# High Resolution Gas Chromatography for Detection of Adulterations of Citrus Cold-Pressed Essential Oils

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Italy is one of the leading countries in the production of citrus oils. The olfactory characteristics of Italian cold-pressed oils are extremely good and, even if sometimes the products are more expensive than those produced in other countries, there is a demand for these oils because of their quality.

However, sometimes the market competition, together with a limited interest of the people who buy the oils with regard to the quality of the products, induces the producers to adulterate the oils with products of low value. It is our belief that Italian oils will only have a special place in the world market as long as their quality and genuineness can be guaranteed. Mainly there are two types of adulterations for cold-pressed citrus essential oils.

One is the rough addition of some natural and/or synthetic products of low value. In such cases the aim is not to try to get a composition of the oil exactly similar to a genuine one, but what is important is "passing certain examinations" like percentage of residue, density, optical rotation, citral content, ester number, UV absorbance, etc.

Some conventional techniques can be useful in detecting these kinds of adulteration. One such method is thin-layer chromatography which facilitates the detection of products added as UV absorbance modifiers.[1,2] Another technique, such as packed column gas chromatography, can be used to detect the addition of the fatty acids of castor oil,[3] which are usually added in order to increase the nonvolatile residue of the essential oil. GC can also be used for the detection of neral and geranial,[4] the ratio of which is very important in order to ascertain whether a lemon oil is genuine, since this ratio is almost always constant and can be taken as a reference parameter.

The second type of adulteration is, from an analytical point of view, most interesting. It includes the sophisticated adulterations which make the oil very similar to a genuine one. In such cases, additions of natural and/or synthetic products of low value are used to get an economic profit while attempting to maintain the qualitative or even the quantitative composition of the natural oil, making the detection of the adulteration very difficult.

High resolution GC, either with conventional stationary phases or with the relatively new chiral phases, is the technique which best helps the analyst in detecting citrus oil adulteration. The information obtained on the volatile fraction of the oils is enough to ascertain whether an oil is genuine or not, and sometimes it is also possible to know what kind of product has been added, and to estimate the level of adulteration.

Before starting a study on adulteration, the analyst has to know the typical values of the genuine oils and the influence of oil isolation methods and production period on their quantitative composition.

## Evaluation of Purity of Essential Oils

The quantitative composition of citrus oils mainly varies according to the production period of the fruit without regard to the industrial isolation procedure used or the cultivation area.[5-10] We have analyzed a large number of oil samples from different productive seasons, and the data obtained showed that the components have very characteristic behaviors which are almost always constant. All of the

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oils analyzed were produced by companies in Sicily or Calabria and were absolutely genuine. We found it essential to study the ratios between certain components which proved to be more useful than just determining the simple minimum and maximum values of components. This technique has helped us to determine the genuineness of an oil.

A typical example of how some components show different values throughout the productive season is lemon oil. This oil is a good example because it is obtained in each month of the year. The oils obtained in winter are of better quality when compared with those of summer. This quality difference is in part due to the high citral value of the winter oil, while oils produced in the summer are deficient in carbonyl compounds. In oils produced in the summer, limonene is found at its lowest level, while β-pinene reaches its maximum during this same time period (Figure 1). Typically, a winter oil possesses a citral content of 3.5 to 4.0%, a limonene content of 66 to 68%, and a β-pinene content that does not exceed 13%. If you consider summer oil, you can generally find only about 3% citral and 60-63% limonene, while the β-pinene content exceeds 15%. Therefore, a lemon oil with the following data cannot be genuine, even if the single values themselves can be considered as being good: citral 3.8%, limonene 61% and β-pinene 16%. This can be the case in a summer oil, that possesses a low combined value of neral and geranial, to which a certain amount of citral was added to make it similar to a winter oil (an oil considered to be of better quality).

A lemon oil (Figure 2) with a percentage of 0.06% of decanal, 0.14% nerol and 12% β-pinene cannot be considered pure, even though the single values are within the minimum and maximum values of genuine oils, because it is not possible for such high values of decanal and nerol to coexist with such a low value of β-pinene. It is more probable that it was an oil that was produced in the period when decanal and nerol show high values, to which orange terpenes were added, thereby resulting in a decrease in the percentage of β-pinene.

An oil with 67% limonene, 0.23% α-terpineol and 0.10% nonanal cannot be genuine even if the single values are in

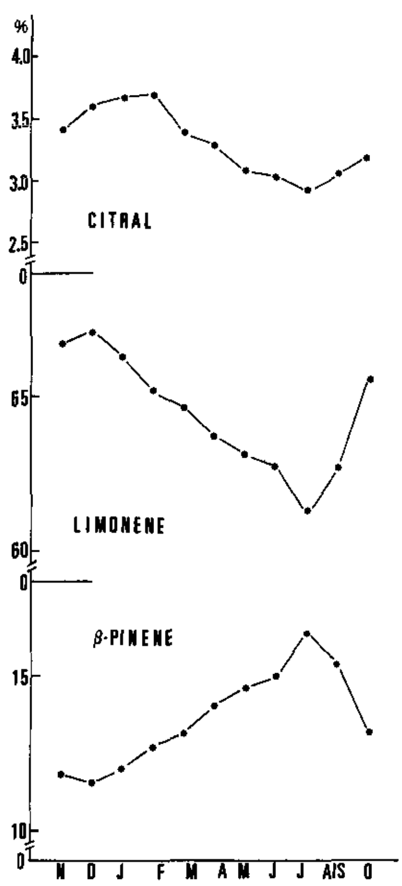


**Figure 1. Average variation in the citral, limonene and β-pinene content for lemon oil for each month of the productive seasons**

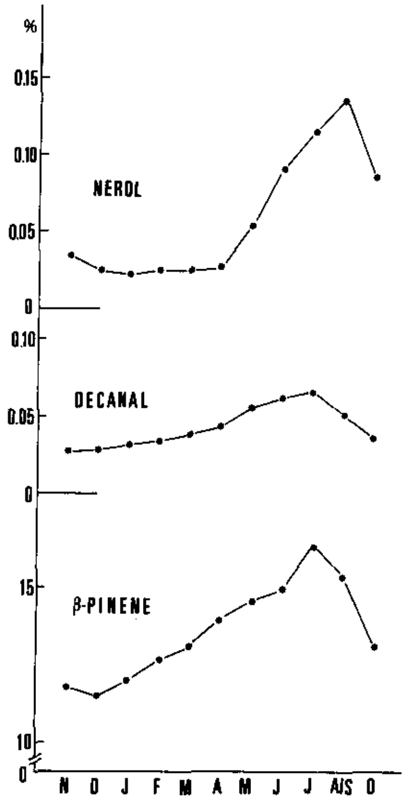


**Figure 2. Average variation in the decanal, nerol and β-pinene content for lemon oil for each month of the productive seasons**

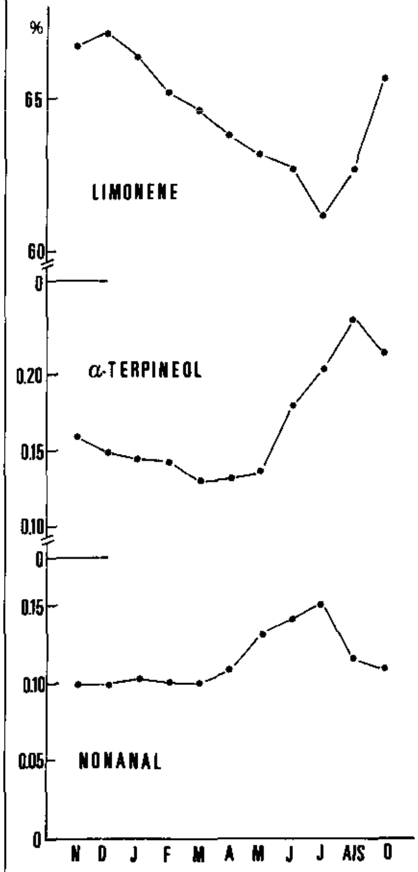


**Figure 3. Average variation in the limonene, α-terpineol and nonanal content for lemon oil for each month of the productive seasons**

the range of values for genuine oils (Figure 3). It is more likely that this oil is a winter oil (with a low content of nonanal and a high content of limonene) to which a distilled oil was added. The reason for this conclusion can be readily understood if it is realized that some monoterpene hydrocarbons could have hydrated during the distillation process in aqueous acid solution, resulting in the increase of some alcohols, such as α-terpineol.

Mandarin oil, which is produced from October to February, has a typical composition which also permits us to ascertain whether an oil is genuine or not. First of all it is important to know that over the productive season all components except limonene tend to decrease. During this same time the relative percentage of limonene increases from 68% in October to 74% at the end of the productive season. This is a time when all the other components show their minimum value. For example (Figure 4), an oil with 73% limonene, 19% γ-terpinene and 0.15 α-terpineol cannot be considered as genuine since such values of α-terpineol and γ-terpinene never occur naturally together with a high content of limonene. Such a sample could be an oil obtained from green mandarins (oil produced from fruit harvested in the first part of the productive season) to which sweet orange oil terpenes were added. This could account for the high level of limonene found.

The study of the genuineness of the oils such as lemon and mandarin is much more precise if more than three parameters are considered.

## Detection of Specific Adulterations

The usual six adulterations which occur for citrus essential oils are:

- Addition of sweet orange oil terpenes to cold-pressed lemon and mandarin oils;
- Addition of reconstituted oils to cold-pressed lemon and mandarin oils;
- Addition of distilled oil to cold-pressed lemon and mandarin oils;
- Addition of "citral" to lemon oil;
- Addition of reconstituted bergamot oil to natural bergamot oil; and
- Addition of sweet orange oil terpenes or lemon oil terpenes to cold-pressed bitter orange oil.

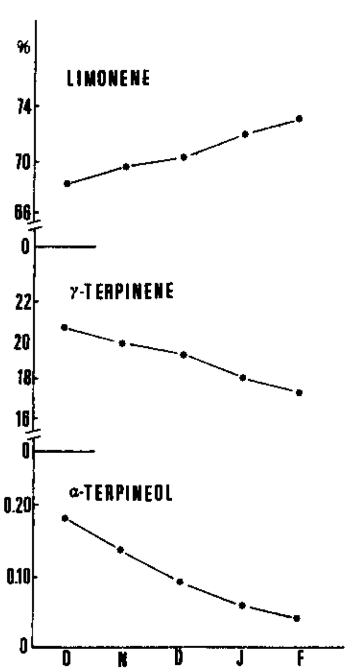


**Figure 4. Average variation in the limonene, γ-terpinene and α-terpineol content for mandarin oil for each month of the productive seasons**

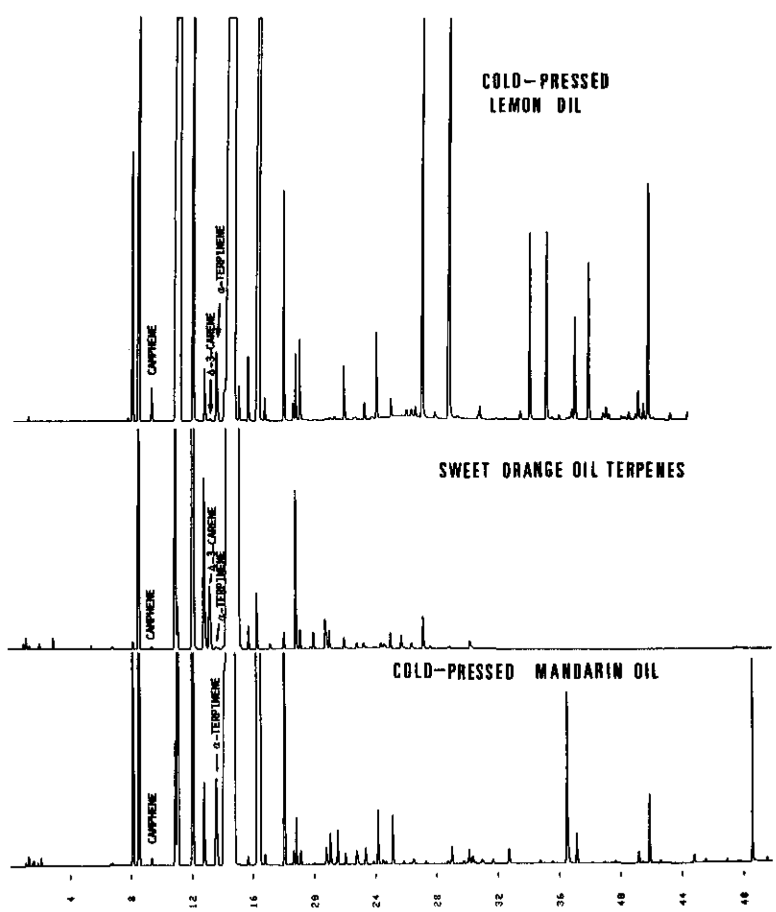


**Figure 5. Chromatograms of lemon oil, mandarin oil and sweet orange oil terpenes.**
**Conditions: glass column, 25 m x 0.32 mm i.d. coated with SE 52 (0.4-0.45 µm); column temp: 60°C (8 min) to 100°C at 3°C/min to 130°C at 2.5°C/min to 180°C at 3°C/min; injector and detector temp: 280°C; carrier gas: hydrogen, 0.4 kg/CM²; injection mode, split; detector FID**

***Detection of the addition of sweet orange oil terpenes to lemon or mandarin oil***—Orange oil terpenes possess almost no carbonyl compounds, esters or sesquiterpenes. If they are added to lemon or mandarin oils, a corresponding decrease in aldehydes, esters and sesquiterpenes occurs; however, this alone is not enough to unequivocally determine that adulteration has taken place.

Orange oil terpenes (Figure 5) are characterized by the existence of about 0.1% of δ-3-carene. This compound is either missing or present only as a trace amount in lemon and mandarin oils. On the contrary, orange oil terpenes are almost completely devoid of α-terpinene and camphene, while in lemon and mandarin oils they are present in levels of 0.20% and 0.06%, and 0.40% and 0.02%, respectively.

The δ-3-carene content, and the ratios of δ-3-carene/camphene and δ-3-carene/α-terpinene are particularly useful for detection of the possible addition of orange oil terpenes to lemon and mandarin oils.

Table I shows the comparison of the 99% confidence limits of δ-3-carene, δ-3-carene/camphene, δ-3-carene/α-terpinene (calculated on about 1,000 samples of genuine lemon oils) and the values of the same parameters calculated on mixtures of lemon oil with additions of 5, 10 and 20% orange oil terpenes. It is evident that even the addition of 5% of orange terpenes makes the quantitative composition of the product different.[11]

Table II shows the comparison of the 99% confidence limits of the same parameters calculated on about 300 genuine mandarin oils and on mixtures of mandarin oils with the addition of 3, 5 and 10% of orange oil terpenes. This trial was made on mixtures of green and red mandarin oils. In this case even the addition of 3% orange terpenes could be detected.[12]

***Detection of the addition of reconstituted oils to genuine lemon or mandarin oil***—The orange oil terpenes, obtained by fractional distillation from orange oil, are used to reconstitute lemon and mandarin oils. At 20°C, lemon oils possess an optical rotation of +57° to +65°; mandarin oils possess an optical rotation of +65° to +75°, while the optical rotation of sweet orange and their terpenes is +98° to +100°. The reconstituted lemon and mandarin oils thus have an optical rotation which is higher than either of the genuine oils. (–)-Limonene (Figure 6), which is a readily available raw material, is generally added at a rate of about 15% to decrease the optical rotation in a reconstituted oil. The ratio of (–)-limonene and (+)-limonene in the genuine lemon oil is about 1.8:98.2, while in genuine mandarin oil it is about 2.2:97.8. In orange oil (–)-limonene is present only as a trace component. Consequently, additions of (–)-limonene to reconstituted oils increase this (–)-limonene to (+)-limonene ratio. As a result the addition of as little as 5% reconstituted oil to

**Table I. Values for the δ-3-carene content, the δ-3-carene/α-terpinene and δ-3-carene/camphene ratios for a number of samples of genuine lemon oil, and samples of lemon oil adulterated with 5, 10 and 20% sweet orange terpenes**

| | Lemon oil 99% confidence limits | | Example 1* | | | Example 2* | | |
|---|---|---|---|---|---|---|---|---|
| | Min | Max | 5% | 10% | 20% | 5% | 10% | 20% |
| δ-3-carene (%) | 0.000 | 0.008 | 0.010 | 0.016 | 0.024 | 0.010 | 0.016 | 0.025 |
| δ-3-carene/α-terpinene | 0.000 | 0.044 | 0.056 | 0.093 | 0.170 | 0.050 | 0.088 | 0.153 |
| δ-3-carene/camphene | 0.005 | 0.129 | 0.182 | 0.320 | 0.510 | 0.160 | 0.286 | 0.510 |

*randomly selected examples (out of thirty examined) of lemon oil adulterated with 5, 10 and 20% orange terpenes

**Table II. Values for the δ-3-carene content, the δ-3-carene/α-terpinene and δ-3-carene/camphene ratios for a number of samples of genuine mandarin oil, and samples of green and red mandarin oils adulterated with 3, 5 and 10% sweet orange terpenes**

| | Mandarin oil 99% confidence limits | | Green mandarin oil with sweet orange terpenes added* | | | Red mandarin oil with sweet orange terpenes added* | | |
|---|---|---|---|---|---|---|---|---|
| | Min | Max | 3% | 5% | 10% | 3% | 5% | 10% |
| δ-3-carene (%) | 0.000 | 0.004 | 0.006 | 0.009 | 0.016 | 0.007 | 0.009 | 0.016 |
| δ-3-carene/α-terpinene | 0.000 | 0.010 | 0.016 | 0.027 | 0.049 | 0.018 | 0.024 | 0.043 |
| δ-3-carene/camphene | 0.000 | 0.260 | 0.380 | 0.501 | 0.987 | 0.424 | 0.592 | 1.083 |

* randomly selected examples out of thirty examined

genuine lemon oil (Table III, Figure 7) or to genuine mandarin oil (Table IV, Figure 8) is detectable.

***Detection of the adulteration of cold-pressed lemon or mandarin oil with distilled oils***—Distilled oils are oils obtained by distillation from the liquids of the screw-pressed residues of cold-extraction and from other waste liquors. The distilled oils differ greatly from the cold-pressed oils because the storage period of the residues (especially for the aqueous acid solution and the fermentation process) and the distillation conditions are parameters which affect the composition of the product.

A comparison of the chromatograms of a cold-pressed lemon oil with that of a distilled lemon oil is given in Figure 9. Similarly, comparison of the chromatograms of a cold-pressed mandarin oil and that of a distilled oil is shown in Figure 10. In the distilled oils the less volatile components,

**Table III. Relative percentage of (–) and (+) limonene in cold-pressed lemon oil reconstituted oil and mixtures of cold-pressed lemon oil with reconstituted oils added at two levels**

| | (–) | (+) |
|---|---|---|
| cold-pressed lemon oil (95 samples) | 1.5-2.1 | 97.9-98.5 |
| reconstituted lemon oil | 14.9 | 85.1 |
| cold-pressed lemon oil 90% reconstituted lemon oil 10% | 3.4 | 96.6 |
| cold-pressed lemon oil 95% reconstituted lemon oil 5% | 2.6 | 97.4 |
| cold-pressed lemon oil 97% reconstituted lemon oil 3% | 2.2 | 97.8 |

**Table IV. Relative percentage of (–) and (+) limonene in samples of cold-pressed mandarin oil, reconstituted oil and mixtures of cold-pressed mandarin oil with reconstituted oils added at two levels**

| | (–) | (+) |
|---|---|---|
| cold-pressed mandarin oil (80 samples) | 1.8-2.6 | 97.4-98.2 |
| reconstituted mandarin oil | 13.4 | 86.6 |
| cold-pressed mandarin oil 90% reconstituted mandarin oil 10% | 3.9 | 96.1 |
| cold-pressed mandarin oil 95% reconstituted mandarin oil 5% | 3.1 | 96.9 |
| cold-pressed mandarin oil 97% reconstituted mandarin oil 3% | 2.5 | 97.5 |

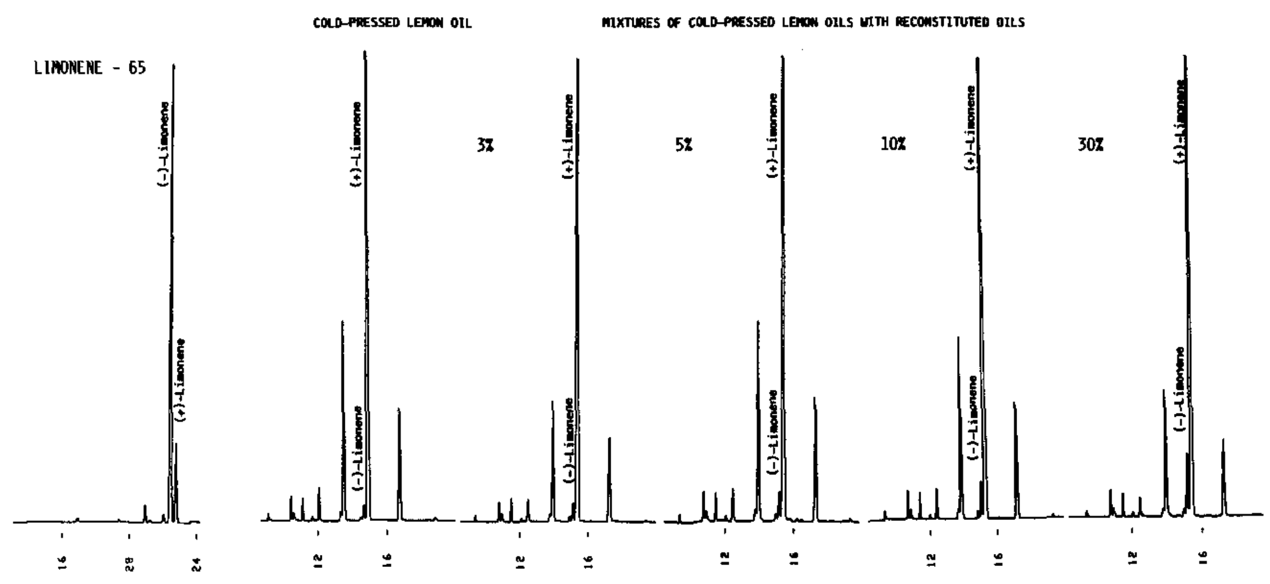


**Figure 6 (left). Chromatogram of a commercial "limonene-65"**
**Conditions: precolumn, 25 m x 0.32 mm i.d. coated with SE 52 (film thickness 0.4-0.45 µm); main column, fused silica 25 m x 0.25 mm i.d. coated with a mixture of (2,3,6-tri-o-methyl)-β-cyclodextrin and OV 1701 (film thickness 0.25 µm) in a ratio of 30% and 70% by weight respectively; temp of the oven 80°C (8 min) to 105°C at 1.2°C/min; injector and detector temp, 250°C; carrier gas, hydrogen, 0.30 kg/cm²; injection mode: split; detector FID**

**Figure 7 (right). Chromatograms of a cold-pressed lemon oil and some mixtures of cold-pressed lemon oil adulterated with reconstituted oils. Temperature of the oven 100°C. Other conditions as in Figure 6**

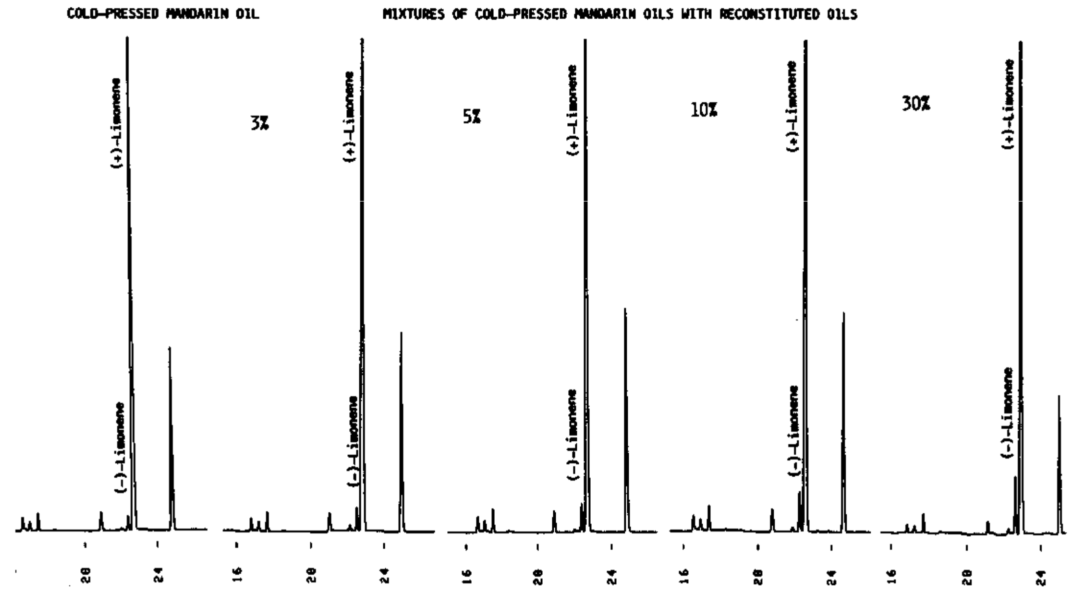


**Figure 8. Chromatograms of a cold-pressed mandarin oil and some mixtures of cold-pressed mandarin oil adulterated with reconstituted oils. Conditions as in Figure 6**

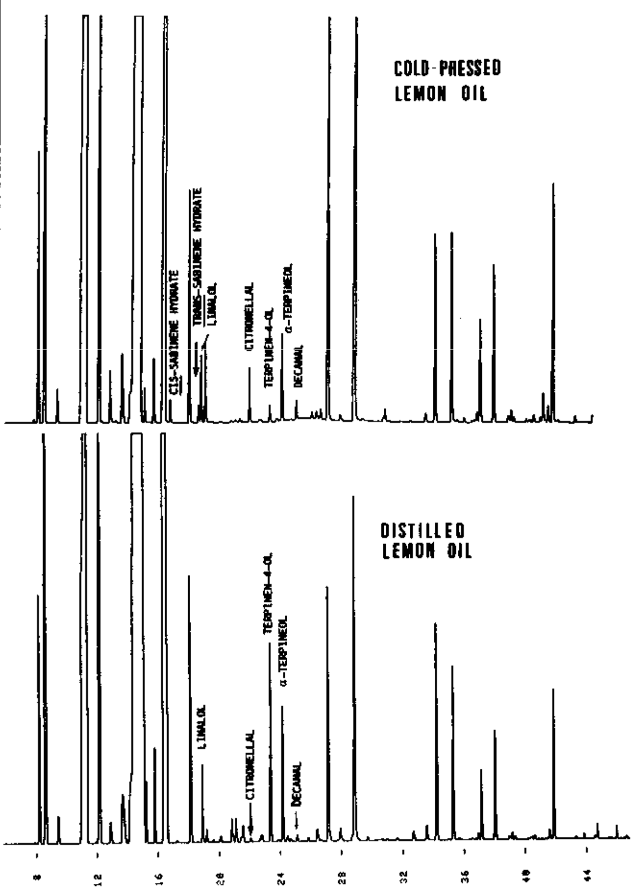


**Figure 9. Chromatograms of a cold-pressed lemon oil and of a distilled lemon oil. Conditions as in Figure 5**

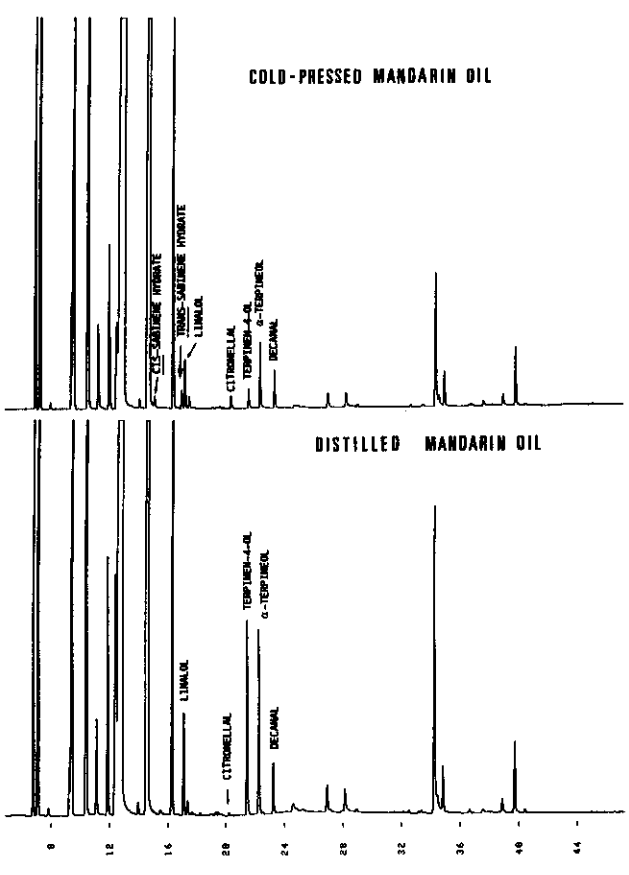


**Figure 10. Chromatograms of a cold-pressed mandarin oil and of a distilled mandarin oil. Conditions as in Figure 5**

like esters and sesquiterpenes, together with citronellal and cis- and trans-sabinene hydrates are found only as minor components. In contrast, some alcohols such as linalool, α-terpineol and particularly terpinen-4-ol are found in proportionally higher levels. The reason for this is that certain monoterpenes can be hydrated in aqueous acid solution.

We found it interesting to study some ratios between certain components, mainly terpinen-4-ol/cis-sabinene hydrate, terpinen-4-ol/trans-sabinene hydrate, terpinen-4-ol/citronellal, terpinen-4-ol/decanal, since they are useful in the detection of the addition of distilled oils to cold-pressed oils.

Tables V and VI list the ratios of the compounds listed

**Table V. Values of some ratios between peak areas of samples of cold-pressed lemon oil and those of the mixtures of these oils to which 5% distilled oil has been added**

| | | Terpinen-4-ol/cis-sabinene hydrate | Terpinen-4-ol/trans-sabinene hydrate | Terpinen-4-ol/citronellal | Terpinen-4-ol/decanal |
|---|---|---|---|---|---|
| 95% confidence limits of the cold-pressed essential oils | **min** | 0.21 | 0.22 | 0.02 | 0.17 |
| | **max** | 1.18 | 1.69 | 0.53 | 1.28 |
| **Mixtures** | | | | | |
| 1 | | 1.00 | **1.73** | 0.30 | 0.72 |
| 2 | | 0.98 | 1.31 | 0.34 | 0.97 |
| 3 | | 1.02 | 1.26 | 0.36 | 1.03 |
| 4 | | **1.25** | 1.48 | 0.37 | 1.12 |
| 5 | | 1.07 | 1.40 | 0.37 | 1.02 |
| 6 | | **1.43** | **1.95** | 0.47 | 1.26 |
| 7 | | 1.05 | 1.18 | **0.56** | **1.74** |
| 8 | | **1.26** | **1.97** | 0.23 | 0.71 |
| 9 | | 0.92 | 1.18 | 0.36 | 1.03 |
| 10 | | 0.93 | 1.19 | 0.39 | 1.12 |
| 11 | | 1.18 | 1.52 | 0.49 | **1.34** |
| 12 | | **1.53** | **2.09** | 0.52 | **1.44** |

The values which exceed the upper 95% confidence limit of the cold-pressed essential oils are in bold

**Table VI. Values of some ratios between peak areas of samples of cold-pressed lemon oil and those of the mixtures of these oils to which 8% distilled oil has been added**

| | | Terpinen-4-ol/cis-sabinene hydrate | Terpinen-4-ol/trans-sabinene hydrate | Terpinen-4-ol/citronellal | Terpinen-4-ol/decanal |
|---|---|---|---|---|---|
| 95% confidence limits of the cold-pressed essential oils | **min** | 0.21 | 0.22 | 0.02 | 0.17 |
| | **max** | 1.18 | 1.69 | 0.53 | 1.28 |
| **Mixtures** | | | | | |
| 1 | | **1.36** | **1.86** | 0.47 | 1.24 |
| 2 | | **1.34** | **1.86** | 0.46 | **1.39** |
| 3 | | **1.57** | **2.14** | **0.55** | **1.57** |
| 4 | | **1.39** | **1.87** | 0.49 | **1.39** |
| 5 | | **1.67** | **2.17** | **0.56** | **1.61** |
| 6 | | **2.07** | **2.95** | **0.74** | **2.07** |
| 7 | | **2.07** | **2.82** | **0.72** | **1.94** |
| 8 | | 1.05 | 1.40 | 0.45 | 1.24 |
| 9 | | **1.21** | 1.57 | 0.49 | **1.35** |
| 10 | | **1.26** | 1.63 | **0.54** | 1.53 |
| 11 | | **1.45** | **1.87** | **0.63** | **1.76** |
| 12 | | **1.67** | **2.10** | **0.71** | **1.97** |

The values which exceed the upper 95% confidence limit of the cold-pressed essential oils are in bold above. Also a comparison of the 95% confidence limits (calculated on about 1,000 cold-pressed lemon oils) with the values of mixtures of 5 and 8% of distilled product can also be found in these same tables. As a result, it can be readily seen that the addition of 5% of a distilled oil to a genuine oil can be detected in about 60% of the cases, while it is almost always possible to detect the addition of 8% of a distilled oil.[13]

Tables VII and VIII show the same ratios of the same constituents, only this time the subject is mandarin oil rather than lemon oil. These tables show the 95% confidence limits (calculated on about 300 cold-pressed oils) with the values of mixtures made from the addition of 5 and 10% distilled oils to cold-pressed oil respectively. The detection of the addition of 5% of a distilled oil to a cold-pressed oil is only possible if it is added to a green mandarin oil (an oil produced from fruit harvested at the beginning of the season) which is rich in alcohols. In most mixtures, the

**Table VII. Values of some ratios between peak areas of samples of cold-pressed mandarin oil and those of the mixtures of these oils to which 5% distilled oil has been added**

| | | Terpinen-4-ol/cis-sabinene hydrate | Terpinen-4-ol/trans-sabinene hydrate | Terpinen-4-ol/citronellal | Terpinen-4-ol/decanal |
|---|---|---|---|---|---|
| 95% confidence limits of the cold-pressed essential oils | min | 0.74 | 0.35 | 0.40 | 0.17 |
| | max | 2.48 | 1.57 | 2.12 | 0.72 |
| **Mixtures** | | | | | |
| 1* | | 1.91 | 1.50 | **2.25** | 0.68 |
| 2* | | 2.16 | 1.06 | **2.30** | **0.80** |
| 3** | | 1.78 | 1.08 | 1.20 | 0.49 |
| 4** | | 2.16 | 1.37 | 2.03 | 0.68 |

* essential oil from green mandarins + distilled oil
** essential oil from red mandarins + distilled oil
The values which exceed the upper 95% confidence limit of the cold-pressed essential oils are in bold

**Table VIII. Values of some ratios between peak areas of samples of cold-pressed mandarin oil and those of the mixtures of these oils to which 10% distilled oil has been added**

| | | Terpinen-4-ol/cis-sabinene hydrate | Terpinen-4-ol/trans-sabinene hydrate | Terpinen-4-ol/citronellal | Terpinen-4-ol/decanal |
|---|---|---|---|---|---|
| 95% confidence limits of the cold-pressed essential oils | min | 0.74 | 0.35 | 0.40 | 0.17 |
| | max | 2.48 | 1.57 | 2.12 | 0.72 |
| Mixtures | | | | | |
| 1* | | 2.20 | **1.79** | **3.12** | **1.07** |
| 2* | | 1.94 | 1.21 | **3.79** | **1.28** |
| 3* | | 1.81 | 1.12 | **3.27** | **1.13** |
| 4* | | **2.50** | **1.92** | **2.78** | **0.87** |
| 5* | | 2.06 | **1.69** | **2.54** | **0.81** |
| 6** | | 2.36 | 1.37 | 1.73 | 0.63 |
| 7** | | **2.95** | 1.55 | **2.32** | 0.72 |
| 8** | | 2.22 | 1.11 | **2.29** | **0.82** |
| 9** | | **3.67** | **2.22** | 2.11 | 0.62 |
| 10** | | 2.46 | **2.19** | **2.27** | 0.60 |
| 11** | | 2.15 | **1.93** | 2.00 | 0.55 |
| 12** | | 2.15 | 2.07 | **2.15** | 0.60 |

* essential oil from green mandarins + distilled oil
** essential oil from red mandarins + distilled oil
The values which exceed the upper 95% confidence limit of the cold-pressed essential oils are in bold

addition of 10% of a distilled oil to a cold-pressed oil can be detected.[14]

Because of the wide range of variability for both cold-pressed and distilled lemon and mandarin oils, it is not possible to establish a limit on addition above which detection can easily be carried out. As a general rule, the oils which are deficient in alcohols (winter lemon oils and red mandarin oils) can be easily mixed with the distilled oils. However, if only one ratio of the components discussed previously exceeds the limits normally found in genuine oils, it is clear that some distilled products have been added.

***Detection of the addition of citral to lemon oil***—"Citral" is a commercially available product which contains about 80 to 97% of neral and geranial. It can be natural or obtained by synthesis. Addition of citral to lemon oil occurs frequently. Such an addition results in an increase in the amount of total carbonyl compounds, a reference quality parameter in these oils.

The detection of "citral" in adulterated lemon oil is possible thanks to its impurities which are components not normally found in genuine oils, or which cause quantitative variations in component ratios outside of the norm expected for genuine oils.[15]

Almost all qualities of "citral" obtained by synthesis have a component not present in genuine lemon oils. This allows the detection of even 0.3% of citral to a genuine oil (Figure 11). This component, indicated in Figure 11 as 1 and whose mass spectrum is shown in Figure 12, has not yet been identified.

***Detection of the addition of reconstituted bergamot oil to genuine bergamot oil***—Because of the wide range of variability of genuine bergamot oils,[16,17] it is very difficult to detect the addition of low levels of a reconstituted bergamot oil to a genuine oil using conventional analytical techniques.

In natural bergamot oil, linalool is present in amounts of 4 to 18%, with an average content of about 10%. Generally to make a reconstituted oil that has a composition very similar to that of genuine oil about 8% linalool is usually used; however, this linalool is usually of a different origin from that of bergamot. As a result, the detection of such an adulteration is possible by the study of the linalool enantiomers.[18]

In natural bergamot oils (+)-linalool is not present or if it is, it is present in amounts that do not ex-

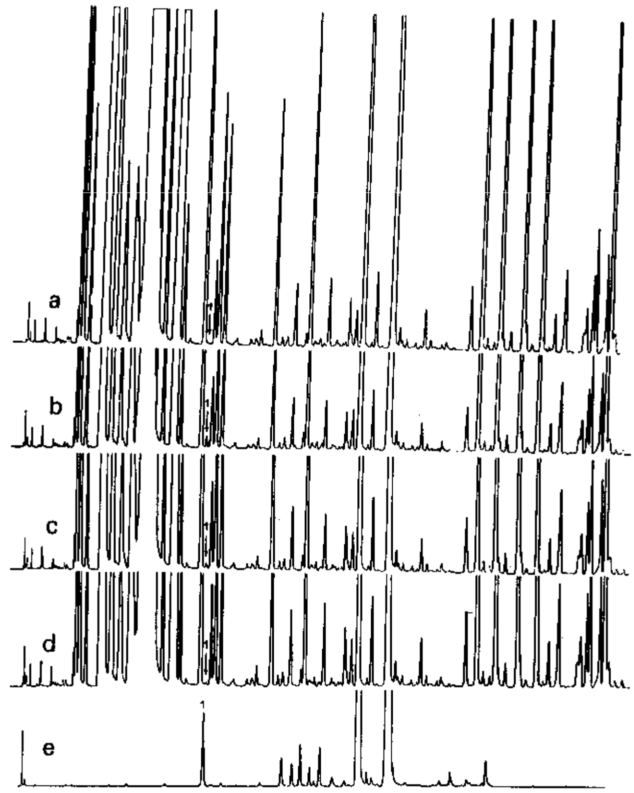


**Figure 11. Chromatograms of (a) a cold-pressed lemon oil, a synthetic citral (97% purity) (e), and mixtures of lemon oil with 2% (b), 0.7% (c), 0.3% (d) citral added. Conditions as in Figure 5**

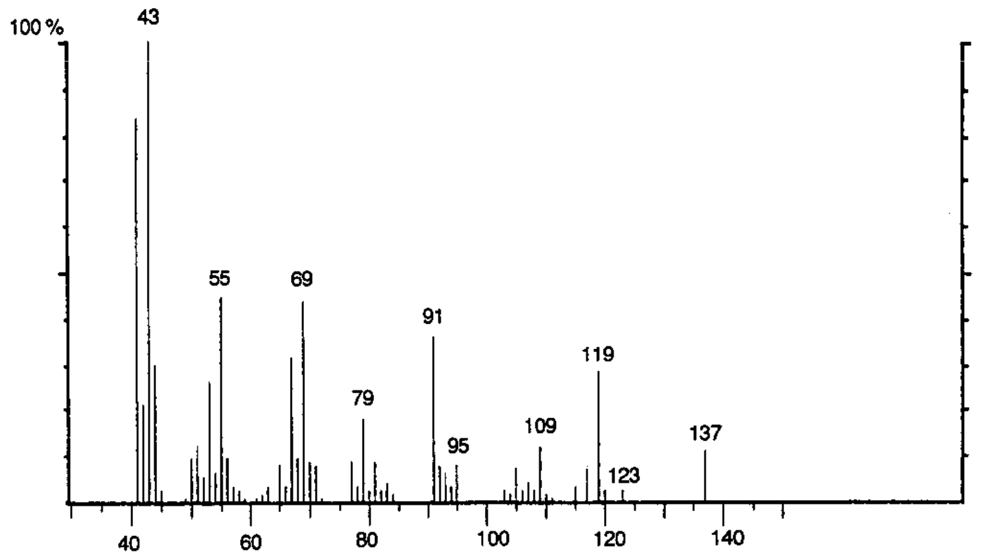


**Figure 12. Mass spectrum of an impurity found in the synthetic citral. GC-MS Conditions: HP Mod 5995C System equipped with HP Software 59970, with a 25 m x 0.32 mm i.d. fused silica capillary column coated with SE 52, column temp 70°C (10 min) to 230°C at 3°C/min; ionizing voltage 70 eV**

ceed 0.5% of the total content of linalool (Figure 13). Linalool samples used to obtain the reconstituted bergamot oils are a racemic mixture (Figure 14). As a result, the reconstituted oil will possess both the linalool enantiomers (Figure 15). Figure 15 and Table IX show that even a 5% addition of a reconstituted bergamot oil to natural bergamot oil can be detected.

***Detection of the addition of sweet orange oil terpenes and lemon oil terpenes to cold-pressed bitter orange oil***—Bitter orange oil has excellent olfactive properties and it sells at high prices. However because of the inconsistency of demand on the national and international markets and a decrease in cultivation, the production of this oil is limited.

The bitter orange fruits that are used for the isolation of oil are sometimes mixed with a small number of sweet orange fruits and, moreover, often the production lines are the same as those used for lemon essential oil. As a result bitter orange oil can be possibly contaminated with either sweet orange oil or lemon oil or both. Moreover, sweet orange terpenes and/or lemon terpenes can also be fraudulently added to bitter orange oil.

In bitter orange oil δ-3-carene is present only as a trace constituent, while in sweet orange terpenes it is found in amounts of 0.1% (Figure 16).[11] Consequently, the δ-3-carene content and the ratios of δ-3-carene/camphene and δ-3-carene/terpinolene are particularly useful for detecting

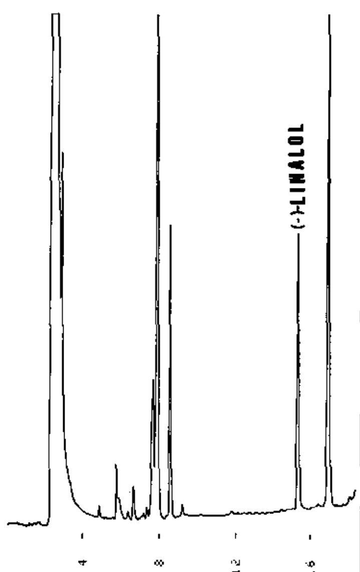


**Figure 13. Chromatogram of a natural bergamot oil. Conditions: fused silica column 25 m x 0.25 mm i.d. coated with a mixture of (2,3,6-tri-o-methyl)-β-cyclodextrin and OV 1701 in a ratio of 30% and 70% by weight, respectively (film thickness 0.25 μm); column temp, from 100°C to 180°C at 1.5°C/min; injector and detector temp, 250°C; carrier gas, hydrogen, 0.30 kg/cm²; injection mode: split; detector FID**

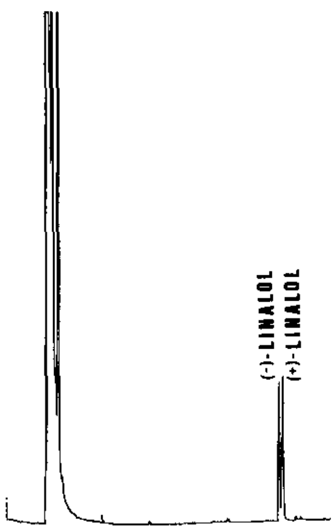


**Figure 14. Chromatogram of a commercial linalool sample. Conditions as in Figure 13**

**Table IX. Relative percentage of (–) and (+) linalool in natural bergamot oil, commercial linalool and mixtures of natural bergamot oil and reconstituted bergamot oil**

| | (–) | (+) |
|---|---|---|
| Natural bergamot oil | 100 | t |
| Commercial linalool | 50 | 50 |
| **Mixtures** | | |
| 75* | 63 | 37 |
| 50* | 73 | 27 |
| 40* | 76 | 24 |
| 25* | 84 | 16 |
| 20* | 88 | 12 |
| 15* | 90 | 10 |
| 10* | 92 | 8 |
| 7* | 93 | 7 |
| 5* | 96 | 4 |
| 5* | 97 | 3 |

* % of reconstituted oil in the analyzed mixtures
t = trace

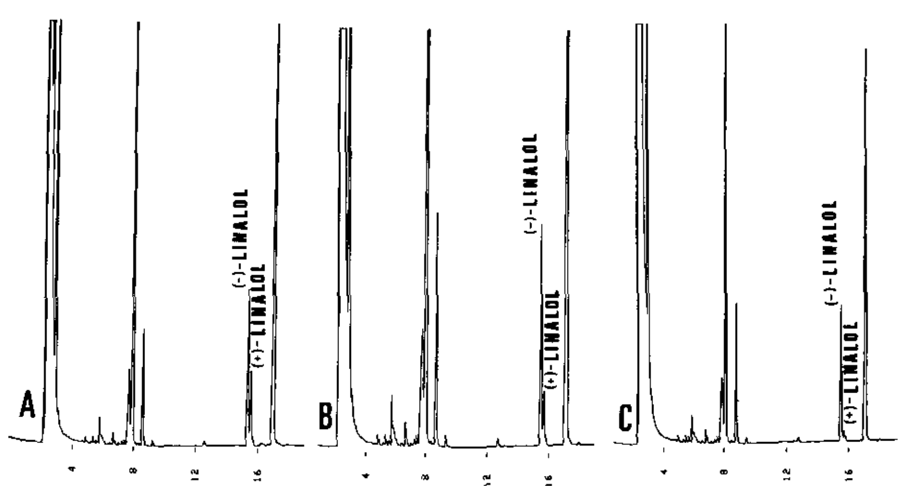


**Figure 15. Chromatograms of mixtures of natural bergamot oils with 60% (A), 30% (B) and 5% (C) of reconstituted oils added. Conditions as in Figure 13**

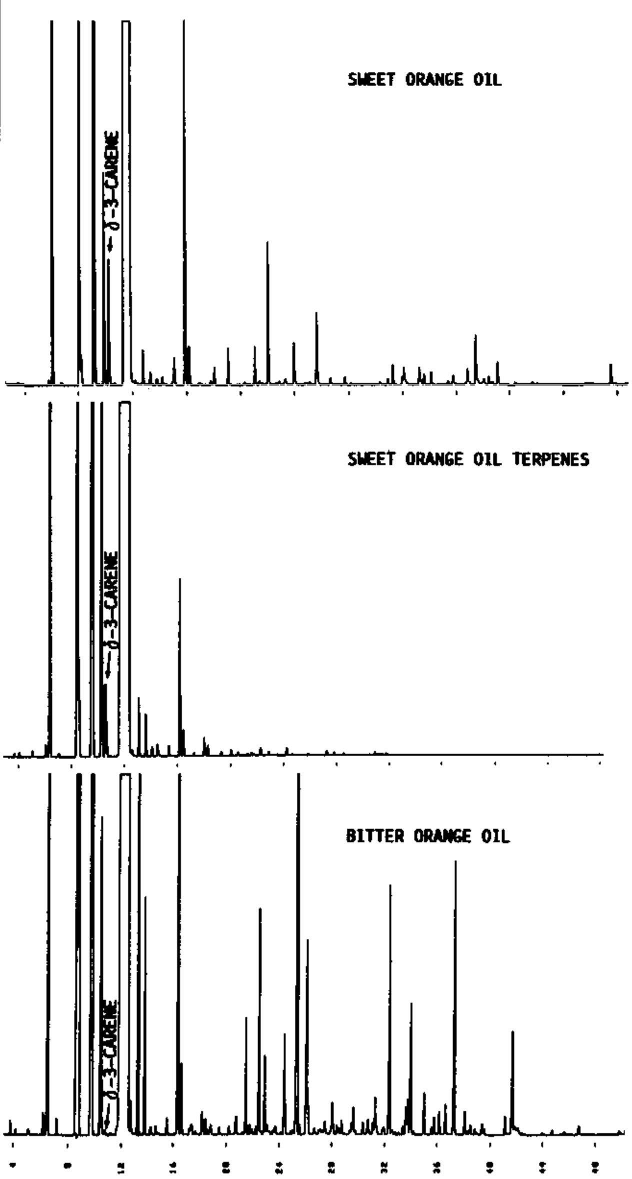


**Figure 16. Chromatograms of a bitter orange oil, sweet orange oil terpenes and sweet orange oil. Conditions as in Figure 5**

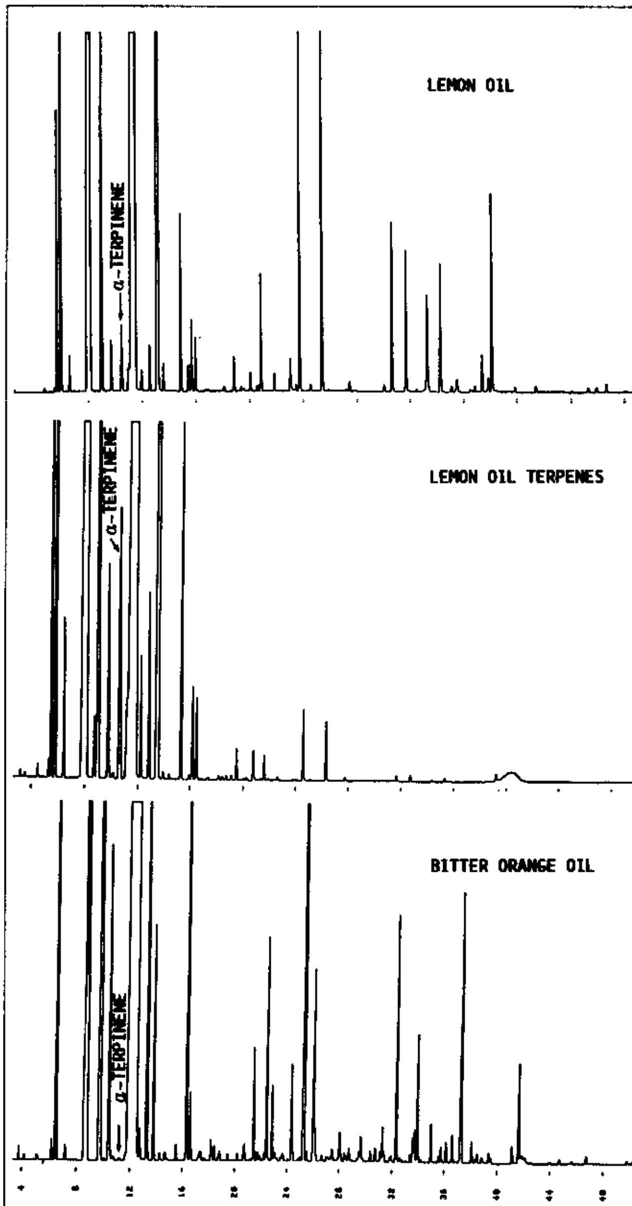


**Figure 18. Chromatograms of a bitter orange oil, sweet orange oil terpenes and lemon oil. Conditions as in Figure 5**

**Table X. Values for the δ-3-carene content, the δ-3-carene/camphene and δ-3-carene/terpinolene ratios for genuine bitter orange oil, and for samples of bitter orange oils with the addition of 3 and 5% sweet orange oil or sweet orange oil terpenes**

| | Bitter orange oils 99% confidence limits | | Mixtures with 3 and 5% of sweet orange oils | | | | Mixtures with 3 and 5% of sweet orange oil terpenes | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | 3% | 3% | 5% | 5% | 3% | 3% | 5% | 5% |
| δ-3-carene (%) | 0.000 | 0.006 | 0.007 | 0.006 | 0.009 | 0.008 | 0.007 | 0.006 | 0.009 | 0.010 |
| δ-3-carene/camphene | 0.000 | 1.092 | 1.000 | 0.857 | 1.500 | 1.143 | 1.000 | 0.857 | 1.125 | 1.111 |
| δ-3-carene/terpinolene | 0.000 | 0.875 | 0.778 | 0.750 | 1.000 | 0.889 | 1.000 | 0.857 | 0.900 | 1.000 |

a possible contamination or the addition of sweet orange terpenes to bitter orange oil.

Table X shows the comparison of δ-3-carene, δ-3-carene/camphene, δ-3-carene/terpinolene levels (calculated on genuine bitter orange oils) at a 99% confidence level, and the value of the same parameters calculated on adulterated bitter orange oils to which 3 or 5% sweet orange terpenes have been added.

From the data presented in Table X and Figure 17, it can be readily seen that the addition of even 5% sweet orange terpenes results in the modification of the quantitative composition of bitter orange oil thus allowing detection of the addition.

In bitter orange oil, α-terpinene is present only in trace amounts, while in lemon oil and lemon terpenes it is normally found in levels of about 0.2% (Figure 18).[10] Because of this the α-terpinene content and the ratios α-terpinene/camphene and α-terpinene/cis-β-ocimene are particularly useful for detecting a possible contamination or addition of lemon oil or lemon terpenes in bitter orange oil. An example of the use of this data can be seen in Table XI. In this table, the α-terpinene, α-terpinene/camphene, α-

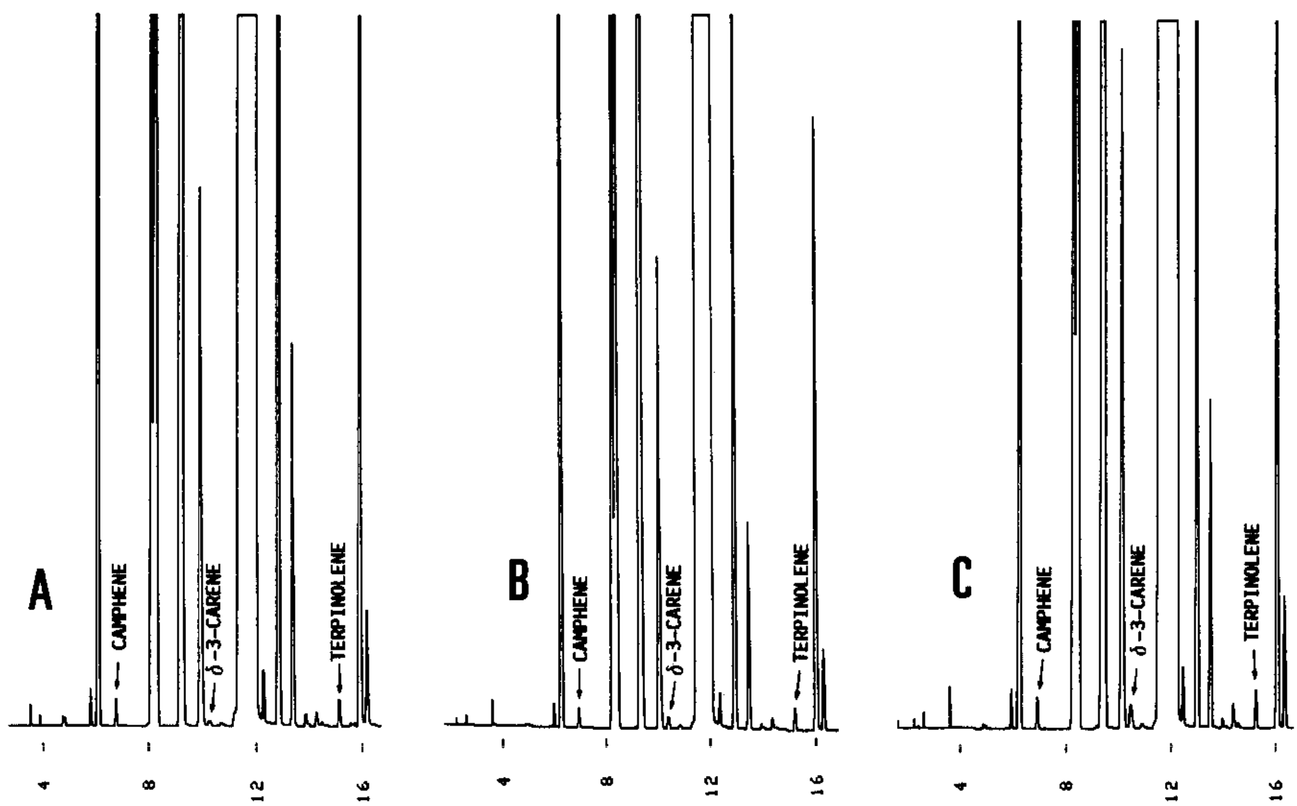


**Figure 17. Chromatograms of a bitter orange oil (A) and of mixtures of bitter orange oil with 3% (B) and 5% (C) of sweet orange oil terpenes added. Conditions as in Figure 5**

**Table XI. Values for the α-terpinene content, the α-terpinene/camphene and α-terpinene/cis-β-ocimene ratios for genuine bitter orange oil, and for samples of bitter orange oil with the addition of 3 and 5% lemon oil or lemon oil terpenes**

| | Bitter orange oils 99% confidence limits | | Mixtures with 3 and 5% of lemon oil | | | | Mixtures with 3 and 5% of sweet lemon oil terpenes | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Min | Max | 3% | 3% | 5% | 5% | 3% | 3% | 5% | 5% |
| α-terpinene (%) | 0.000 | 0.005 | 0.009 | 0.008 | 0.011 | 0.013 | 0.011 | 0.011 | 0.017 | 0.017 |
| α-terpinene/camphene | 0.000 | 0.814 | 1.000 | 0.889 | 1.100 | 1.300 | 1.100 | 1.222 | 1.417 | 1.700 |
| α-terpinene/cis-β-ocimene | 0.000 | 0.429 | 0.409 | 0.348 | 0.440 | 0.619 | 0.611 | 0.624 | 0.809 | 1.809 |

terpinene/cis-β-ocimene levels (calculated on genuine bitter orange oils) at a confidence level of 99% can be found. Also values of the same parameters can be calculated on mixtures of bitter orange oil to which 3 or 5% of lemon oil or lemon terpenes have been added. A review of the data presented in Table XI and Figure 19 shows that the addition of 3% lemon oil or lemon terpenes is detectable.

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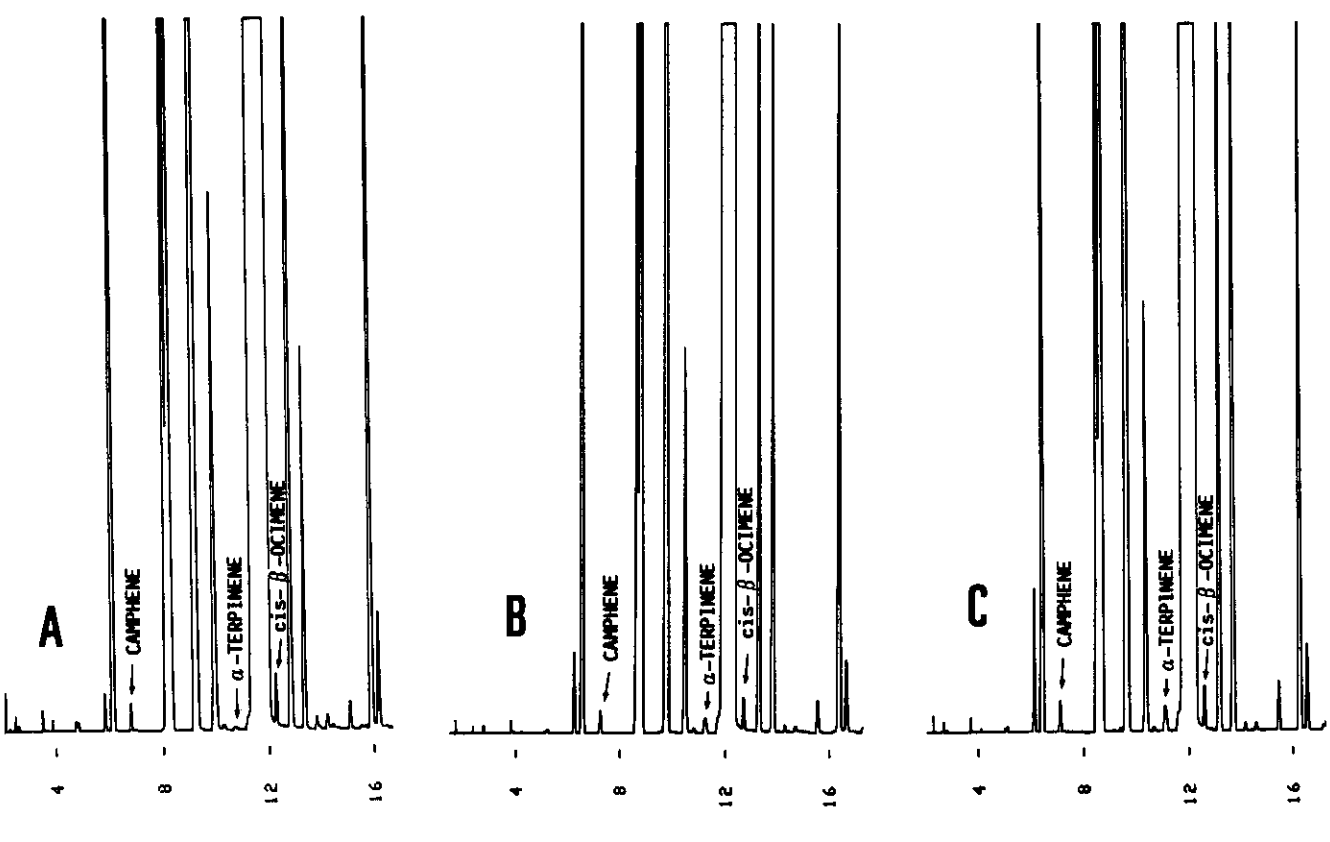


**Figure 19. Chromatograms of a bitter orange oil (A) and of mixtures of bitter orange oil with 3% (B) and 5% (C) of lemon oil terpenes added. Conditions as in Figure 5**