their linear retention indices, determined in relation to a homologous series of n-alkanes (C8-C32), with those from pure standards or reported in literature. Comparison of fragmentation patterns in the mass spectra with those stored on databases and MS data of our collection was also performed.

***Multivariate analysis:*** Principal component analysis (PCA) was performed to gain an overview of how the samples were correlated to each other with regard to equilibrium volatile headspace concentration. Correlation matrix was applied in multivariate analysis with Minitab software release 14, so that the data was autoscaled by variable to give the same weight to all components.

## Results and Discussion

In the present work, forty-nine volatile components were identified and quantified in six commercial essential oils produced in different regions of the world, as listed in Table 1. Moreover, the total amount of hydrocarbons, oxygenated compounds, monoterpenes, sesquiterpenes, carbonyl compounds, alcohols, esters and aldehydes shown by the various cold-pressed lemon oil were given in Table 1. The linear retention indices (LRI) and aroma descriptors for the identified flavour components are also shown in Table 1. The quantitative data were divided according to the production areas. The identified volatile flavor compounds were found to be in agreement with that reported by previous researchers [15-17]. In the proposed studies, the chemical composition and aroma active compounds of cold-pressed lemon essential oil were isolated by specific techniques. The present work was conducted to find the possible relationship between the most important aroma active class of compounds in lemon essential oil and geographical region of growing and production of those oils. Therefore, the major volatile flavor composition of all cold -pressed lemon oils was analyzed. Fig. 1 also shows the gas chromatograms obtained from qualitative analysis of Spanish cold-pressed lemon oil. The components identified represented 93-99% of the total volatile fraction of cold-pressed lemon oils. The results were almost similar to that of obtained from Italian and Uruguayan industrial genuine oils [16-20].

One of the most important volatile flavor classes identified in cold-pressed lemon oils was shown to be the aldehyde compounds. Aldehydes are the most aroma-active compounds and the tope note of perfumes and the fresh and fruity lemon flavour in beverages come from these compounds. Citral (neral plus geranial) as one of major aldehyde compounds representative of lemon aroma were identified in all cold-pressed lemon oils analyzed in this study. The content of citral (neral plus geranial) in lemon flavour determines the lemon oil flavor peculiarity and the market value of lemon flavour oil. The highest content of aldehyde compounds was observed in the lemon oil from Ivory Cost (Table 1). Since the type and concentration of aldehyde compounds

**Table 1.** Volatile composition of cold-pressed peel oils from Spain, Ivory Cost, South America, Argentina, Uruguay and Italy.

| Component | LRI* (HP-5) | Spain | Ivory Cost | South America | Argentina | Uruguay | Italy | Aroma characteristic (12, 13) |
|---|---|---|---|---|---|---|---|---|
| Tricyclene | 920 | 0.02 | 0.01 | 0.01 | - | 0.01 | 0.01 | - |
| α-Thujene | 926 | 0.78 | 0.70 | 0.69 | 0.66 | 0.04 | 0.43 | - |
| α-Pinene | 932 | 3.35 | 2.83 | 2.91 | 2.94 | 1.89 | 1.95 | Pine-like, resinous, sweet |
| Camphene | 947 | 0.15 | 0.12 | 0.12 | 0.13 | 0.06 | 0.06 | Oily, camphoraceous |
| Sabinene + β-Pinene | 974 | 18.23 | 15.53 | 16.24 | 17.30 | 14.31 | 13.07 | Oily, green/ resinous, woody |
| Myrcene | 992 | 1.92 | 1.82 | 1.80 | 1.73 | 1.49 | 1.44 | Musty, wet soil |
| α-Phellandrene | 1004 | 0.13 | 0.16 | 0.15 | 0.15 | 0.07 | 0.05 | - |
| α-Terpinene | 1017 | 0.48 | 0.59 | 0.36 | - | 0.19 | 0.19 | Lemony, citrus-like |
| p-Cymene | 1025 | - | - | - | 0.45 | 0.15 | 0.13 | - |
| Limonene | 1034 | 51.31 | 47.45 | 50.46 | 52.23 | 67.02 | 65.23 | Citrus-like, fresh |
| (*Z*)-p-Ocimene | 1039 | 0.11 | 0.09 | 0.12 | 0.09 | 0.07 | 0.07 | Herbaceous, flowery, sweet |
| *cis*-Limonene oxide | 1135 | 0.09 | 0.02 | 0.02 | - | 0.01 | 0.01 | Citrus-like |
| *trans*-Limonene oxide | 1139 | 0.08 | 0.02 | 0.01 | - | 0.01 | 0.01 | Citrus-like |
| Camphor | 1145 | 0.02 | 0.01 | - | - | - | - | - |
| (*E*)-p-Ocimene | 1049 | 0.17 | 0.17 | 0.20 | 0.15 | 0.12 | 0.13 | - |
| γ-Terpinene | 1060 | 13.38 | 13.70 | 12.77 | 12.52 | 8.81 | 9.54 | Lemony, lime-like |
| *cis*-Sabinene hydrate | 1068 | - | 0.05 | 0.06 | - | 0.05 | 0.04 | - |
| α-Terpinolene | 1088 | 0.75 | 0.87 | 0.70 | 0.71 | 0.35 | 0.38 | Citrus, pine |
| *trans*-Sabinene hydrate | 1099 | 0.02 | 0.03 | 0.03 | - | 0.03 | 0.03 | - |
| Linalool | 1101 | 0.19 | 0.21 | 0.15 | 0.13 | 0.12 | 0.11 | Floral, sweet, citrus |
| Nonanal | 1106 | 0.09 | 0.08 | 0.13 | 0.12 | 0.12 | 0.11 | Piny, floral, citrus |
| Citronellal | 1155 | 0.12 | 0.11 | 0.18 | 0.08 | 0.08 | 0.09 | Powerful, floral, lemon |
| Terpinen-4-ol | 1178 | 0.19 | 0.30 | 0.04 | 0.07 | 0.06 | 0.04 | Woody, earthy |
| α-Terpineol | 1192 | 0.41 | 0.65 | 0.33 | 0.27 | 0.19 | 0.17 | Floral, lilac-like |
| Decanal | 1207 | 0.08 | 0.07 | 0.07 | 0.04 | 0.04 | 0.04 | Beefy, musty, cucumber |
| Nerol | 1233 | - | 0.07 | - | - | 0.02 | 0.04 | Sweet, fruity, floral |
| Neral | 1243 | 0.68 | 1.42 | 1.01 | 0.96 | 0.81 | 0.83 | Lemony, citrus |
| Geraniol | 1253 | - | 0.04 | - | - | 0.04 | 0.02 | Floral, rose like |
| Geranial | 1273 | 1.09 | 2.17 | 1.84 | 1.53 | 1.38 | 1.39 | Citrus-like, flowery, fruity |
| Undecanal | 1309 | 0.04 | 0.03 | 0.03 | 0.03 | 0.02 | 0.02 | Pleasant waxy, floral |
| Tetradecanal | 1308 | - | 0.01 | - | - | 0.01 | 0.01 | Fresh, herbaceous |
| Citronellyl acetate | 1355 | 0.06 | 0.07 | 0.07 | 0.03 | 0.02 | 0.03 | Fresh, rosy, fruity |
| Neryl acetate | 1366 | 0.93 | 0.97 | 0.92 | 0.80 | 0.33 | 0.40 | Fruity, floral, very sweet |
| Geranyl acetate | 1386 | 0.80 | 0.69 | 0.69 | 0.35 | 0.31 | 0.42 | Dry, herbaceous |
| (*E*)-β-Caryophyllene | 1422 | 0.38 | 0.57 | 0.37 | 0.38 | 0.24 | 0.23 | Citrus-like, fresh |
| α-Humulene | 1456 | 0.03 | 0.05 | 0.03 | - | 0.02 | 0.34 | Woody |
| (*E*)-β-Farnesene | 1459 | 0.40 | 0.07 | 0.04 | 0.03 | 0.05 | 0.04 | Sweet, fruity |
| *cis*-β-Santalol | 1462 | 0.03 | - | 0.03 | 0.02 | - | - | - |
| Germacrene-D | 1485 | - | 0.03 | - | - | 0.01 | 0.01 | - |
| Valencene | 1495 | 0.03 | 0.08 | 0.12 | 0.02 | 0.02 | 0.03 | Sweet, woody, citrus |
| Bicyclogermacrene | 1498 | - | 0.10 | 0.08 | 0.10 | 0.07 | 0.07 | - |
| α-Bisabolene | 1504 | 0.12 | 0.17 | 0.10 | 0.12 | 0.04 | 0.04 | Woody, dry, mild |
| β-Bisabolene | 1511 | 1.32 | 1.60 | 1.09 | 0.97 | 0.44 | 0.51 | - |
| Spathulenol | 1581 | 0.08 | 0.01 | 0.02 | 0.03 | - | - | - |
| α-Bisabolol | 1689 | 0.06 | - | 0.04 | 0.03 | 0.02 | 0.02 | - |
| Nootkatone | 1809 | - | 0.01 | 0.01 | - | - | 0.02 | Green, grapefruit |

* Linear retention index identified by GC-MS equipped with HP 5 column.

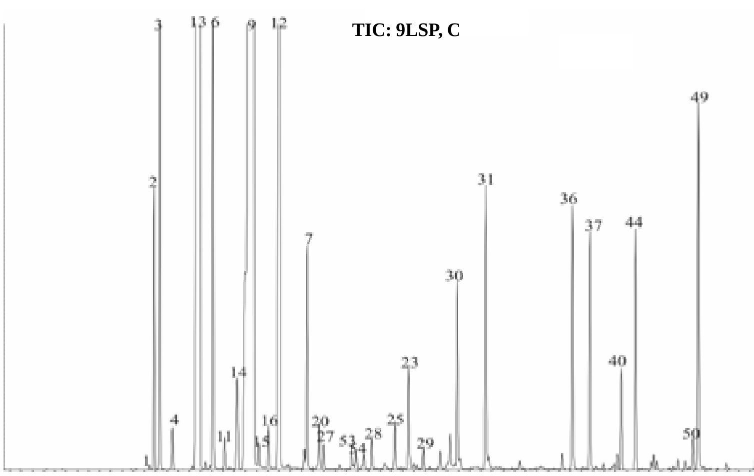


***Figure 1.*** Gas chromatograms obtained from qualitative analysis of Spanish cold-pressed lemon oil.

present in lemon flavor play a critical role for the industry, the data indicated that the cold-pressed lemon essential oil from Ivory Cost was the most suitable fragrance due to its high aldehyde content. Esters are the second important group of flavour compounds due to their high aroma potency. The lemon essential oils from Spain had the highest concentration of ester compounds (Table 1).

Among the cold-pressed lemon oils, the Uruguayan essential oil showed the least content of all major classes of flavor compounds except for alcohol compounds; while the highest concentration level of all chemical classes (i.e. oxygenated compounds, monoterpenes, sesquiterpenes, carbonyl compounds, alcohols, esters and aldehydes) of volatile flavor compounds identified in this study. Some of alcohol compounds have aroma activities but they are usually produced via the oxidation of monoterpenes. Some of alcohol compounds are responsible for the off flavour in the products. The cold-pressed essential oil from Spain had the highest alcohols content, thus indicating that Spain lemon oil was more exposed to the oxidation compared to the other cold-pressed lemon oils (Table 1). High quantity of monoterpenes indicates the high risk of oxidation. Among the monoterpenes present in citrus flavors, limonene is composed as a major monoterpene hydrocarbon which is not or very small aroma active. The highest quantity of monoterpenes was shown in the cold-pressed lemon oil from Uruguay (Table 1). This observation showed that Uruguay lemon flavor had higher risk of oxidation compared to the other cold-pressed lemon oils. Usually, that kind of oils are using to produce 5 or 10 fold concentrated lemon oil. The resulting oil had high quantities of aldehydes and smaller quantities of limonene.

Three cold-pressed lemon oils collected from Uruguay and Italy showed very similar flavour compound profile. On the other hand, for the samples provided by South America and Argentina gave basically similar results. However, the quantitative information summarized in Table 1 and Fig. 2 was not enough to evaluate the similarities and differences between the cold-pressed lemon oils considered in this study. A multivariate pattern recognition approach should be more effective in recognizing differences among the samples analyzed. The results showed the comparison between the major chemical classes of volatile chemical compounds of eighteen cold-pressed lemon oils (Fig.2). According to those results, the lemon essential oil from Ivory Cost had the highest quantities of aldehydes which are identified as one of the most important aroma active compounds in cold-pressed lemon oil. As shown in Fig. 2, the lemon essential oils from Spain, South America and Ivory Cost cold-pressed lemon essential oils had higher content of esters than lemon essential oils from Argentina, Uruguay and Italy. Sesquiterpenes are major group of flavor compounds in citrus flavors in term of quantity. As shown in Fig. 2, the lemon oil from Ivory Cost showed the highest quantity of Sesquiterpenes. Those results indicated that the lemon essential oil from Ivory Cost had the highest quantities of all three most important classes of flavor compounds found in cold-pressed lemon oil.

On the other hand, the presence of highest content of oxygenated flavor compounds in lemon essential oil from Ivory Cost might be an indication of chemical oxidation of essential oil. PCA score plots were used to determine whether six different cold-pressed lemon oils could be grouped into different classes (Fig. 3). To focus on the differences among the cold-pressed lemon oils

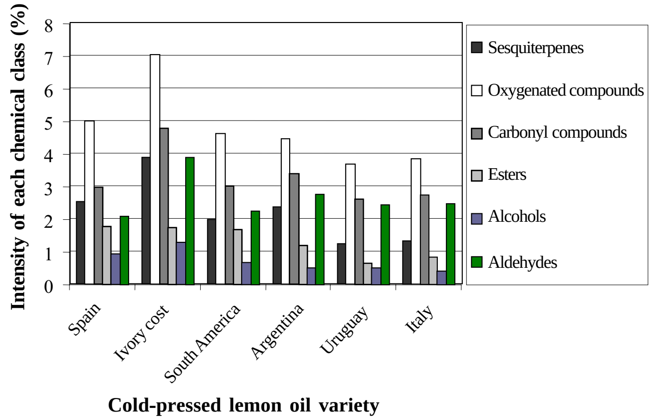


***Figure 2.*** Comparison between the major chemical classes of volatile flavor compounds of eighteen cold-pressed lemon oils.

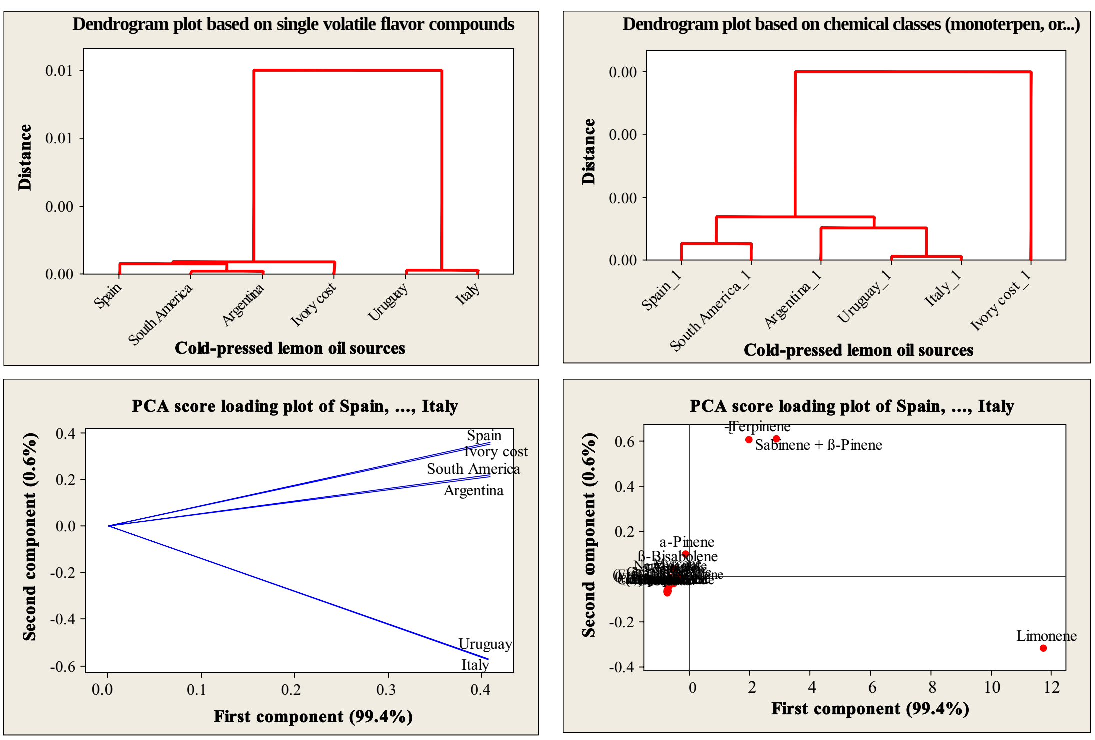


***Figure 3.*** PCA scatter plots and cluster dendrograms discriminating cold-pressed lemon oils and their volatile flavour compositions into different classes.

and target volatile compounds, cluster observation and cluster variable dendrograms were constructed using the nearest neighbour (Fig. 3). The results indicated that first two principal components explained 99.4% and 0.6% of the total variability, respectively. Despite first two principal components, which showed 100% of total variation, the remaining principal components didn't account for any variability and were probably unimportant.

Except for some monoterpene hydrocarbons namely sabonene, β-pinene, limonene and γ-terpinene, the other target volatile compounds could be classified in one group in PC1, because the coefficients of these volatile compounds were the same negative sign located in PC 1 (Fig. 3). In most cases, the differentiation or closeness between the volatile flavour compounds directed in negative side of PC 1 was dependent on their chemical classes. The second principal component (PC 2) accounted for very small proportion (0.7%) of the total variability. As shown in Fig. 3, most of target volatile flavour compounds identified in cold-pressed lemon oils namely alcohol (i.e. linalool, terpinen-4-ol, α-terpineol and geraniol), ester compounds (i.e. citronellyl acetate, neryl acetate and geranyl acetate) and aldehyde compounds (i.e. octanal decanal, citronellal, nonanal, neral and geranial) were classified with the same negative sign in PC 2; while α-pinene, β-bisabolene and some other monoterpene hydrocarbons identified were placed in the positive side of PC 2 (Fig. 3).

PCA score plot significantly differentiated the cold-pressed lemon oils from each other (Fig. 3). The PCA analysis separated the Urguayan and Italian oils from the other tested cold-pressed lemon oils. Because all the samples have been processed with the same technology at the same season, quantitative differences in the essential oil compositions might be due to the genetic origin and the micro ecological environment.

## Conclusions

In the present study, high resolution gas chromatography (HRGC) equipped with mass spectrometer (MS) and flame ionization detector (FID) was employed for the qualitative and quantitative analyses of volatile flavour compounds of various cold-pressed lemon oils collected from different geographical regions. Multivariate analysis techniques aided in the interpretation of chemical data obtained for volatile components of lemon commercial oils. Differences among individual components provided less useful information, mainly because there is often a wide variation in the volatile fraction composition from different samples of the same oils; since multivariate analysis involves variability of several or all of the compounds; it seems that the discrimination results were less affected by such variation. PCA score plot significantly differentiated the Urguayan and Italian oils from the other tested cold-pressed lemon oils. Although the same technology was applied to process all the cold-pressed lemon oils, but quantitative differences in the essential oil compositions might be due to the genetic origin and the microecological environment.

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