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Trabajos originales

# Influence of Rootstock on Essential Oil Composition of Mandarins

Lilian PEDRUZZI [1], Ana Cristina dos SANTOS [1], Luciana Atti SERAFINI [1] and Patrick MOYNA [1,2]

[1] *Instituto de Biotecnologia, Universidade de Caxias do Sul, CEP 95001-970; Caxias do Sul, RS, Brazil.*

[2] *Cátedra de Ciencia y Tecnologia de Alimentos; Facultad de Química, Universidad de la República; CP 11800; Montevideo, Uruguay.*

**SUMMARY. The peel and leaf essential oils obtained by hydrodistillation from grafted mandarins grown in Rio Grande do Sul (Southern Brazil) were studied to establish the influence the roostock has, if any, on grafted plants, comparing against the same plants grown from seedlings.**

*RESUMEN.* "Influencia del pie de injerto en la composición del aceite esencial de mandarina". Se estudiaron los aceites esenciales de cáscaras de frutos y hojas de mandarinas injertadas, cultivadas en Rio Grande do Sul (Brasil), para establecer la influencia del pie, comparándolas con la misma variedad creciendo de semillas.

## INTRODUCTION

Brazil is one of the most important citrus fruit producers in the World, with most of the commercially important citrus species and varieties under cultivation. Mandarins are extensively planted in the southern state of Rio Grande do Sul, which accounts for 10% of the total *Citrus* production of Brazil [1].

The regions of Vale do Cai (Montenegro, Sao Sebastiao de Cai, etc., Fig. 1) are large volume producers of mandarins *(Citrus deliciosa* Tenore). Commercial plantations in the region are established on the use of *Poncirus trifoliata* L. rootstocks [2] . This is based on the improved characteristics observed in the fruit production of the grafted plants, in particular cold hardiness, when compared to the same varieties grown from seed [3].

As part of a wider study on the brazilian *Citrus* essential oil compositions [4], it was thought important to establish the influence, if any, of the rootstock and grafting materials on the final oil compositions. The *Poncirus* rootstock changes several morphological and physiological characteristics of the Mandarins, improving their fruit yields and quality [3] and changes in the essential oils was considered as another possibility.

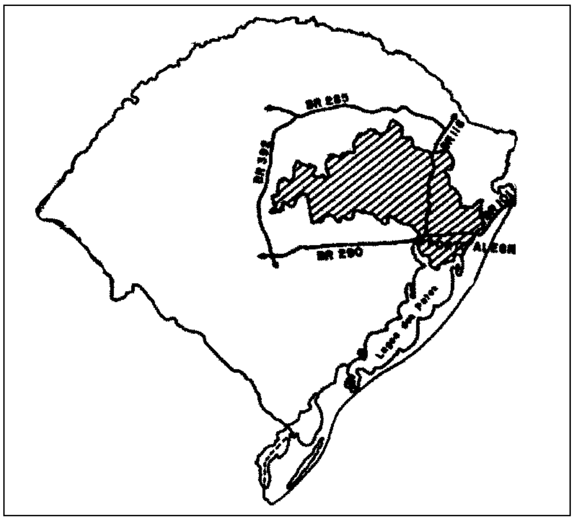


**Figure 1**. Vale do Cai region in Rio Grande do Sul, Brazil.

### *Experimental*

*Plant Material*

Fruits and leaves were collected from plants growing in Sao Sebatiao de Cai. The same trees were always used for the collections. The trees went unharvested during the whole experimental period. The age of the seed grown Mandarin tree could be established to be 15 years, and the

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* Author to whom correspondence should be addressed.



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other trees were selected to be 14 years old [3]. The age of the *Poncirus* tree could be established at 12 years [3]. The fruits were hand peeled, and the materials (peels, leaves) weighted fresh. The peels and leaves from each collection were processed the day of picking.

*Oil Isolation and Analysis*

The essential oil was isolated from the fresh leaves or peels by a 1 h hydrodistillation using a Clevenger-type apparatus. The oil was dried over anhydrous $Na_2SO_4$. Care had to be taken in the case of leaf oils to ensure adequate collection, as it is slightly more dense than water. GC analysis were carried out on a Hewlett Packard 6890 Series gas chromatograph, equipped with FID detector and a Chemstation data processor. Two bonded phase capillary columns were used: an HP-5 (30 m x 0.32 mm i.d.; 0.25 µm film thickness) and an HP-Innowax (30 m x 0.32 mm i.d; 0.50 µm film thickness). The oven temperature was programmed as follows: 40 °C (8 min), 40-180 °C (3 °C/min), 180-230 °C (20 °C/min); 230 °C (20 min); injector temperature, 250 °C; detector temperature, 275 °C. The same temperature programme was used for the HP-Innowax column. Other conditions used in both cases: injection mode, split; split ratio, 1:50; carrier gas, $H_2$ (34 Kpa); volume injected, 0.1 µL, of a 1/10 dilution in hexane.

The GC-MS analysis were run on a Shimadzu QP1100 and a Hewlett Packard 6890/5973 MS (both with Wiley spectral data) [5]. Both were equipped with the same stationary phases used in the GC-FID analysis, using interface temperature 280(C; injection mode, split, split ratio, 1:100; carrier gas He (1.0 mL/min); lineal velocity 36 cm/s; ionization energy 70 eV, acquisition mass range 40-350u; solvent cut 3.5 min; volume injected 0.4 µL of the oil diluted in n-hexane (1:10).

The retention indices [6] were determinated by co-injection of n-alkane standard solution ($C_9$-$C_{26}$, Aldrich, USA) on both phases. The constituents of the oil were identified by comparison of their mass spectral data and retention indices in both columns with corresponding data of authentic compounds and with the MS libraries and literature data [7-9].

## RESULTS AND DISCUSSION

The yields of essential oils obtained by hydrodistillation of peels and leaves are shown in Table 1.

In Table 2 the compositions of peel oils for the grafted Mandarin (Cai variety), *Poncirus trifoliata* and Cai Mandarin scion are shown. Samples from two succesive harvests were used for *Poncirus.*

| Sample | date | yield v/w % |
|---|---|---|
| Cai peel oil | Mar-01 | 0.35 |
| | Mar-02 | 0.41 |
| Seed grown peel | Mar-01 | 0.4 |
| Poncirus peel | Mar-01 | 0.15 |
| Cai leaves | Mar-01 | 0.48 |
| | Mar-02 | 0.52 |
| Seed grown leaves | Mar-01 | 0.6 |
| Poncirus leaves | Mar-01 | not observable |

**Table 1**. Mandarin esential oil yields.

In Table 3 the compositions of the essential oils of the grafted mandarin and Cai mandarin scion leaves (Petitgrain oils) are shown. *Poncirus* yields no Petitgrain oils through this procedure.

As can be observed in Tables 1, 2 and 3, the yields for peel and Petitgrain oils are quite similar for both the grafted and the seed mandarin varieties, but have wide discrepancies with the rootstock plant. Even in rough a figure as v/w % yields, those corresponding to both the seed grown and grafted mandarin oils are similar, and widely different from that measured for *Poncirus*. The mandarin and grafted mandarin peel and leaf oils have the same components and similar compositions to those of the same varieties described in the literature [4,10-16].

The main components are limonene and γ-terpinene in the peel oils and methyl-N-methylanthranilate, γ-terpinene, limonene and p-cymene in the leaf oils. The rootstock plant has a different composition for its peel oil, and did not yield measurable amounts of “petitgrain” oil following our technique. The main constituents of the peel oil are limonene, β-myrcene, α-phellandrene, β-phellandrene, Methyl-N-methylanthranilate, Z-B-ocymene, γ-terpinene, and linalool or linallyl acetate depending on the season. There are some quite distinctive components in this oil, as is the case of phenylacetonitrile. The overall composition is similar to that reported in the literature [17,18], which is based on rather outdated methodologies.

Although the gross identity of the seed grown mandarin could be established, it must be borne in mind that citrus plants usually have a greater variability when grown from seed than when grown as grafts [19]. The slight differences

| Peak N° | Compound | seed grown Cai 2001 Apr | grafted Cai 2001 Apr | *Poncirus* 2001 Mar | *Poncirus* 2001 Dec |
|---|---|---|---|---|---|
| 1 | tricyclene | | | tr | 0.24 |
| 2 | α-Pinene | 1.39 | 1.21 | 1.13 | 0.5 |
| 3 | α-Thujene | 0.55 | 0.44 | 0.11 | tr |
| 4 | α-fenchene | | | 0.08 | |
| 5 | Camphene | tr | tr | | |
| 6 | β-Pinene | 1.3 | 1.05 | 2.55 | 2.65 |
| 7 | Sabinene | | 0.16 | 0.97 | 1.16 |
| 8 | α-phellandrene | | | 7.78 | 12.44 |
| 9 | γ-3-carene | | tr | | |
| 10 | β-Myrcene | 1.46 | 1.59 | 17.99 | 16.01 |
| 11 | α-Terpinene | 0.52 | 0.4 | | |
| 12 | Limonene | 64.55 | 71.4 | 36.25 | 34.5 |
| 13 | β-phellandrene | | 0.25 | 7.78 | 12.44 |
| 14 | Z-B-ocymene | | | 6.42 | 1.83 |
| 15 | γ-Terpinene | 18.86 | 15.5 | 3.74 | 1.48 |
| 16 | E-B-ocymene | | tr | | |
| 17 | p-cymene | 1.08 | 0.46 | 0.53 | tr |
| 18 | α-Terpinolene | 0.92 | 0.76 | | |
| 19 | Octanal | | 0.28 | 0.29 | tr |
| 20 | *cis*-3-hexanoyl-acetate | | | 0.64 | tr |
| 21 | 6-methyl-5-hepten-2-one | | 0.19 | | |
| 22 | hexanol | | tr | | |
| 23 | p-cymenene | | tr | 0.13 | tr |
| 24 | Nonanal | | tr | | |
| 25 | 1,3,8-p-menthatriene | | tr | tr | 0.23 |
| 26 | *cis*-Sabinene hydrate | | tr | tr | 0.4 |
| 27 | Citronellal | | tr | | |
| 28 | Decanal | | 0.11 | | |
| 29 | Linalool | 1.07 | 0.7 | 0.71 | 3.49 |
| 30 | 1-Octanol | | 0.13 | 0.21 | 1.46 |
| 31 | Linalyl acetate | | tr | 3.11 | 0.3 |
| 32 | 4-Terpineol | 0.88 | 0.7 | | |
| 33 | Caryophyllene | | tr | 0.29 | 1.23 |
| 34 | *cis*-p-menthen-2-en-1-ol | | tr | | |
| 35 | Neral | 0.3 | tr | 0.27 | 0.65 |
| 36 | α-Humulene | | tr | | |
| 37 | α-Terpineol | 1.79 | 1.56 | | |
| 38 | Neryl acetate | | tr | | |
| 39 | Geranial | | tr | | |
| 40 | Bicyclogermacrene | | 0.16 | | |
| 41 | Geranyl acetate | | 0.35 | 0.55 | 1.24 |
| 42 | Cadinene | | tr | | |
| 43 | Geranio | | tr | | |
| 44 | phenylacetonitrile | | | 1.35 | 4.33 |
| 45 | Caryophyllene-oxide | | tr | 0.41 | tr |
| 46 | Germacrene-D | | | 0.12 | 0.31 |
| 47 | α-farnesene | | | 0.12 | 0.96 |
| 48 | Germacrene-B | | | 0.75 | 0.8 |
| 49 | Methyl N-methyl anthranilate | 1.92 | 2.05 | 7.31 | 2.94 |
| 50 | Methyl-N-dimethyl anthranilate | | tr | | |
| 51 | Thymol | 0.94 | 0.14 | | |
| 52 | Carvacrol | | 0.23 | | |
| 53 | Methyl anthranilate | | tr | | |
| 54 | Sinensal | | 0.06 | | |
| **Total identified compounds** | | **96.7** | **99.82** | **96.70** | **93.2** |
| **Grouped components** | | | | | |
| | Hydrocarbons | 90.63 | 93.38 | 86.74 | 86.78 |
| | oxygenated | 4.98 | 4.45 | 6.19 | 7.54 |
| | Oxygenated compounds | 8.37 | 4.46 | 3.07 | 9.46 |
| | Anthranilates | 1.92 | 2.05 | 7.31 | 2.94 |
| | Aldehydes and Ketones | 0.3 | 0.64 | 0.56 | 0.65 |
| | Alcohols and phenols | 4.68 | 3.47 | 0.92 | 4.95 |

**Table 2**. Percentage compositions of single components and classes of substances in peel oils for year 2001.

| **Compound** | **Cai leaves March 2001** | **Seed Cai leaves March 2001** |
|---|---|---|
| Tricyclene | tr | |
| α-Pinene | 0.21 | tr |
| α-Thujene | 0.09 | tr |
| α-fenchene | tr | nd |
| Hexanal | tr | nd |
| β-Pinene | 0.37 | tr |
| Sabinene | 0.23 | tr |
| γ-3-carene | tr | nd |
| β-Myrcene | 0.29 | tr |
| α-Terpinene | tr | tr |
| Limonene | 5.19 | 2.57 |
| β-phellandrene | tr | tr |
| γ-Terpinene | 14.17 | 8.68 |
| (*E*)-β-Ocimene | 0.38 | tr |
| p-cymene | 1.99 | 1.16 |
| α-Terpinolene | 0.54 | 0.31 |
| Octanal | tr | nd |
| 6-methyl-5-hepten-2-one | tr | tr |
| z-3-hexenol | tr | nd |
| Nonanal | tr | tr |
| 1,3,8-menthatriene | tr | tr |
| Citronellal | tr | tr |
| Decanal | tr | tr |
| Linalool | 0.47 | 0.38 |
| 1-Octanol | tr | tr |
| Linalyl acetate | 0.07 | nd |
| 4-Terpineol | 0.2 | 0.13 |
| α-Humulene | 0.08 | tr |
| β-Terpineol | 0.31 | 0.31 |
| Caryophyllene-oxide | tr | tr |
| Methyl N-methyl anthranilate | 74.68 | 85.8 |
| Thymol | 0.23 | 0.18 |
| **Total identified compounds** | **99.5** | **99.52** |
| **Grouped components** | | |
| Hydrocarbons | 23.54 | 12.72 |
| Oxygenated compounds | 1.28 | 1 |
| Anthranilates | 74.68 | 85.8 |
| Aldehydes and Ketones | tr | tr |
| Alcohols | 1.21 | 1 |

**Table 3**. Compositions of leaf essential oils (Petitgrain oils).

with the compositions described in the literature could be due to this, as well as to the influence of other cultivation factors [19]. In our case, the seed grown plant was growing in a garden outside the commercial plantation.

Grafted plants tend to show differences to the same seed grown plants, in growth behaviour, in overall plant structure, vigour, fruiting density, cold hardiness, resistance to certain diseases, tolerance to salt, mineral element concentrations [19-21]. In spite of these large changes, little is known about differences in secondary metabolite compositions, which could be considered to be more susceptible to the rootstock influences. In Citrus it has been established that there are changes in the growth hormone concentrations [22], in overall physicochemical and organoleptic characteristics of the fruit juices [23,24], in minor changes in flavonoid composition in grafted lemons [25], but very small variations in the essential oils in the cases of lemons [26] and bergamots [27].

The scion and grafted mandarin oils show a typical high concentration of limonene, as well as high percentages of γ-terpinene which are much lower in *Poncirus*, and an important percentage of α-terpineol which is not present in the rootstock. Typical components for *Poncirus* α- and β-phellandrene, α-terpinene) are traces or not present in mandarin oils, and myrcene is 10 times more abundant. The concentrations in the grafted and seed mandarin oils for the components typical for the rootstock oil, showed no variations or influence from the rootstock.

In the case of petitgrain oil components, anthranylates and hydrocarbons are the two main groups of constituents in both grafted and seed varieties, with oxygenated compounds (mostly alcohols) as a minor fraction. The yields for *Poncirus* petitgrain were negligible following our technique.

Our results agree with those reported in the literature [26,27] in the sense of the rootstock having little or no influence on the essential oil compositions of the grafted plant.

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