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# Chemical variability of peel and leaf essential oils in the *Citrus* subgenus *Papeda* (Swingle) and relatives

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**Abstract:** Citrus fruits of the subgenus *Papeda* include little-exploited wild forms with a potentially large and original aromatic diversity. The essential oils obtained from peels and leaves of the following seven species of the *Citrus* subgenus *Papeda*, namely: *C. hystrix*, *C. micrantha*, *C. macroptera*, *C. ichangensis* (3 accessions), *C. latipes* (2 accessions), *C. junos* & *C. macrophylla*, were investigated by GC, GC-MS and <sup>13</sup>C NMR spectrometry in order to describe the chemical variability. A total of 60 compounds were identified in peel oils, accounting for 91.6 to 100% of the total peel oil composition. Limonene was the major component in almost all samples, excepted for *C. micrantha* & *C. hystrix* oils where  $\beta$ -pinene dominated. Concerning the leaf oils, 76 compounds were identified, accounting for 93.6% to 99.3% of the total composition. Among them, the major components were: citronellal for *C. hystrix* & *C. micrantha*; sabinene, linalool and  $\beta$ -pinene for *C. macroptera*; (E) and (Z) ocimene for two accessions of *C. ichangensis*, and sabinene for the third accession; limonene, linalool and citronellal for one accession of *C. latipes*, and  $\gamma$ -terpinene, geranial, neral and  $\beta$ -pinene for the other one;  $\gamma$ -terpinene,  $\beta$ -phellandrene and p-cymene for *C. junos* and finally geranial, neral and limonene for *C. macrophylla*. We highlighted here a strong chemical diversity not only among the sections of the subgenus *Papeda*, but also between the species of these sections, and even at an intraspecific level.

**Keywords:** *Papeda*; Citrus; essential oil; chemical composition; diversity

## 1. Introduction

Citrus trees are native to Southeast Asia and their exceptional diversity is the result of both migration and geographical isolation during its evolution time [1] (Wu et al. 2018, Nature). The current cultivated forms are the result of crosses between species that evolved in Southeast Asia [2–5] (Ollitrault et al. 2003; Nicolosi, 2007; Garcia-Lor et al. 2012; Wu et al. 2014). There are also Oceanic species such as the taxa *Eremocitrus* and *Microcitrus*. The main characteristic of citrus is the presence of highly aromatic essential oils in tissue storage cells of the fruit, leaf and flower (petals). These essential oils are complex mixtures that can contain hundreds of compounds with a very wide chemical variability particularly prized by the aromatic and cosmetic industry [6] (Demarcq et al. 2021 JAFC). The composition of essential oils of the large majority of citrus fruits grown for consumption is very well documented [7] (“Citrus oils” book Dugo & Mondello 2011). A large part of the aromatic diversity found in citrus fruits is however under-exploited. For example, citrus fruits of the *Papeda* subgenus are largely unknown.

Swingle (1943) considered that there were 2 subgenera in Citrus: *Papeda* and *Citrus* [8]. In the subgenus *Papeda*, he defined 2 sections (*Papeda* and *Papedocitrus*) with respectively 4 species (*hystrix*, *macroptera*, *micrantha* and *celebica*) listed in the first one and 2 species (*C. ichangensis* (*Ichang papeda*) and *C. latipes* (*Khasi papeda*)) in the second one. The section *Papedocitrus* is considered as intermediate between the two sub-genera *Papeda* and *Citrus* (Swingle 1943). In the chapter entitled "Botany of citrus and its wild relatives" Swingle & Reece (1967) described the subgenus *Papeda* as follows: "pulp-vesicles containing numerous droplets of acrid oil; petioles long and very broadly winged, but not cordate, often nearly as broad as the leaf blades; stamen usually free...flowers larger and petioles very long, 1.75-3 longer than broad" [9]. Recently, exploration of the Citrus genome by molecular markers and sequencing has demonstrated that *Papeda* is a non-homogeneous group actually consisting of two very distinct (polyphyletic) genetic groups, one with *C. hystrix* (or *C. micrantha*) as a reference and the other with *C. cavaleriei* (or *C. ichangensis*) [1,10] (Wu et al. 2018; Talon et al. 2020). These two genetic groups are considered to be two ancestral species that have generated through outcrossing with other ancestral species (*C. maxima*, *C. reticulata*, *C. medica*) some cultivated varieties such as Yuzu (*C. reticulata* x *C. ichangensis*) or Alemow (*C. micrantha* x *C. medica*) [1,11,12] (Ollitrault et al. 2012; Curk et al. 2016; Wu et al. 2018). Ollitrault et al. (2020) [13] proposed a new classification taking into account phylogenetic relationships and sexual compatibility and give the correspondence with the former classifications built by Tanaka [14], Swingle and Reece [9], Zhang and Mabberley [15]. In the *Papeda* group, there are two true species, the first one is *C. cavaleriei* H. Lév. ex Cavalerie from West-central and Southwestern China, which includes *C. ichangensis* Swingle and *C. latipes* (Swingle) Tanaka. The second one is *C. micrantha* Wester from Southern Philippines, which includes two varieties *micrantha* (Biasong) and *microcarpa* (Samuyao) and *Combava* (*C. hystrix* DC) but named by Zhang & Mabberley, *C. hystrix* DC (2008) [15]. This new classification confirms in some ways that of Swingle and Reece who had divided the *Papeda* group into two sections. It should be noted that the classification of Melanesian *papeda* (including *Eremocitrus* and *Microcitrus*) has not been considered in this phylogenomic taxonomy. In terms of genetic diversity, there are very few studies concerning the *Papeda* group of Citrus. Those concerning *C. macroptera* [16] (Malik et al. 2013) and *C. cavaleriei* (or *C. ichangensis*) [17] (Yang et al. 2017) show nevertheless a great intraspecific genetic diversity.

Data concerning the chemical composition of peel and leaf oils from Citrus classified as *Papeda* are scarce. The literature is mainly focused on cultivated hybrids, as *C. junos* known as Yuzu [18,19] (Uehara et al., 2000; Huang and Pu). Leaf oil composition was also reported in *C. ichangensis* [20–22] (Zhang et al food chem 2017; Liu plos one 2013, Zhang 2020). *C. hystrix* is also well described in the literature [21–24] (Dugo & DiGiacomo 2002, Waikedre, Zhang 2017, Zhang 2020), while *C. macroptera* leaf oil was only described by Huang et al. [19] and Waikedre et al. [24]. *C. latipes* and *C. macrophylla* were described only once in the same publication [25] (Hijaz et al., 2016). For *C. macrophylla* it could be due to its only used as a rootstock for citrus cultivation [26] (Ollitrault and Luro 2001). To our knowledge, there is no chemical data concerning *C. micrantha* in the literature.

The chemical composition of peel and leaf essential oils determined by (i) Gas Chromatography associated by retention indices (RI) calculated for polar and apolar columns (GC-FID) with (ii) Gas Chromatography-mass spectrometry (GC-MS) provide abundant information, not only for metabolism-related research, but also for chemo-taxonomy. Thus, several papers developed this approach [27,28]

(Xi 2015; Jing 2015). The chemotaxonomy of Mangshanyegan (*C. nobilis*), was determined by comparison of volatile profiles of fruits and leaves and those of 29 other genotypes of Citrus, Poncirus, and Fortunella [20] (Liu Plos one 2013). The chemical components identified in the peels of 66 citrus germplasms from four Citrus species (mandarin, orange, pomelo and lemon) were used for biomarker mining, thirty potential biomarkers were identified and four compounds ( $\beta$ -elemene, valencene, nootkatone, and limettin) were validated as biomarkers after a study involving 30 Citrus germplasms [29] (Zhang et al., 2019). However, Luro et al. [30] found that the diversity based on leaf oil compositions from *Citrus medica* L. did not agree with the molecular diversity and was unsuitable for in-traspecific phylogenetic studies (phytochem 2012).

In this context, the aim of this study was to investigate the diversity of chemical composition of peel and leaf oils from Citrus belonging to the subgenus Papeda present in the INRAE-CIRAD CRB citrus germplasm bank (Corsica, France). All accessions are fully indexed in a plot with identical climatic and agronomic conditions of growing [31] (Luro et al. 2017 AGRUMED). These conditions are suitable to study the relation-ship between chemistry and taxonomy and to produce a reference data of Papeda peel and leaf oils composition. We analyzed ten samples from seven species: firstly 3 Papeda species (*C. hystrix*, *C. micrantha* and *C. macroptera*) and 2 Papedocitrus species (three ac-cessions of *C. ichangensis* and two accessions of *C. latipes*) to characterize the two sec-tions of the subgenus Papeda; and secondly 2 relatives species (*C. junos* and *C. macro-phylla*), in order to discuss the inheritance of chemical characters.

## 2. Results and Discussion

In total, 60 compounds were identified in peel oils, accounting for 91.6% to 100% of the total oil composition (Table 1), whereas 76 compounds were identified in leaf oils, accounting for 93.6% to 99.3% (Table 2).

### 2.1. Peel oils

Among the ten studied accessions, only nine peel oil samples were obtained by hydro-distillation of peels because one accession of *C. ichangensis* didn't produce a suf-ficient amount of fruits.

All the peel oil samples were widely dominated by monoterpene hydrocarbons, mostly because of the abundance of limonene (20.7 – 81.4%), as described in the litera-ture [23] (Dugo & Di Giacomo, 2002). Despite this common characteristic, several chemical profiles were observed.

#### 2.1.1. Section Papeda

*C. hystrix* & *C. micrantha*, showed low contents of limonene (respectively 25.2 and 20.7%) associated with higher contents in  $\beta$ -pinene, which was the major component (35.0 and 33.4%). While *C. hystrix* oil showed a large amount of sabinene (22.7%), *C. micrantha* oil contained a noticeable quantity of oxygenated monoterpenes with cit-ronellol (6.8%),  $\alpha$ -terpineol (6.6%), terpinen-4-ol (3.8%), citronellyl acetate (3.1%) and other smaller components. According to the literature, this low content of limonene is usual in *C. hystrix*, the review of Lawrence [32] give the following main components :  $\beta$ -pinene (20.4

– 42.2%), sabinene (13.0 – 25.9%), citronellal (3.4 – 16.8%), limonene (2.8 – 14.2%), terpinen-4-ol (3.8 – 8.9%) and  $\alpha$ -terpineol (1.7 – 7.4%) (Dugo & Di Giacomo, 2002, chapter 14 ).

*C. macroptera* oil exhibited limonene (53.8%), sabinene (12.4%) and beta-pinene (3.9%) associated with monoterpene alcohols such as linalool (11.8%) and terpinen-4-ol (4.3%). As observed for *C. hystrix* and *C. micrantha*, the percentage of oxy-genated monoterpenes was elevated (18.6%). This composition of *C. macroptera* peel oil is different than the one described by Rana & Blazquez [33] with 55.3% limonene, 4.7% of (E)- $\beta$ -caryophyllene and 3.5% geraniol and the other one described by Miah et al. [34] with 73.5% limonene, 3.4%  $\delta$ -cadinene and 3.0%  $\alpha$ -terpineol.

### 2.1.2. Section Papedocitrus

*C. ichangensis* and *C. latipes* oils were characterized by high percentages of limonene varying between 42.3 and 66.9% . However, a strong chemical variability was observed for the Papedocitrus section. The accession ich-3 of *C. ichangensis* exhibited a usual composition dominated by monoterpene hydrocarbons: limonene (58.2%), sabinene (9.6%),  $\beta$ -phellandrene (8.8%) and p-cymene (4.4%) and mostly terpinen-4-ol (7.3%) for the oxygenated compounds. Conversely, the accession ich-2 showed an atypical composition characterized by a ratio 1/1 of monoterpenes (42.8% of hydro-carbons and 1.9% of oxygenated monoterpenes) and sesquiterpenes (33.4% of hydro-carbons and 7.3% of oxygenated sesquiterpenes) and a noticeable quantity of acyclic compounds (6.0%). Moreover, the percentages of  $\beta$ -bisabolene (18.4%) and intermedeol (4.7%) were remarkable, likewise other sesquiterpenes identified in smaller proportions such as trans- $\alpha$ -bergamotene (3.2%),  $\gamma$ -muurolene (3.1%), valencene (2.7%) and (E)-nerolidol (1.6%). Although, a recent study revealed that among several *C. ichangensis* peel oils, one of them contained higher amounts of sesquiterpenes than monoterpenes [21] (Zhang et al., 2017). In this study,  $\alpha$ -cadinene,  $\beta$ -bourbonene and the acyclic esters butyl butanoate and ethyl hexanoate were reported in *C. ichangensis* peel oil. Another study described the composition of *C. ichangensis* peel oil, with higher percentage of limonene (61.0 – 70.4%) but similar amounts of  $\beta$ -bisabolene (9.3 – 13.0%) and (E)-nerolidol (3.1 – 3.9%) [20] (Liu et al, 2013). Our results agreed with published results about the presence of both acyclic compounds and a large range of sesquiterpene hydrocarbons in *C. ichangensis*.

The two accessions of *C. latipes* peel oil lat-1 and lat-2 exhibited a slightly difference in the percentage of limonene (66.9 vs. 50.4%) and also differed by the presence of myrcene in noticeable amount (18.8% and 1.6% respectively). No chemical data were found about peel oil of *C. latipes* in the literature.

### 2.1.3. Relatives

Peel oils of *C. junos* and *C. macrophylla* were characterized by high contents of limonene, respectively 79.9 and 81.4%, and  $\gamma$ -terpinene, respectively 8.8 and 5.0%. The chemical composition we observed for *C. junos* is similar than those described by Dugo & Di Giacomo [23] with a percentage of limonene varying between 60.4 and 82.4%, mainly associated with  $\gamma$ -terpinene (7.6 – 10.7%) and linalool (0.9 – 5.6%). No data were found about peel oil composition of *C. macrophylla*.

These compositions widely dominated by limonene are frequently observed in many Citrus species such as *C. reticulata* Blanco [35] (around 70%) and *C. sinensis*, *C. aurantium*, *C. paradisi*, *C. aurantifolia* [23] (around 90%) (Lota et al., 2000 and Dugo et Di Giacomo 2002). Citron (*C. medica*) peel oils are known to contain variable amounts of limonene (39.5 – 94.3%), either as the only major component or associated with geraniol/neral or  $\gamma$ -terpinene [36] (Venturini et al., 2010). In the same way, lemon

(*C. limon*) peel oils can contain uneven quantities of limonene (38.1 – 95.8%), in association or not with other major components like  $\gamma$ -terpinene, linalool,  $\beta$ -pinene [37] (Lota et al, 2002). Nevertheless, the proportion of limonene in peel essential oil of citrons (*C. medica*) and lemons (*C. limon*) is lower (between 40 and 50%) [23,30] (Luro et al. 2012; Dugo et Di Giacomo 2002 ). The two Citrus x Papeda hybrids, Alemow and Yuzu, have a higher proportion of limonene in the leaf essential oil than their two respective Citrus parents. These are 2 cases of transgressive inheritance already observed in a clementine x mandarin population [38] (Tomi et al. 2008).

In our sampling, Papedocitrus (*C. ichangensis* and *C. latipes*) peel oils constituted an intermediate between low amounts of limonene observed in Papeda section (around 25%) and high percentages (around 80%) in the relative species, *C. junos* & *C. macrophylla*.

## 2.2. Leaf oils

The ten leaf oil samples exhibited a chemical composition dominated by mono-terpenes, as usually found in Citrus leaf essential oils [23] (Dugo & Di Giacomo. 2002). However, we observed a tremendous quantitative variability among the major components: sabinene (0 – 44.6%),  $\beta$ -pinene (0 – 15.7%), (Z)- $\beta$ -ocimene (tr – 18.2%), (E)- $\beta$ -ocimene (0.2 – 62.7%),  $\gamma$ -terpinene (0 – 28.2%), linalool (0.2 – 24.6%), citronellal (0 – 78.1%), neral (0 – 18.9%), geranial (0 – 24.7%).

*C. hystrix*, *C. micrantha* and *C. macrophylla* were dominated by oxygenated mono-terpenes, whereas *C. junos*, *C. ichangensis* (3 accessions) and *C. macroptera* were dominated by monoterpene hydrocarbons. The last species, *C. latipes*, exhibited a ratio between hydrocarbon/oxygenated terpenes close to 1.

The yields of the ten leaf oil samples varied drastically between 0.015 to 0.18 % (Table 2). For example, the three samples of *C. ichangensis* exhibited very different yields and obviously, a strong intraspecific variability.

### 2.2.1. Section Papeda

Combava (*C. hystrix* DC.) & Biasong (*C. micrantha* Wester)

*C. hystrix* and *C. micrantha* leaf oils exhibited a close chemical composition strongly dominated by citronellal (respectively 78.1 and 76.1%) and its derivatives, citronellol (3.4 and 4.4%) and citronellyl acetate (0.7 and 5.1%).

Similar compositions were previously reported for *C. hystrix* oils: citronellal between 58.9 and 81.5%, citronellol between 6.0 and 8.2% and citronellyl acetate between 0.9 and 5.1% [23] (Dugo & Di Giacomo. 2002). The recent review about *C. hystrix* revealed that some authors described leaf oils with 1.4 to 72.5% citronellal, while others described leaf oil dominated by limonene (40.7 – 83.9%) [39] (Agouillal et al, 2017). A New-Caledonian study showed a drastically different chemical composition of *C. hystrix* leaf oil dominated by terpinen-4-ol (13.0%),  $\beta$ -pinene (10.9%),  $\alpha$ -terpineol (7.6%) and citronellol (6.0%) with a very low content in citronellal (2.7%) [24] (Waikedre et al.. 2010). Finally, the study of Zhang et al. [22] show four accessions of *C. hystrix*, with different major components: one of them with citronellal and geranyl acetate, another one with geranial and geranyl acetate, and two with

geranyl acetate associated with either citronellal or geranial (Zhang et al. 2020). To our knowledge, there is no existing description of *C. micrantha* oil in the literature.

It's interesting to note that in many phylogeny studies, *C. micrantha* and *C. hystrix* are grouped together [40] (Froelicher 2011) or even formed a separate cluster [41,42] (Nicolosi et al., 2000 ; Penjor et al., 2013). In other studies without *C. hystrix*, *C. micrantha* is still grouped with another lime, the Mexican lime [1,43] (Wu et al., 2018 ; Carbonell-Caballero et al., 2015). These phylogenetic studies seem indicate that in this case, genetics and chemistry agreed to consider *C. micrantha* and *C. hystrix* as related species. The recent publication of Ollitrault et al. (2020) [13] even consider them as synonyms.

Melanesian papeda (*C. macroptera* Montr.)

The leaf oil of *C. macroptera* is characterized by large amounts of sabinene (32.4%),  $\beta$ -pinene (15.7%) and linalool (18.2%), as well as significant percentages of (E)- $\beta$ -ocimene (8.6%) and terpinen-4-ol (3.8%).

Two articles reported the chemical composition of this essential oil. The first one reported was characterized by the pre-eminence of sabinene (20.9%) associated with geranyl acetate (15.5%),  $\beta$ -phellandrene (9.1%), geranial (8.7%), (E)- $\beta$ -ocimene (8.0%) and neral (6.8%) [19] (Huang & Pu. 2000), whereas hydrocarbons were the main components in the second one:  $\beta$ -pinene (33.3%),  $\alpha$ -pinene (25.3%), p-cymene (17.6%) and (E)- $\beta$ -ocimene (6.7%), this time with very few sabinene (4.8%) and no geranyl acetate [24] (Waikedre et al).

So, the composition described herein produced a new chemical composition, suggesting a significant variability in this species, as previously observed with DNA molecular markers [16] (Malik et al, 2013).

## 2.2.2. Section Papedocitrus

Ichang papeda (*C. ichangensis* Swingle)

The essential oils of *C. ichangensis* showed a significant intraspecific diversity. Two accessions, "ich-1" and "ich-2", were characterized by a dominance of the two (E) / (Z)- $\beta$ -ocimenes, in variable amounts 18.2% / 62.7% and 13.0% / 32.4% respectively. The first accession ich-1 also showed appreciable amounts of linalool (9.3%) and linalyl acetate (10.8%). Moreover, these ocimene-type oils contained an appreciable amount of alismol (1.7 and 1.6%), an unusual sesquiterpene in Citrus genus. Indeed, occurrences of alismol in Citrus oils have already been found in kumquats (*Fortunella* genus) [44] (Sutour et al.. 2015) and in *C. x jambhiri* [45] (Kasali et al.. 2009). The third accession ich-3 is completely different and is dominated by sabinene (44.6%), associated with  $\beta$ -phellandrene (11.7%) and terpinen-4-ol (8.4%).

In a recent study, Zhang et al. [22] reported the chemical composition of ten accessions of *C. ichangensis* (Zhang et al., 2020). Five over ten oil samples exhibited (E)- $\beta$ -ocimene as major component, associated with linalyl acetate in four accessions and  $\alpha$ -pinene in another one, whereas two over ten oil samples were dominated by sabinene, associated with  $\gamma$ -terpinene and limonene. In our sampling a similar ratio: 2/1 between these two compositions was observed. The three other accessions of *C. ichangensis* of this study are mostly composed of  $\gamma$ -terpinene for two of them, and linalyl acetate for the last one. Moreover, the authors indicated that percentages of sesquiterpenes hydrocarbons such as (E)- $\beta$ -caryophyllene, (E)- $\beta$ -farnesene,  $\beta$ -elemene or germacrene D were punctually high, in the same way as in our sampling.

Another Citrus oil known to contain appreciable amount of ocimene is a lemon named “Poire du Commandeur” or “Peer lemon” (*C. lumia*), a supposed pumme-lo/mandarin hybrid [12] (Curk et al. 2016) characterized by high contents in  $\beta$ -pinene (41.4%) and (E)- $\beta$ -ocimene (15.8%), associated with linalool (11.2%), limonene (8.6%) and sabinene (4.8%) [37] (Lota et al., 2002).

The strong intraspecific diversity observed at the level of aromatic compounds is in agreement with the high genetic diversity of this taxa observed at the DNA level [17] (Yang et al. 2017).

Khasi papeda (*C. latipes* (Swingle) Tanaka)

The two accessions were characterized by a ratio hydrocarbon/oxygenated ter-penes close to 1 but with drastically different chemical compositions. The first oil sam-ple lat-1 was dominated by limonene (41.0%), associated with linalool (24.6%) and cit-ronellal (14.1%), while the second one lat-2 was characterized by an association  $\gamma$ -terpinene (19.5%), geranial (15.6%) neral (11.6%) and  $\beta$ -pinene (9,7%). We observed that citronellal (14.1%), citronellol (1.8%) and citronellyl acetate (1.0%), the main components of *C. hystrix* and *C. micrantha*, presented a noticeable amount in lat-1 ac-cession.

The only description found in the literature gave neral as major component (24.6%) , followed by an unusual high amount of undecanal (19.6%),  $\beta$ -phellandrene (11.4%), limonene (10.5%) and linalool (7.6%) [25] (Hijaz et al, 2016).

### 2.2.3. Relatives

Yuzu (*C. junos* Sieb. Ex Tan)

Fresh leaves of *C. junos* produced an essential oil composed of  $\gamma$ -terpinene (28.2%), p-cymene (11.4%),  $\beta$ -phellandrene (11.2%) and linalool (10.4%). This oil also showed appreciable amounts of p-cymenene (6.2%), (E)- $\beta$ -ocimene (5.0%),  $\alpha$ -pinene (4.8%), limonene (4.7%) and  $\beta$ -pinene (4.1%). It could be pointed out that this oil also exhibited 2,5-dimethoxy-p-cymene (1.4%), a compound not identified in others Papeda acces-sions .

The chemical composition of *C. junos* leaf oil is known to present a great chemical variability [23] (Dugo & Di Giacomo. 2002. chapter 14). A complete study concerning chemical composition of 110 Citrus species shows the intraspecific variability of *C. ju-nos* oils [19] (Huang & Pu. 2000). Ten cultivars of *C. junos* were investigated, showing very different profiles dominated by (i) methyl-N-methyl anthranilate (a compound rather known to be found in high contents in Citrus *reticulata* Blanco mandarins), or (ii)  $\gamma$ -terpinene, in proportions varying from 22.6 to 53.2%. Three accessions of this study exhibited a composition very similar to ours, with  $\gamma$ -terpinene (25.7 – 26.6%), p-cymene (11.5 – 12.8%),  $\beta$ -phellandrene (8.2 – 12.0%) and linalool (5.8 – 8.1%). An-other study gives a near chemical composition with the same major components but in a different ranking with 25.4% linalool, 15.6%  $\gamma$ -terpinene, 11.2%  $\beta$ -phellandrene and 9.5% p-cymene [46] (Kamiyama, 1970).

This chemical composition dominated by the association  $\gamma$ -terpinene/p-cymene/linalool is frequently reported for mandarin leaf essential oils such as wase (*C. unshiu*), fuzhu (*C. unshiu*), owari (*C. unshiu*), kunembo (*C. nobilis*), szibat (*C. suhuiensis*) and sunki (*C. sunki*) [47] (Lota et al., 2001). This characteristic of the chemical profile of Junos could be inherited from its male relative, the mandarin.

Alemow (*C. macrophylla* Wester)

The leaf oil of *C. macrophylla* was characterized by large amounts of geranial (24.7%), neral (18.9%), limonene (17.7%) and in smaller proportions  $\gamma$ -terpinene (6.2%), *p*-cymene (4.3%), linalool (4.3%) and citronellal (3.5%).

A chemical composition reported in literature showed the same major components but in a different ranking: limonene (31.4%), geranial (22.8%), neral (16.1%) and citronellal (13.9%), followed by  $\delta$ -3-carene (3.5%) and  $\alpha$ -terpinene (3.4%) [25] (Hijaz et al., 2016).

This type of composition dominated by the association geranial/neral/limonene is also found in leaf essential oils of citrons and some limes [37] (Lota et al., 2002). This characteristic of the chemical profile of Alemow might be inherited from its citron male relative.

### 3. Materials and Methods

#### 3.1. Plant Material

According to the Swingle and Reece systematics (1967), ten accessions were selected to represent the diversity of the subgenus *Papeda*: three *Ichang papeda* (*C. ichangensis* Swing.) and two *Khasi papeda* (*C. latipes* (Swing.) Tan.) for section *Papedocitrus*, *Biasong* (*C. micrantha* Wester), *Combava* (*C. hystrix* D.C.) and *Melanesian papeda* (*C. macroptera* Montr.) for section *Papeda*. Two other *Citrus* species related to *Papeda* have been added: Alemow (*C. macrophylla* Wester; *C. micrantha* x *C. medica*) and Yuzu (*C. junos* Sieb. ex Tan.; *C. ichangensis* x *C. reticulata*) (Table 1). All the trees are maintained in the INRAE-CIRAD citrus collection (certified as Biological Resource Center (BRC) citrus NF96-600) localized in San Ghjulianu (France, Corsica): latitude 42°17'N; longitude 9°32'E; Mediterranean climate; average: rainfall and temperature: 840 mm and 15.2°C per annum, respectively; soil derived from alluvial deposits and classified as fersiallitic; pH range 6.0–6.6 [31] (Luro et al. 2017).

About 100 g of fruit peels and 200 g of leaves were randomly picked all around the tree. The fresh materials were submitted to water distillation for 3 hours using a Clevenger type apparatus. Peel oil yields being influenced by the presence of variable amounts of albedo during the peeling of the epicarp, they were not calculated. Distillation yields of leaf oils were calculated using the weight of essential oil/weight of fresh leaves ratio. Each sample was analyzed by gas chromatography with two columns and gas chromatography coupled with mass spectrometry (GC-MS) in order to determine the chemical composition. To avoid any misidentification, some samples, selected on the basis of the chromatogram profile, were analyzed with carbon-13 nuclear magnetic resonance (<sup>13</sup>C NMR) following a methodology developed in our laboratory [48] (Tomi et al., 1995).

#### 3.2. Gas Chromatography (GC) Analysis

GC analyses were performed on a Clarus 500 FID gas chromatograph (PerkinElmer, Courtaboeuf, France) equipped with two fused silica gel capillary columns (50 m x 0.22 mm, film thickness 0.25  $\mu$ m), BP-1 (polydimethylsiloxane) and BP-20 (poly-ethylene glycol). The oven temperature was programmed from 60 to 220 °C at 2°C/min and then held isothermal at 220°C for 20 min, injector temperature: 250°C; detector temperature: 250°C; carrier gas: hydrogen (1.0 mL/min); split: 1/60. The relative pro-

portions of the oil constituents were expressed as percentages obtained by peak area normalization, without using correcting factors. Retention indices (RIs) were determined relative to the retention times of a series of n-alkanes with linear interpolation ('Target Compounds' software of PerkinElmer). The EO samples (50 mg) were diluted in 1 mL deuterated chloroform (CDCl<sub>3</sub>).

### 3.3. Mass Spectrometry

The EOs were analyzed with a PerkinElmer TurboMass detector (quadrupole, Perkin Elmer, Courtaboeuf, France), directly coupled to a PerkinElmer Autosystem XL (PerkinElmer), equipped with a fused silica gel capillary column (50 m x 0.22 mm i.d., film thickness 0.25 μm), BP-1 (polydimethylsiloxane). Carrier gas: helium at 0.8 mL/min; split: 1/75; injection volume: 0.5 μL; injector temperature: 250°C; oven temperature programmed from 60 to 220°C at 2°C/min and then held isothermal (20 min); ion source temperature: 250°C; energy ionization: 70 eV; electron ionization mass spectra were acquired over the mass range 40–400 Da. Oil samples were diluted in deuterated chloroform with 50 mg of essential oil in 1 mL of CDCl<sub>3</sub>.

### 3.4. NMR Analysis

<sup>13</sup>C NMR analyses were performed on an AVANCE 400 Fourier Transform spectrometer (Bruker, Wissembourg, France) operating at 100.623 MHz for <sup>13</sup>C, equipped with a 5 mm probe, in CDCl<sub>3</sub>, with all shifts referred to internal tetramethylsilane (TMS). <sup>13</sup>C NMR spectra were recorded with the following parameters: pulse width (PW): 4 μs (flip angle 45°); acquisition time: 2.73 s for 128 K data table with a spectral width (SW) of 220,000 Hz (220 ppm); CPD mode decoupling; digital resolution 0.183 Hz/pt. The number of accumulated scans ranged from 2000–3000 for each sample (around 40 mg of oil in 0.5 mL of CDCl<sub>3</sub>). Exponential line broadening multiplication (1.0 Hz) of the free induction decay was applied before Fourier Transformation.

### 3.5. Identification of Individual Components

Identification of the components was based on: (i) comparison of their GC retention indices (RIs) on polar and apolar columns, determined relative to the retention times of a series of n-alkanes with linear interpolation ('Target Compounds' software of PerkinElmer), with those of authentic compounds [49] (McLafferty & Stauffer, 1988); (ii) computer matching against commercial mass spectral libraries [50,51] (McLafferty & Stauffer, 1994; König et al., 2001) and by comparison of spectra with literature data [52,53] (Joulain & König, 1998; Adams, 2007) and (iii) comparison of the signals in the <sup>13</sup>C NMR spectra of EOs with those of reference spectra compiled in the laboratory spectral library, with the help of a laboratory-made software [48,54,55] (Tomi et al., 1995; Tomi & Casanova, 2006; Bighelli & Casanova, 2009). In the investigated samples, individual components were identified by NMR at contents as low as 0.5%.

## 4. Conclusions

The two sections of Papeda are clearly distinguishable in the compositions of both the leaf and the fruit peel essential oils. This supports the classification of Swingle who proposed two sections in Papeda. Furthermore, as stated in this classification, the section Papedocitrus is an intermediate

between the two subgenera *Papeda* and *Citrus*, and certain aromatic compounds, such as limonene content, seem to confirm this status.

A great chemical diversity was observed in leaf oils and peel oils of representatives of *Papeda* and *Papedocitrus* sections. However, for some of them (*Biasong* and *Combava*) the chemical profiles are very close, reflecting a close genetic relationship. On the other hand, the 3 representatives of *C. ichangensis* present very different profiles as between the 2 of *C. latipes*. The two *Citrus* x *Papeda* hybrids demonstrate that crosses between these two taxa can create a high variability in the aromatic composition of essential oils. Prospects for exploiting this aromatic diversity are offered by the combination of these little-known citrus fruits with field crop species.

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**Table 1.** Chemical composition of peel essential oils of nine *Papeda* oil samples

N°	RI <sub>A</sub>	RI <sub>P</sub>	Name	<i>hys</i>	<i>mic</i>	<i>mapt</i>	<i>lat-2</i>	<i>ich-3</i>	<i>lat-1</i>	<i>ich-2</i>	<i>jun</i>	<i>maph</i>
1	923	1022	$\alpha$ -thujene	0.2	0.1	0.1	0.4	0.1	1.0	-	0.4	0.3
2	931	1020	$\alpha$ -pinene	3.0	2.4	1.3	2.2	2.1	2.4	-	1.6	1.0
3	945	1070	camphene	0.2	0.3	-	tr	-	tr	-	-	-
4	966	1127	sabinene	22.7	1.0	12.4	0.1	9.6	0.3	0.1	0.1	0.1
5	972	1116	$\beta$ -pinene	35.0	33.4	3.9	3.5	0.7	1.9	-	0.8	0.6
6	977	1221	butyl butyrate	-	-	-	-	-	-	2.4	-	-
7	981	1166	myrcene	0.9	1.0	2.1	1.6	2.1	18.8	0.2	1.9	1.6
8	996	UD	hexyl acetate	-	-	-	0.3	-	-	-	-	-
9	998	1170	$\alpha$ -phellandrene	-	0.1	-	0.2	0.3	-	-	0.4	-
10	1010	1185	$\alpha$ -terpinene	-	0.4	0.3	0.1	0.1	0.2	-	0.2	tr
11	1013	1276	<i>p</i> -cymene	0.7	0.8	1.1	7.0	4.4	6.5	0.3	1.3	2.6
12	1022	1215	$\beta$ -phellandrene *	0.3	1.0	0.5	3.9	8.8	0.2	-	2.7	0.1
13	1022	1205	limonene *	25.2	20.7	53.8	66.9	58.2	50.4	42.3	79.9	81.4
14	1026	1237	( <i>Z</i> )- $\beta$ -ocimene	-	-	-	-	tr	-	-	-	0.5
15	1037	1255	( <i>E</i> )- $\beta$ -ocimene	-	0.4	0.2	0.2	0.1	0.1	-	0.4	0.8
16	1049	1251	$\gamma$ -terpinene	-	1.3	1.0	10.1	1.0	16.2	-	8.8	5.0
17	1062	1446	<i>cis</i> -linalool oxide THF form	0.5	0.3	1.2	tr	tr	-	-	-	0.1
18	1075	1474	<i>trans</i> -linalool oxide THF form	0.3	0.2	0.6	-	-	-	-	-	0.1
19	1079	1288	terpinolene	-	1.1	0.3	0.5	0.3	0.7	-	0.4	0.2
20	1086	1551	linalool	0.9	2.2	11.8	0.3	0.7	0.1	0.4	1.0	0.3
21	1111	1565	<i>cis-p</i> -menth-2-en-1-ol	tr	-	0.2	-	0.3	-	-	-	-
22	1133	1574	isopulegol	-	1.5	-	-	-	-	-	-	-
23	1133	1483	citronellal	3.4	1.5	-	-	-	-	-	-	tr
24	1144	1567	isoneopulegol	-	1.0	-	-	-	-	-	-	-
25	1160	1672	cryptone	-	-	-	0.1	0.4	-	-	-	-
26	1163	1604	terpinen-4-ol	1.2	3.8	4.3	0.5	7.3	0.1	tr	0.1	0.1
27	1175	1699	$\alpha$ -terpineol	0.8	6.6	0.4	0.7	0.5	0.2	0.3	0.1	0.2
28	1173	1415	butyl hexanoate	-	-	-	-	-	-	1.5	-	-
29	1175	1417	hexyl butyrate	-	-	-	-	-	-	0.8	-	-
30	1199	1837	<i>trans</i> -carveol	0.2	-	-	tr	-	-	0.6	-	tr
31	1212	1769	citronellol	0.1	6.8	-	-	-	-	-	-	-
32	1217	1683	neral	-	-	-	-	-	-	-	-	0.5
33	1237	1851	geraniol	tr	1.0	0.1	-	-	-	-	-	-
34	1245	1753	geranial	-	-	-	-	-	-	-	-	0.6
35	1309	2275	limonene-1,2-diol	-	-	-	0.1	tr	-	0.6	-	-
36	1333	1697	$\alpha$ -terpinyl acetate	-	-	-	0.3	-	-	-	-	-
37	1334	1664	citronellyl acetate	-	3.1	-	-	-	-	-	-	-
38	1361	1759	geranyl acetate	1.1	2.1	-	tr	0.2	tr	-	-	0.1
39	1369	1611	hexyl hexanoate	0.1	-	-	-	-	-	0.5	-	-
40	1370	1614	butyl octanoate	-	-	-	-	-	-	0.8	-	-
41	1375	1492	$\alpha$ -copaene	0.3	0.2	0.4	-	-	-	0.1	-	tr
42	1387	1591	$\beta$ -elemene	0.2	0.2	0.4	-	tr	-	-	-	0.1
43	1410	1569	<i>cis</i> - $\alpha$ -bergamotene	-	-	-	-	-	-	0.3	-	tr
44	1417	1597	( <i>E</i> )- $\beta$ -caryophyllene	0.1	0.1	1.7	-	-	0.1	-	-	0.3
45	1417	1572	$\alpha$ -santalene	-	-	-	-	0.5	-	-	-	-
46	1432	1586	<i>trans</i> - $\alpha$ -bergamotene	-	-	-	-	-	-	3.2	-	0.6
47	1447	1667	( <i>E</i> )- $\beta$ -farnesene	-	-	-	0.2	-	-	1.8	0.1	tr

48	1469	1688	$\gamma$ -muurolene	-	-	-	-	-	-	3.1	-	-
49	1475	1708	germacrene D	-	0.2	0.3	-	0.1	0.7	-	-	0.6
50	1481	1718	$\beta$ -selinene	-	tr	-	-	-	-	2.0	-	-
51	1488	1718	valencene	-	-	-	0.1	-	-	2.7	-	-
52	1490	1723	$\alpha$ -selinene	tr	tr	0.1	-	-	-	0.7	-	-
53	1495	1750	( <i>E,E</i> )- $\alpha$ -farnesene	-	1.7	-	-	-	-	-	-	0.1
54	1500	1727	$\beta$ -bisabolene	-	-	-	-	-	-	18.4	-	0.9
55	1505	1758	$\gamma$ -cadinene	-	-	-	-	-	-	1.1	-	-
56	1513	1757	$\delta$ -cadinene	0.1	0.3	0.5	tr	-	-	-	-	0.1
57	1548	2043	( <i>E</i> )-nerolidol	-	tr	0.2	-	0.9	-	1.6	-	-
58	1550	1826	germacrene B	-	0.3	-	-	-	-	-	-	tr
59	1611	2254	alismol	-	0.3	0.1	-	-	-	1.0	-	0.1
60	1641	2229	intermedeol	-	-	-	-	-	-	4.7	-	-
Monoterpene hydrocarbon				88.2	63.7	76.9	96.7	87.7	98.7	42.8	98.7	94.3
Oxygenated monoterpene				8.5	30.1	18.6	2.0	9.4	0.4	1.9	1.2	2.0
Sesquiterpene hydrocarbon				0.7	3.2	3.4	0.3	0.6	0.7	33.4	0.1	2.6
Oxygenated sesquiterpene				0.0	0.3	0.3	0.0	0.9	0.0	7.3	0.0	0.1
Acyclic compound				0.1	0.0	0.0	0.3	0.0	0.0	6.0	0.0	0.0
TOTAL				97.5	97.3	99.3	99.3	98.6	99.9	91.5	100.0	98.9

Order of elution and relative percentages of individual components are given on an apolar column (BP-1) excepted those with an asterisk (\*) for which percentages were taken on polar column (BP-20); RI<sub>A</sub>, RI<sub>P</sub>: retention indices measured on apolar and polar capillary columns respectively; tr: trace level (<0.05%); *hys*: *C. hystrix*, *mic*: *C. micrantha*, *mapt*: *C. macroptera*, *lat*: *C. latipes*, *ich*: *C. ichangensis*, *jun*: *C. junos*, *maph*: *C. macrophylla*.

**Table 2.** Chemical composition of leaf essential oil of ten *Papeda* oil samples.

N°	RI <sub>A</sub>	RI <sub>P</sub>	Name	<i>hys</i>	<i>mic</i>	<i>mapt</i>	<i>ich-1</i>	<i>ich-2</i>	<i>ich-3</i>	<i>lat-1</i>	<i>lat-2</i>	<i>jun</i>	<i>maph</i>
1	923	1022	α-thujene	tr	-	0.3	-	tr	0.5	-	0.8	2.0	0.2
2	931	1020	α-pinene	0.1	-	2.0	tr	tr	2.2	0.1	2.0	4.8	0.5
3	965	1342	6-methyl-5-hepten-2-one	-	-	-	-	-	-	-	0.3	-	0.5
4	966	1127	sabinene	3.0	tr	32.4	-	tr	44.6	0.2	1.4	0.7	0.1
5	972	1116	β-pinene	0.5	0.1	15.7	-	tr	1.6	0.3	9.7	4.1	0.5
6	981	1166	myrcene	0.5	0.4	1.8	1.7	1.1	2.3	0.7	0.8	1.4	0.6
7	998	1170	α-phellandrene	-	-	0.2	0.1	0.1	1.3	-	0.3	1.7	-
8	1006	1153	δ <sup>3</sup> carene	tr	-	1.9	0.4	0.7	-	-	-	-	tr
9	1010	1185	α-terpinene	0.1	-	1.0	-	-	2.2	-	0.3	0.8	tr
10	1013	1276	<i>p</i> -cymene	tr	-	0.1	-	tr	0.2	0.2	5.1	11.4	4.3
11	1022	1215	β-phellandrene *	0.1	-	0.7	0.1	0.1	11.7	tr	3.4	11.2	-
12	1022	1205	limonene *	2.4	0.1	2.7	0.4	0.1	3.1	41.0	4.0	4.7	17.7
13	1026	1237	( <i>Z</i> )-β-ocimene	tr	0.1	1.8	13.0	18.2	0.7	0.1	0.1	0.2	0.3
14	1037	1255	( <i>E</i> )-β-ocimene	0.2	0.6	8.6	32.4	62.7	3.7	2.2	3.0	5.1	0.6
15	1049	1251	γ-terpinene	0.5	-	1.6	tr	-	3.5	0.1	19.5	28.2	6.2
16	1057	1467	<i>trans</i> -sabinene hydrate	tr	tr	0.2	-	-	0.6	-	-	tr	tr
17	1062	1446	<i>cis</i> -linalool oxide THF form	tr	tr	1.1	-	tr	0.2	0.1	tr	-	0.2
18	1073	1442	<i>p</i> -cymenene	-	-	-	-	tr	-	tr	-	6.2	-
19	1075	1474	<i>trans</i> -linalool oxide THF form	tr	-	0.6	-	tr	0.1	tr	-	-	0.1
20	1079	1288	terpinolene	0.1	tr	0.7	0.3	tr	0.8	tr	0.9	2.0	0.2
21	1086	1551	linalool	3.4	1.2	18.2	9.3	0.2	1.0	24.6	6.1	10.4	4.3
22	1087	1550	<i>cis</i> -sabinene hydrate	-	-	-	-	-	0.5	-	-	-	-
23	1111	1565	<i>cis-p</i> -menth-2-en-1-ol	-	-	0.2	-	-	0.5	-	-	-	-
24	1117	1375	allo-ocimene	-	-	-	0.4	0.4	-	-	-	-	-
25	1126	1630	<i>trans-p</i> -menth-2-en-1-ol	-	-	0.1	-	-	0.3	-	-	0.6	-
26	1133	1574	isopulegol	0.8	0.9	-	-	-	-	0.2	-	-	0.1
27	1133	1483	citronellal	78.1	76.1	-	-	-	0.3	14.1	1.0	-	3.5
28	1145	1567	isoneopulegol	0.3	0.3	-	-	-	-	tr	-	-	-
29	1159	UD	isogeranial	-	-	-	-	-	-	-	0.2	tr	0.4
30	1163	1604	terpinen-4-ol	0.3	-	3.8	tr	tr	8.4	-	0.4	0.2	0.2
31	1175	1699	α-terpineol	0.1	-	0.2	3.1	tr	0.3	0.1	0.3	0.1	0.6
32	1212	1769	citronellol	3.4	4.4	-	-	-	0.1	1.8	0.2	-	0.1
33	1212	1804	nerol	0.1	-	-	0.8	-	0.1	0.1	2.3	-	0.2
34	1215	1597	thymyl methyl oxide	-	-	-	-	-	-	-	-	0.3	-
35	1217	1683	neral	tr	-	-	-	-	0.1	0.8	11.6	-	18.9
36	1237	1851	geraniol	0.6	1.2	-	2.3	tr	0.3	0.1	0.4	-	0.6
37	1241	1560	linalyl acetate	-	-	-	10.8	-	-	0.1	-	-	0.2
38	1245	1753	geranial	0.1	-	-	-	tr	0.2	1.0	15.2	-	24.7
39	1268	2192	thymol	-	-	-	-	-	-	-	-	1.1	-
40	1303	1697	methyl geranate	-	-	-	-	-	0.7	-	-	-	-
41	1334	1664	citronellyl acetate	0.7	5.1	-	-	-	0.3	1.0	-	-	0.4
42	1335	1472	δ-elemene	-	0.3	-	-	-	-	-	0.3	0.1	0.1
43	1343	1728	neryl acetate	0.1	tr	-	1.2	-	0.2	0.1	1.1	-	0.5
44	1361	1759	geranyl acetate	1.2	2.9	-	2.4	-	5.0	0.1	0.1	-	2.1
45	1375	1492	α-copaene	0.2	0.3	-	0.1	tr	-	tr	-	-	0.1
46	1387	1591	β-elemene	tr	0.5	-	-	tr	-	tr	0.3	tr	0.3

47	1399	1872	2,5-dimethoxy-para-cymene	-	-	-	-	-	-	-	-	1.4	-
48	1417	1597	(E)- $\beta$ -caryophyllene	1.1	0.8	0.5	1.4	-	-	2.9	0.1	0.1	2.7
49	1427	1638	$\gamma$ -elemene	tr	-	-	-	0.3	-	-	-	tr	-
50	1432	1586	trans- $\alpha$ -bergamotene	0.1	-	-	0.1	0.1	-	1.1	-	-	0.6
51	1447	1667	(E)- $\beta$ -farnesene	-	0.1	-	0.1	0.3	-	-	tr	tr	tr
52	1449	1667	$\alpha$ -humulene	0.1	0.1	0.1	0.2	0.2	-	0.2	0.1	tr	0.3
53	1469	1688	$\gamma$ -muurolene	-	0.1	-	0.2	0.3	-	-	-	-	tr
54	1471	1668	guaia-6,10(14)-diene	-	-	-	0.3	0.4	-	-	-	-	-
55	1475	1708	germacrene D	0.1	0.2	-	-	-	0.1	0.6	1.2	tr	0.9
56	1481	1718	$\beta$ -selinene	-	-	-	0.4	0.5	-	tr	-	-	tr
57	1490	1723	$\alpha$ -selinene	-	-	-	tr	0.3	-	-	-	-	-
58	1490	1732	bicyclogermacrene	0.3	0.2	0.4	-	-	0.1	0.3	0.1	0.1	0.4
59	1495	1750	(E,E)- $\alpha$ -farnesene	0.2	0.8	0.3	-	0.4	0.1	-	-	-	0.2
60	1500	1727	$\beta$ -bisabolene	0.1	-	-	0.7	1.2	-	1.5	0.1	-	0.9
61	1513	1757	$\delta$ -cadinene	0.3	0.3	0.1	0.5	0.3	-	tr	-	tr	0.2
62	1534	2079	$\beta$ -elemol	tr	0.1	-	-	-	-	-	1.1	-	-
63	1548	2043	(E)-nerolidol	0.2	tr	0.3	0.5	0.6	1.3	tr	0.3	tr	tr
64	1549	1825	germacrene B	-	0.5	-	0.4	0.6	tr	-	0.4	tr	0.2
65	1563	2121	spathulenol	-	-	0.1	0.1	tr	0.1	0.1	0.1	-	0.7
66	1570	1978	caryophyllene oxide	tr	-	-	1.8	-	-	0.2	-	-	0.4
67	1592	2033	humulene oxide II	-	-	-	0.3	0.2	-	-	-	-	tr
68	1611	2254	alismol	-	0.1	-	1.7	1.6	0.1	-	0.4	-	0.3
69	1616	2197	eremoligenol	-	-	-	0.4	-	-	0.1	0.3	-	-
70	1618	2176	$\gamma$ -eudesmol	-	tr	-	0.1	-	-	tr	0.6	-	-
71	1625	2169	$\tau$ -cadinol	-	-	0.1	0.3	0.1	-	-	0.2	tr	0.1
72	1634	2225	$\beta$ -eudesmol	-	-	-	0.7	0.8	-	0.2	0.6	tr	-
73	1639	2216	$\alpha$ -eudesmol	-	-	-	1.1	0.1	-	tr	0.5	-	-
74	1651	2145	$\beta$ -bisabolol	-	-	-	0.4	-	-	-	-	-	-
75	1668	2215	$\alpha$ -bisabolol	-	-	-	0.4	0.5	-	0.1	-	-	0.1
76	2098	2610	(E)-phytol	-	0.3	0.7	3.0	1.4	tr	1.1	0.2	0.1	0.5
Monoterpene hydrocarbon				7.4	1.3	71.5	48.6	83.4	78.3	44.9	51.2	84.4	31.1
Oxygenated monoterpene				88.9	92.2	24.4	30.0	0.2	19.1	44.1	39.1	14.1	57.2
Sesquiterpene hydrocarbon				2.5	4.2	1.3	4.3	4.8	0.3	6.6	2.4	0.3	7.0
Oxygenated sesquiterpene				0.2	0.2	0.6	7.7	3.9	1.5	0.6	4.1	0.0	1.5
Oxygenated diterpene				0.0	0.3	0.7	3.0	1.4	0.0	1.1	0.2	0.1	0.5
TOTAL				99.0	98.3	98.5	93.6	93.8	99.3	97.2	97.1	98.9	97.3
Yields (% ; w/w)				0.05	0.02	0.02	0.18	0.17	0.08	0.08	0.03	0.04	0.10

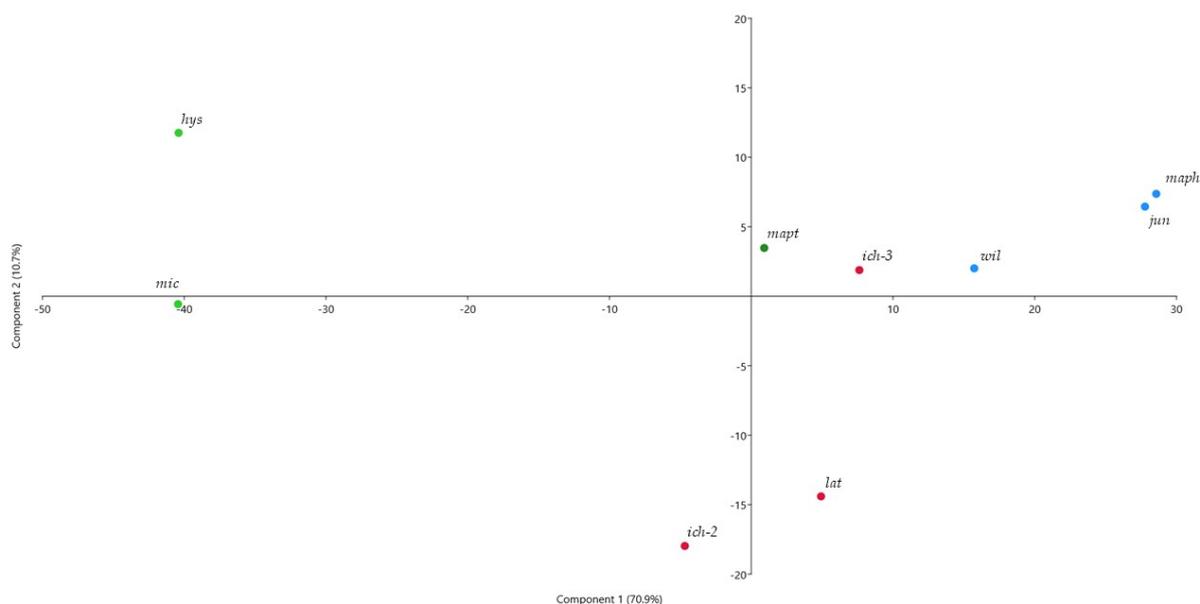
Order of elution and relative percentages of individual components are given on an apolar column (BP-1) excepted those with an asterisk (\*) for which percentages were taken on polar column (BP-20); RI<sub>A</sub>, RI<sub>P</sub>: retention indices measured on apolar and polar capillary columns respectively; tr: trace level (<0.05%); *hys*: *C. hystrix*, *mic*: *C. micrantha*, *mapt*: *C. macroptera*, *lat*: *C. latipes*, *ich*: *C. ichangensis*, *jun*: *C. junos*, *maph*: *C. macrophylla*.

**Table 3.** List of studied species and accessions.

Scientific name	Common name	Sample	ICVN
<i>C. hystrix</i> DC.	Combava	<i>hys</i>	0100630
<i>C. macroptera</i> Montr.	Melanesian	<i>mapt</i>	0100686
<i>C. micrantha</i> Wester	Biasong	<i>mic</i>	0101140
<i>C. ichangensis</i> Swingle	Ichang papeda	<i>ich-1</i>	0100687
		<i>ich-2</i>	0110241
		<i>ich-3</i>	0110240
<i>C. latipes</i> (Swingle)	Khasi papeda	<i>lat-1</i>	0110243
		<i>lat-2</i>	0100844
<i>C. junos</i> Siebold ex	Yuzu	<i>jun</i>	0100988
<i>C. macrophylla</i> Wester	Alemow, Kolo	<i>maph</i>	0110058

ICVN: International Citrus Varietal Numbering

**Figure 1.** Principal Component Analysis performed on peel oil samples (components higher than 2%). Green: *hys*: *C. hystrix*, *mic*: *C. micrantha*, *mapt*: *C. macroptera*; Red: *lat*: *C. latipes*, *ich*: *C. ichangensis*; Blue: *wil*: *C. wilsonii*, *jun*: *C. junos*, *maph*: *C. macrophylla*.



**Figure 2.** Three-dimensional Principal Component Analysis of leaf oil samples (components higher than 2%). Green: *hys*: *C. hystrix*, *mic*: *C. micrantha*, *mapt*: *C. macroptera*; Red: *lat*: *C. latipes*, *ich*: *C. ichangensis*; Blue: *wil*: *C. wilsonii*, *jun*: *C. junos*, *maph*: *C. macrophylla*.

