



Bitter orange peel essential oil: a review of the different factors and chemical reactions influencing its composition

Marion Gaff, Martine Esteban-Decloux, Pierre Giampaoli

► To cite this version:

Marion Gaff, Martine Esteban-Decloux, Pierre Giampaoli. Bitter orange peel essential oil: a review of the different factors and chemical reactions influencing its composition. *Flavour and Fragrance Journal*, 2020, 35 (3), pp.247-269. 10.1002/ffj.3570 . hal-03667282

HAL Id: hal-03667282

<https://agroparistech.hal.science/hal-03667282>

Submitted on 13 May 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Bitter orange peel essential oil: a review of the different factors and chemical reactions influencing its composition

Journal:	<i>Flavour and Fragrance Journal</i>
Manuscript ID	Draft
Wiley - Manuscript type:	Review
Date Submitted by the Author:	n/a
Complete List of Authors:	Gaff, Marion; AgroParisTech, INRA, Université Paris-Saclay, Ingénierie Procédés Aliments Decloux, Martine; AgroParisTech, INRA, Université Paris-Saclay, Ingénierie Procédés Aliments Giampaoli, Pierre; AgroParisTech, INRA, Université Paris-Saclay, Ingénierie Procédés Aliments
Keywords:	bitter orange peel, Citrus aurantium L., essential oil, extraction method, ripening stage

SCHOLARONE™
Manuscripts

Bitter orange peel essential oil: a review of the different factors and chemical reactions influencing its composition

Short title: Composition of bitter orange peel essential oil: a review

Marion GAFF^a, Martine ESTEBAN-DECLoux^a, Pierre GIAMPAOLI^{a,*}

^a Unité Mixte de Recherche Ingénierie Procédés Aliments, AgroParisTech, INRA, Université Paris-Saclay, F-91300 Massy, France.

* Corresponding author:

AgroParisTech. 1 av des Olympiades 91300 Massy, France

pierre.giampaoli@agroparistech.fr

Tel : +33 1 69 93 50 23

Abstract

This review summarizes the findings and discusses the results on the different factors that can influence the bitter orange peel oil composition. Small changes in composition can result in noticeable modifications of the odour profile of the essential oil. The analysis method appears to have a crucial effect on the number of compounds identified in the essential oil. Growing location, cultivar, ripening stage, storage conditions, and extraction methods are reported to impact the bitter orange essential oil. Bitter orange peel oil should be stored at -21°C to prevent changes in the composition and formation of artefacts when storing for a period of several months. The influence of cultivars should not be neglected, since a few cultivars differ a lot from the main cluster of *Citrus aurantium* L.. The geranyl pyrophosphate forms most of the terpenes present in the bitter orange, especially limonene which is the majority compound, by the action of the terpene synthases during growing of the fruit. The extraction method can also be responsible for some differences in the composition due to the absence of water-soluble compounds in cold-pressed oils and to acid-catalysed reactions in hydrodistilled oils such as the decomposition of the linalyl acetate forming many different terpene derivatives.

Keywords:

bitter orange peel; *Citrus aurantium* L.; essential oil, extraction method, ripening stage

1. INTRODUCTION

Bitter oranges, also called sour oranges or bigarades, belong to the genus *Citrus* in the family Rutaceae. The bitter orange tree was used as the main rootstock for citrus until the tristeza disease appeared.¹ The fruits from bitter orange tree are cultivated for the preparation of marmalade,^{1,2} and peels, leaves and flowers are used for the extraction of essential oil.

The major part of the production of bitter orange peel oil is used in perfumery, combined with other natural or synthetic aromas. The rest is used in pharmacology and aromatherapy, in the cosmetic and detergent industry as a fragrance component, but also in food industry as a flavouring agent. For instance, it can be used as an aroma for soft and alcoholic beverages.³ It was also reported that bitter orange peel oil can be used for its antioxidant properties^{4,5} and its antimicrobial activity.⁶

Essential oils (EO) are mostly obtained by hydrodistillation.⁷ Citrus essential oils are an exception, because on an industrial scale they are obtained by cold pressing. Nevertheless, many authors studied bitter orange peel oils extracted by hydrodistillation on a laboratory scale.

In 1991, Boelens⁸ published a review on the chemical composition of citrus oils, which listed only six articles dealing with bitter orange peel oils. Boelens can be acknowledged for highlighting that some erroneous data were published by Shaw,⁹ Lawrence,¹⁰ and Swaine and Swaine¹¹ due to the misunderstanding of publications originally from Ortiz *et al.*¹² and Maekawa *et al.*¹³ Since then, numerous articles have been published on the composition of bitter orange peel oil, and thanks to the advances in analytical chemistry many more volatile compounds have been identified. Many of these articles try to assess the influence of different factors on the bitter orange oil composition, such as the growing location, the cultivars, the ripening stages, the storage conditions and the extraction methods. The aim of the present review is to confront all the conclusions that were drawn on the subject and to explain the chemical or botanical mechanisms behind those changes in the composition of the bitter orange peel oil.

2. BITTER ORANGE TAXONOMY

The most common taxonomic systems (i.e. botanical classification) used for the genus *Citrus* are the Tanaka system¹⁴ which lists 162 species and the Swingle system¹⁵ which lists only 16 species.¹⁶ These two systems do not always classify citrus in the same way because of the ability of the *Citrus* species to hybridize easily and create interspecific hybrids that can be considered as new species.¹⁷

According to Tanaka system, bitter oranges are classified into four botanical species: *Citrus aurantium* L. which has a wide number of cultivars (i.e. cultivated varieties), and *Citrus myrtifolia* Raf., *Citrus natsudaoidai* Hay., and *Citrus neo-aurantium* Tan., which have a very small number of cultivars (Table 1). In total, there are more than 80 cultivars of bitter orange. All the cultivars of *Citrus myrtifolia* Raf., *Citrus natsudaoidai* Hay., and *Citrus neo-aurantium* Tan belonging to bitter oranges are listed in Table 1, while only a few examples of cultivars of *Citrus aurantium* L. are given.

3. GENERAL COMPOSITION OF BITTER ORANGE ESSENTIAL OIL

Bitter orange peel oils, like other citrus peel oils, are composed of two fractions: a non-volatile fraction and a volatile fraction.

The non-volatile fraction (1% to 15% of the essential oil) is constituted of waxes, oxygen heterocyclic compounds (coumarins, psoralens, polymethoxylated flavones), fatty acids, hydrocarbons, sterols (also known as steroid alcohols), and carotenoids.^{18,19} Obviously, the non-volatile residue is almost absent in oils obtained by hydrodistillation.^{19,20}

The volatile fraction of bitter orange essential oil, which represents 85% to 99% of the EO, is constituted of hydrocarbon monoterpenes and sesquiterpenes as well as oxygenated compounds derived from the following hydrocarbons: alcohols, aldehydes, ketones, esters, ethers and oxides.⁷ All the scientific papers agree on the fact that the majority component of bitter orange EO is limonene.

According to the ISO / FDIS 9844 standard requirements²¹ the volatile composition of the cold-pressed essential oil of *Citrus aurantium* L. should follow what is stated in Table 2 column 3. According to the ISO standard 9844, the bitter orange essential oil contains at least the 10 following volatile compounds: α -pinene, β -pinene, myrcene, limonene, linalool, linalyl acetate, geranyl acetate, β -caryophyllene, germacrene D, (*E*)- β -nerolidol.²¹ For those 10 volatile compounds the quantity is expressed as a relative percentage of the total peak area. Octanal and decanal are also mentioned as compounds potentially present in bitter orange oil, which means that all in all only 12 volatile compounds are mentioned as components of bitter orange oil in ISO standard 9844.²¹ The non-volatile compounds are not mentioned. Some analysis conditions of the essential oil are given in the ISO standard 9844 for quick and easy quality control, but they are not normative.

But actually, the composition of the bitter orange oil reported in all the studies tends to show that many more compounds can be found. Most of the authors identified more than 20 volatile compounds (Table 2, Table 3, Table 4, Table 5 and Table 7), with a maximum identification of 64 volatile compounds by Dugo *et al.*¹⁹

4. FACTORS INFLUENCING THE COMPOSITION OF THE VOLATILE FRACTION OF THE BITTER ORANGE PEEL OIL

The different factors that can influence the bitter orange oil composition are the growing locations (i.e. geographic origin), the cultivars, the ripening stages, the storage conditions, the extraction methods.²² The influence of the rootstock and the plant age are not detailed in this article, but they might as well impact the essential oil composition as indicated by Boussaada and Chemli²³ and Kale and Adsule². Nevertheless, no literature was found on the subject as far as bitter orange peel oil is concerned.

4.1 Growing location

The world major producing countries of bitter orange oil are Brazil, Ivory Coast, Spain, Dominican Republic, Italy, Argentina, Haiti, France, Egypt, Tunisia, Morocco, Uganda,

Paraguay, and China.^{24,25} The different growing locations of bitter orange EO that were studied in the literature are: Brazil,^{26,27} China,²⁸ Cuba,²⁹ Egypt,³⁰ France,^{26,31} Greece,^{5,26,32} Iran,^{7,33,34} Italy,^{6,35–40} Ivory Coast,⁴¹ Japan,^{42–46} Pakistan,¹⁸ Spain,^{35,47–49} Tunisia,^{4,23,50,51} Turkey,^{52,53} United States,¹² Venezuela,⁵⁴ West Indies,⁵⁵ and several from unspecified geographical origins.^{23,56–58} Even if Argentina, Haiti, Morocco, Uganda, and Paraguay are in the top fifteen of the world major producing countries of bitter orange oil,^{24,25} to our knowledge the composition of the bitter orange peel cultivated in those countries has never been studied in the literature. Actually, the composition of a bitter orange essential oil from Argentina was reported by De La Torre and Sardi⁵⁹, but as stated by Dugo *et al.*⁶⁰ the composition is too different from a bitter orange oil to be genuine. Abderrezak *et al.*⁶¹ reported the analysis of a bitter orange peel essential oil from Algeria, but 0.25% limonene is not compatible with the chemotype of a bitter orange peel oil. Thus, these two studies were not taken into account in the present review.

Some authors tried to compare bitter orange peel oils from different origins using previous studies of different authors.^{41,52} It can be very tricky and conclusions should be taken with cautiousness because many factors that could influence the bitter orange oil composition are unknown. As a consequence, when combining results from different authors, the growing location may not be the only parameter under review.

For instance, Dugo⁴¹ compared the composition of bitter orange peel oils from Italy, Spain, Ivory Coast and Brazil, but the cultivars were not specified for the Italian, the Ivorian, and the Brazilian EO, whereas Lota *et al.*³¹ highlighted that the cultivar can influence a lot the composition of bitter orange peel oil. Moreover, the differences in the percentages of linalool and linalyl acetate were attributed to the different growing locations whereas these two percentages are more likely to be representative of the ripening stage of the bitter oranges (which are not specified) than of their growing location. Indeed, linalyl acetate and linalool drastically decrease when citrus are ripening.⁶² Finally, designating more precise locations would be more relevant, as it would be hard to conclude on the influence of a growing location as big as Brazil.

Boussaada *et al.*²⁶ compared the composition of bitter orange peel oils from Chania (Greece), Roxani (Greece), Corsica (France), and Brazil. They mentioned being cautious in comparing the essential oil from trees of approximately the same age, disease-free and submitted to the same horticultural conditions. Nevertheless, none of the cultivars was specified. And once more, the name of the location where the trees were grown in Brazil, was not stated.

Kirbaslar and Kirbaslar⁵² compared the volatile composition of their Turkish EO with the Spanish and Italian EO of Boelens and Jimenez.³⁵ They concluded that percentages of nonanal, tetradecanal, myrtenol, neral, nootkatone and octanol were higher than 0.01% in Italian and Spanish oils, whereas these compounds were not detected in Turkish oil. Actually, the fact that fewer compounds were detected could possibly be due to an analysis method with a more important dilution of the EO. Indeed, Kirbaslar and Kirbaslar⁵² did a split injection of 1 µL of Turkish EO diluted 1/20 in hexane (no mention of the split ratio used, neither if the dilution was by weight or by volume), and they identified between 23 and 27 compounds in their samples, which is rather low compared to other studies (Tables 4, 5 and 7).

Abderrezak et al.⁶¹ claimed that they did a comparative study of the compositions of essential oils of citrus growing in different countries, but they did not take into account the extraction method used by the different authors and compared cold-pressed oils with hydrodistilled oils. Not to mention that the cultivars and the analysis methods were also different between the EO.

Boelens and Jimenez³⁵ are the only authors who accurately assessed the impact of growing location on the bitter orange EO composition, using bitter oranges of the same cultivar, the same year of harvest, with a similar extraction method (cold-pressing) and the same chromatographic method. Though, there were a few errors in this article in the table listing the chemical composition of Mediterranean orange oils: (1) octanol and decanol as well as octyl acetate and decyl acetate were classified in aliphatic aldehydes, hopefully not counted in this class, but a separate class for aliphatic alcohols and esters should have been created, (2) the total of aliphatic aldehydes of bitter orange oil from Italy is 0.631, not 0.531, (3) the total of monoterpenes of bitter orange oil from Spain is 97.406, not 96.85, (4) the total of monoterpenes of bitter orange oil from Italy is 96.873, not 96.83. The conclusions drawn by these authors were that α -pinene, β -pinene, sabinene, aldehydes, and monoterpenoid alcohols and esters were more concentrated in Sicilian bitter orange oil than in Andalucian oil, whereas limonene, sesquiterpenes, nerolidol and nootkatone were less concentrated. These conclusions are based on the analysis of only one sample of each growing location. More studies on the impact of growing location on the bitter orange EO composition would be needed to be able to affirm that these conclusions could be generalized to the influence of coastal climate (Sicilian) and desert-like climate (Andalucian).

Thus, not much can be concluded for sure on the influence of the country in which bitter orange trees are grown on the composition of the essential oil.

4.2 Cultivars

The study by Ortiz *et al.*¹² was meant to compare essential oils obtained by the same laboratory extraction method, from numerous cultivars of bitter orange harvested in the same period (June-July), all cultivated in the Citrus Research Centre at Riverside in California.

Ortiz *et al.*¹² are not the only authors who rigorously examined the influence of the cultivar on the volatile composition of the bitter orange peel oil. More recently, Lota *et al.*³¹ compared the composition of 30 different cultivars of bitter orange EO while making sure that the growing location (Corse, France), the cultural conditions, as well as the laboratory extraction method were the same, so that the only parameter under review during the study was the cultivar. They drew the conclusion that the 26 cultivars of *Citrus aurantium* L. that they studied exhibited a weak chemical variability, except for the Espagne and the Gou Tou cultivar, and were characterized by limonene as majority component. Actually, limonene was also the majority component for the Espagne and the Gou Tou cultivar, but to a far less extent (72.9% peak area for the Espagne cultivar and 84.2% peak area for the Gou Tou cultivar versus between 87.6% to 95.1% peak area for the 24 other *Citrus aurantium* L. cultivars). The fact that the peel EO of the Gou Tou cultivar differed from most of the *Citrus aurantium* L. is probably linked to its supposed natural hybrid origin (possible hybrid between *Citrus*

aurantium L. and *Citrus grandis*).^{63,64} The peel EO of the Tosu cultivar belonging to the species *Citrus neo-aurantium* Tan. was similar to the Gou Tou cultivar: a lower percentage of limonene and a higher percentage of β -pinene than the group of 24 cultivars of *Citrus aurantium* L.. As far as *Citrus myrtifolia* Raf. is concerned, the peel EO of the Large Chinotto cultivar had a profile similar to the 24 cultivars of *Citrus aurantium* L.. The peel EO of the monovarietal *Citrus natsudaoidai* Hay. also had a profile close to the 24 cultivars of *Citrus aurantium* L., despite the fact that it does not contain α -pinene.

4.3 Ripening stages

Several studies concerning the influence of the ripening stages of bitter oranges on peel oil composition have been reported in the literature.^{7,30,33,39,51,56,65} Citrus are non climacteric, which means that once fruits are harvested, the maturation process stops and they do not ripen further.¹ The colour of the peel is an indicator of the maturity of the fruit. When the bitter orange is immature the peel is dark green. As it ripens, it passes through light green, then yellow, and finally orange at the mature stage. The evolution of the gas chromatography percentages of the compounds during the growing and the ripening of bitter oranges are listed in Table 2. Only the compounds listed in the ISO standard 9844 are taken into account.

First of all, one should be careful with the wording chosen by the authors of the studies dealing with ripening stages, when in need to compare those results. When Ashour and El-Kebeer³⁰ referred to immature fruits, they meant fruits which have reached the size of a bitter orange but were still green in colour, whereas when Rowshan and Najafian⁷ referred to immature fruits, they meant green fruits of the size of a lemon, that is to say smaller than a bitter orange. Thus, the immature stage of Ashour and El-Kebeer³⁰ seems to be equivalent to the semi-mature stage of Rowshan and Najafian⁷. Moreover, the size criteria chosen by Rowshan and Najafian⁷ and by Ashour and El-Kebeer³⁰ to determine maturity were rather imprecise since no real measurement was provided. Ashour and El-Kebeer³⁰ called setting fruits, green fruits that were really small in size. According to the analysis results of the setting fruits, it can be assumed that setting fruits were even less mature than the immature stage stated by Rowshan and Najafian⁷. Azhdarzadeh and Hojjati³³ compared unripe peels and ripe peels with no further details on the maturity stage (colour, size of the fruits, etc...). From the analysis results of the unripe peels, one can hypothesize that they were slightly more ripe than the setting fruits of Ashour and El-Kebeer³⁰ and less ripe than the immature fruits of Rowshan and Najafian⁷. Bourgou *et al.*⁵¹ defined the immature, semi-mature and mature fruits by their colour, just as Dugo *et al.*³⁹ used the colour to define ripe and unripe fruits. Once more, neither of them gave any details on the precise colour, which could have been measured with a colorimeter for instance, nor on the size measurement of the fruit.

Boussaada and Chemli⁶⁵ found that the percentage of limonene rises at its maximum in the middle of the productive period when fruits are normally harvested, and then decreases in the end of the productive period to arrive at a percentage that is approximately the same as in the beginning (Table 2). Rowshan and Najafian⁷ observed that the highest percentage of limonene was obtained for the semi-mature stage (green colour, size of a bitter orange) and slightly decrease at the mature stage (orange colour). The lowest percentage of limonene is at the immature stage (green colour, size of a lemon). Ashour and El-Kebeer³⁰ found that during the

ripening of the fruits (from immature to ripening fruits, no changing in size but changing of colour), the percentages of limonene slightly decreased from 95.25% to 92.68%. The different authors, except Bourgou *et al.*⁵¹, agree on the fact that limonene is formed during the growing of the bitter orange and slightly decreases during the ripening of the bitter orange. Actually, the analysis results of the semi-mature bitter orange peel obtained by Bourgou *et al.*⁵¹ seem suspect: limonene percentage of only 67.90% and products of hydration and oxidation percentages (1,8-cineole, (Z)-sabinene hydrate, octanol, (Z)-linalool oxide, terpinen-4-ol) higher than at the immature and the mature stage.

The compositions of unripe and ripe bitter orange peel obtained by Dugo *et al.*³⁹ are very close to one another, apart from linalyl acetate, (E)- β -ocimene, (E) and (Z)-linalool oxide which are higher in unripe peel.

In Boussaada and Chemli⁶⁵ study, the linalool percentage gradually decreases along the productive period. Rowshan and Najafian⁷ noticed that linalool percentage was maximum at the immature stage and deeply decreases as the fruit grows and ripens. It is suggested that limonene is formed during growing by decomposition of the linalool and the linalyl acetate.^{47,65}

Ashour and El-Kebeer³⁰ noticed that during growing of bitter oranges (from setting fruits, which are really small in size, to immature fruits, which reached the size of the bitter orange but are still dark green in colour) β -pinene, ocimene, linalool, linalyl acetate, and α -terpineol decreased, while limonene increased from 46.19% to 95.25%. Ocimene and linalool were not present any more in the ripening fruit.³⁰

As far as β -pinene is concerned, Rowshan and Najafian⁷ came to the same conclusion as Ashour and El-Kebeer³⁰: β -pinene decreased during growing. But contrary to Ashour and El-Kebeer³⁰, Rowshan and Najafian⁷ found that α -pinene and myrcene decreased during growing.

Boussaada and Chemli⁶⁵ concluded that β -pinene and α -terpinolene percentages showed a slight increase throughout the productive season. But, is this slight increase (from 0.2% to 0.3% and from 0.4% to 0.6% respectively) sufficient to be significant? The percentages of the other monoterpenes were considered as being constant from unripe to ripe citrus oil. α -terpineol, geraniol, nerol and geranyl acetate percentages were said to be fairly constant in all the oils from unripe and ripe fruits. Actually, geraniol varies from 0.1% to 0.2% and geranyl acetate from 0.2% to 0.3%. Boelens and Jimenez⁵⁶ also came to the conclusion that α -terpineol, geraniol, and nerol were fairly constant during the ripening of bitter oranges, whereas geranyl acetate and neryl acetate increased from 0.06% to 0.13% and from 0.02% to 0.04% respectively.

As far as octanal and decanal are concerned, Azhdarzadeh and Hojjati³³, Ashour and El-Kebeer³⁰, Boelens and Jimenez⁵⁶, Rowshan and Najafian⁷, as well as Dugo *et al.*³⁹ to a lesser extent, all agree that these two aldehydes increase during the ripening of bitter oranges.

Boelens and Jimenez⁵⁶ pointed out that the oxygen-containing sesquiterpenes (nerolidol, farnesol, nootkatone and α -selinenone) appear to be formed during the ripening.

All those authors drew conclusions on the evolution of the peel oil composition during ripening based on comparisons of relative percentages of peak area. None of them quantified using external or internal standard, or standard additions.

4.4 Storage conditions

Boelens and Jimenez⁵⁶ studied the impact of one to two weeks storage on picked ripe fruits. They observed that linalool, linalyl acetate, nerolidol, nootkatone and α -selinenone kept on increasing after picking up the fruits, and attributed the formation of these compounds to enzymatic reactions. In plants, endogenous glycosidases are involved in enzymatic hydrolysis of the glycosidic bond of glycosidic aroma precursors, releasing free aroma compounds.⁶⁶

Njoroge *et al.*⁴³ studied the impact of storage conditions (temperature and time of storage) on the volatile composition of Daidai (*Citrus aurantium* L. var. *cyathifera* Y. Tanaka) cold-pressed peel oil (Table 3). Artefact compounds may be formed during storage of essential oils upon oxidation, heat or chemical interactions between the compounds that are initially present in the oil.⁴³ No changes were found over time when cold-pressed Daidai oil was stored at -21°C. Changes occurred in the Daidai oil stored several months at 5°C and 20°C. The longer the oil was stored, the more important the changes in the composition were. Limonene, myrcene, γ -terpinene and linalyl acetate percentages decreased when the temperature and the time of storage increased. The decreases of myrcene and γ -terpinene were observed when stored at 20°C or when stored 12 months at 5°C (Table 3). Under the same conditions, terpinolene, *p*-cymene, α -terpineol, thujol and octyl acetate slightly increased, while (*Z*)-carveol, limonene-10-yl acetate, (*E*)-*p*-mentha-2,8-dien-1-ol, linalool, nonyl acetate, decyl acetate, β -caryophyllene, (*E*)- β -farnesene, α -humulene sharply increased. Germacrene D decreased until disappearing. Some compounds that did not exist in the fresh oil were formed upon storage: carvone, sabinene hydrate, (*Z,E*)-farnesol, and several oxygen derivatives of the farnesene group. Their relative percentages increased with time and temperature. Surprisingly neither linalool oxides nor limonene oxides were identified in the oils after long storage periods.

4.5 Extraction methods

In the literature, the extraction methods used to obtain peel oil from citrus are: cold pressing, hydrodistillation, supercritical carbon dioxide⁵⁷ or solvent⁶⁷ extraction. The most common methods are cold pressing and hydrodistillation, and the result of extraction is not called essential oil when using supercritical carbon dioxide or solvent extraction.

4.5.1 Cold pressing

On an industrial scale, bitter orange essential oils are obtained by cold pressing. All the different mechanical systems for cold extraction of citrus oils are based on the following steps.^{68,69} First, mechanical action on fruits or on peels breaks essential oil glands (utricles) and releases the EO. Then, the citrus oil is dispersed in water which acts as a vehicle agent. In most cases, the aqueous phase is continuously recycled to minimize the loss of oxygenated compounds. Finally, the emulsion is centrifuged to obtain pure EO. The water-soluble compounds are lost. The different methods are Sfumatrice, Torchi, Pelatrice, Brown Oil

Extractor and FMC (Food Machinery Corporation, now called JBT).⁷⁰ Sfumatrice machines work on peels without pulp; now these are not widely used and are replaced by Torchi, which work on the entire fruits. Pelatrice machines work on the entire fruit, and the EO is obtained by rolling the fruit over spikes. The Brown Oil Extractor is quite similar to the Pelatrice method.⁷¹ FMC machines also process the entire fruit: the extraction of juice and EO are performed simultaneously. The aqueous phase that carries the EO is easily recycled when oil is produced using the Pelatrice and FMC processes. The ability to recycle the aqueous phase is more difficult for Sfumatrice and Torchi machines because of the rapid increase of pectins in the aqueous phase.^{41,72}

On a laboratory scale, three different types of cold extraction were found in the bibliography. (1) Lota⁷³ used a semi-automatic method (« Roze-Schwob ») consisting of two jaws that rotate the bitter orange and a sharp point which breaks the oils glands.³ The dispersion that was released (mix of EO and aqueous phase) was then centrifuged to obtain pure EO. (2) Kirbaslar and Kirbaslar,⁵² Njoroge *et al.*,^{42,43} and Sawamura⁴⁵ applied manual pressure on the flavedo to release the oil. Njoroge *et al.*^{42,43} added brine to the oil. Then, the oil was centrifuged and dried over anhydrous sodium sulphate. (3) Boelens and Jimenez^{35,56} extracted the peel oil by needle puncturing followed by centrifugation.

Tables 4 and 5 compare the volatile composition of bitter orange essential oils obtained by cold pressing on an industrial scale and a laboratory scale respectively. The compositions reported in table 4 and 5 are not exhaustive since some authors found more than 50 volatiles, but focused on the compounds that are found in bitter orange EO according to the ISO standard.

Among all the gas chromatographic analysis results of cold-pressed bitter orange oil that can be found in the literature, the only ones which are in accordance with the ISO standard 9844 are Dugo *et al.*^{19,36} and Mondello *et al.*^{37,40} Veriotti and Sacks⁵⁸ are the ones who identified the lowest number of compounds in cold-pressed bitter orange oil. The chromatographic method used is to blame: the lowest volume injected and the highest split of all the studies on bitter orange, when specified (Table 4).

4.5.2 Hydrodistillation

Hydrodistillation is used to extract bitter orange essential oil on a laboratory scale for analysis and quantification of oil contained in the peels. To our knowledge, all the bitter orange hydrodistilled oils were obtained using a Clevenger type apparatus. The Table 6 gives exhaustive details on the different methods used to obtain bitter orange essential oil with a Clevenger apparatus.^{5-7,18,23,26,32,50,54,65}

In general, authors give very few information on the methods employed for peel hydrodistillation. Gorgorcena and Ortiz⁴⁹ also used hydrodistillation to extract essential oil from bitter orange but they were not recorded in table 6 because no details are given on the method. The study by Gonzalez de *et al.*⁵⁴ is quite terse. The methods used are not detailed and only seven compounds were identified in this Venezuelan oil, but this can probably be attributed to the analysis method not being adapted since very few compounds were also found in the other *Citrus* species of this study. The essential oils can be obtained by

hydrodistillation using dried or fresh peels. The mass of bitter orange peel used for one extraction varies quite a lot: from 35 g of fresh peels to 100 g of dried peels. The time of hydrodistillation varies from 1.5 hours⁴ to 6 hours.¹⁸ The only article which specifies the distillate flow rate (3 to 3.5 mL/min) is Sarrou *et al.*⁵ It can be assumed that the extractions by Protopapadakis and Papanikolaou³² and Rowshan and Najafian⁷ were done with a distillate flow rate of 2 to 3 mL/min, since these authors claim that they followed the method described in the European Pharmacopoeia⁷⁴. And, when specified, after extraction the essential oil is dried over Na₂SO₄ anhydrous and stored in sealed vials between -20°C and 6°C.

To compare the yields expressed in different units in the articles, they were all converted in g/100g dry matter using values found in the literature. Dried bitter orange peels contain 90% dry matter and fresh bitter orange peels contain 30% dry matter. According to the ISO standard 9844, the bitter orange essential oil density ranges from 0.84 to 0.86 g/mL, so the density of the different oils obtained was considered as being 0.85 g/mL. The yields obtained vary from 0.21 to 1.12 g/100g dry matter (Table 6). These important differences in yield obtained could be due to different factors: (1) part of the essential oil may be lost during drying of peel, (2) the mass of peel from which the oil is extracted is too small to scale up (concerning Sarrou *et al.*⁵), (3) the time of hydrodistillation is too short to extract the whole quantity of essential oil contained in the peels, (4) the cultivars of bitter oranges are different, (5) the ripening stage may be different, (6) the growing location is different.

Concerning Azhdarzadeh and Hojjati³³, Boussaada and Chemli^{23,65}, Boussaada *et al.*²⁶ and Bourgou *et al.*⁵¹, the yields given in the article were expressed for 100 g of fresh peel but it was not specified if the figures given were in mL or in g. Thus, they could not be converted in g/100g dry matter.

The extraction method influences not only the yields but also the composition of the EO. Indeed, the hydrodistillation requires heating during several hours and also the citrus peels, when in water, form an aqueous acidic solution. According to Boelens,⁸ the pH of bitter orange peels is between 2.5 and 4. Temperature, time and pH are the three main factors that cause degradation of terpenes, elimination, rearrangement and cyclization of alcohols and esters.

Table 7 compares the volatile composition of bitter orange essential oils obtained by hydrodistillation to the ISO standard obtained by cold pressing. The compositions of hydrodistilled oils reported in table 7 are not exhaustive, but focused on the 12 compounds that are mentioned in the ISO standard. The germacrene D percentage in hydrodistilled oils appears to be always lower than the ISO standard. The linalyl acetate percentage is always lower than the ISO standard (Table 7). This can be imputed to the decomposition of the linalyl acetate present in peels during hydrodistillation due to low pH and heating. Except for Hosni *et al.*,⁵⁰ the linalool percentage in hydrodistilled oils is always higher than the ISO standard. This can be attributed to the formation of linalool during hydrodistillation. Thus, it can be assumed that linalyl acetate is converted partially in linalool during hydrodistillation. Indeed, linalyl acetate undergoes thermal degradation that generates linalyl carbocation. In aqueous acid solution, the linalyl carbocation is hydrated to give linalool.⁷⁵

5. CHEMICAL REACTIONS HAPPENING DURING GROWING, RIPENING, STORAGE OF THE BITTER ORANGE AND DURING OIL EXTRACTION BY HYDRODISTILLATION

Adapted from the literature,^{3,47,75-90} the figure 1 is a review of the different reactions that can happen during growing, ripening and storage of the bitter orange and during hydrodistillation due to heating and acid pH.

Most of the terpene derivatives are produced during hydrodistillation by degradation of linalyl acetate, via rearrangements and cyclization of the linalyl carbocation.⁷⁵ During hydrodistillation, the major mechanism by which oxygen is added to the terpenes produced by degradation of linalyl acetate, is not oxidation but acid-catalysed reactions in aqueous acid conditions (due to the acidity of bitter oranges).⁸⁷ Baxter *et al.*⁸⁴ studied the products of the reactions of linalool, geraniol, nerol as well as their acetate esters in aqueous acid conditions (pH = 2.3) at 24°C during 10 to 20 days. Actually, 10 days is too long to be able to identify the different intermediate products of reaction. Cori *et al.*⁸⁹ studied the kinetics of those reactions and concluded that the hydration of the linalyl carbocation in linalool is faster than the allylic rearrangement of the linalyl carbocation in geranyl and neryl carbocation and than the cyclization in terpinyl carbocation. Solvolysis of linalyl acetate is 120 times faster than solvolysis of geranyl acetate and of neryl acetate.⁸⁸ The mechanisms of reaction appear to be different as well. The linalyl acetate forms the linalyl carbocation that is more stable than the geranyl and the neryl carbocation because it is a tertiary carbocation. Meanwhile the geranyl acetate and the neryl acetate proceed by deacylation to form directly geraniol and nerol respectively. Morin and Richard⁷⁵ studied the kinetics of linalyl acetate degradation simulating hydrodistillation by heating under reflux at different pH: 2, 4, 7 and 9 during periods of time varying between 0.3 to 6 hours. At pH 2 during 6 hours, the products the most abundant were terpenes: terpinolene (20.2%), (*E*)-ocimene (13.8%), α -terpinene (coeluted with 1,4-cineole 13.1%), limonene (9.3%), γ -terpinene (7.2%)... Many alcohols were also produced in smaller quantities, among which: α -terpineol (3.9%), (*Z*)- β -terpineol (0.4%) and terpinene-4-ol (1.1%), as well as linalool oxides (0.13%).

Another proof of the role of the acid pH was given by Boelens and Jimenez.⁵⁶ Those authors showed that, if the sampling was performed with a syringe rinsed with an ethanol solution of triethanolamine, it was possible to avoid the following reactions which are due to acid pH of bitter orange peels: linalyl acetate degradation into monoterpenes, formation of α -terpineol by cyclisation, as well as allylic rearrangement of linalool and linalyl acetate to form nerol and geraniol and their acetates.

In 1966, Attaway *et al.*⁹¹ stated that linalool could form limonene during growing, but it is not the only precursor⁶² and the most likely hypothesis at present is that limonene is derived from geranyl pyrophosphate (GPP) (also known as geranyl diphosphate) by the action of enzymes called terpene synthases or cyclases.⁹² Terpenes are synthesized by the condensation of isopentenyl pyrophosphate (IPP) (also known as isopentenyl diphosphate) and dimethylallyl pyrophosphate (DMAPP) (also known as dimethylallyl diphosphate) which forms GPP. IPP

and DMAPP can be formed through two different pathways which are located in two different cellular compartments: the cytosolic mevalonate pathway and the plastic methylerythritol phosphate pathway. GPP is the precursor of monoterpenes such as ocimene and limonene. Monoterpene synthase genes were characterized in citrus^{93–95}. A high expression of those genes appears to have an important role in the formation of citrus essential oils.⁹² Indeed, terpenes synthases are responsible for the synthesis of monoterpenes.

When submitting β -pinene to hydrodistillation in harsh acid conditions during 3 hours using a Clevenger apparatus, Haro-Guzmán and Huet obtained important quantities of α -terpineol, terpinolene and 1,4-cineole, as well as lesser quantities of 1,8-cineole and *p*-cymene.⁹⁶ Muneyuki *et al.* reviewed the acid catalysed rearrangements of α -pinene and β -pinene.⁹⁷ Under acidic conditions, the degradation of pinenes mainly gave α -terpineol, and smaller amounts of α -fenchol and borneol. When pH is even lower, some hydrocarbons such as limonene, terpinolene, camphene and fenchene can be formed as well.

The major hydration products of acid-catalysed reaction of sabinene and α -thujene are: terpinen-4-ol, γ -terpinene, α -terpinene, terpinolene, sabinene hydrate and 1,4-terpin.⁸²

In plants, not all aroma compounds are found in a free state. Some are glycosidically bound aroma compounds: they are called aroma precursors since they are able to release free odorous aroma compounds when the glycosidic bond is hydrolysed,⁹⁸ but they are not odorous as such, when glycosidically bound. Glycosidic aroma precursors are preferential storage and transport forms probably due to the toxicity of the free form in the cells above a certain concentration.⁶⁶ Deglycosylation occurs by enzymatic or acid hydrolysis of the glycosidic bond⁹⁹ during fruit maturation, spontaneously when the pH is acid enough or because of heat treatment. Thus, it can be assumed that due to the acidity of bitter orange peels in aqueous solution,⁸ hydrodistillation of peels induces deglycosylation and release of aroma precursors, but no literature was found on this point.

Oxidations of the terpenes and the sesquiterpenes can happen in the presence of dioxygen. Boelens and Sindreu⁴⁷ stated that nerolidol can form various sesquiterpenes such as germacrene D, α -humulene, caryophyllene, α -selinene that can oxidise in α -selinenone, and valencene that can oxidise in nootkatone.

Ikeda *et al.*⁷⁶ were the first authors to assume that when γ -terpinene is exposed to air at room temperature it could possibly form *p*-cymene as γ -terpinene concentration decreases while *p*-cymene concentration increases. Kimura *et al.*⁷⁷ studied the impact of storage on lemon oil and on different compounds: citral, limonene, β -pinene, and γ -terpinene, in acidic conditions at 38°C. They found out that citral and β -pinene concentrations significantly decreased after 60 days of storage at these temperature and pH conditions, while α -terpineol and *p*-cymene which are more stable increased, due to low degradation of limonene and γ -terpinene respectively. As the storage time increases, the concentration of *p*-cymene increases.¹⁰⁰ In 2014, Lorjaroenphon *et al.*⁸⁵ studied the degradation products of citral when stored three days in citric acid solution. They proposed rearrangement pathways for citral in aqueous acidic

solution very similar to the ones by Clark *et al.*⁸⁷ dating from 1977. These reaction schemes are more detailed than the ones presented by Kimura *et al.*⁷⁷

6. CONCLUSION

More than 50 studies have been published on the composition of the bitter orange essential oils. Many factors can influence its composition. Up until now, the following factors have been discussed in the literature: growing location, cultivars, ripening stage, storage conditions, and extraction methods.

First of all, it may be underlined that only one article did a true quantification of the compounds of a sample of bitter orange essential oil using internal standards.⁵⁵ All the studies that compared different samples of bitter orange essential oils draw conclusions based on raw GC percentages. It ensues from the comparison of the number of compounds identified in each study, that some authors should have injected a volume of essential oil more important or have lowered the split, in order to identify more compounds. When trying to identify more compounds, authors should be cautious when it comes to the saturation of limonene, or inject the limonene separately for its quantification.

It results from the examination of the compositions of the bitter orange peel oils published in the literature, that 50 out of the 53 cold-pressed peel oils extracted from *Citrus aurantium* L. reported in this review would not be identified as bitter orange peel oil according to the ISO standard 9844, because they do not comply with the normative ranges of values for the 10 compounds of the standard. The only three results of analysis of cold-pressed oils which are compliant with the ISO standard 9844 are Dugo *et al.*,^{19,36} the Maroc cultivar from Lota *et al.*,³¹ and Mondello *et al.*^{37,40} Moreover, the means of the percentages of limonene, linalyl acetate, and germacrene D of the 24 cultivars of *Citrus aurantium* L. which have a similar chemotype according to Lota *et al.*³¹ are not compliant with the standard. Hence, the ISO standard 9844, may consider broadening some of its normative ranges of values.

Bitter orange peel essential oil coming from some of the major producing countries such as Argentina, Haiti, Morocco, Uganda, and Paraguay have not been studied yet in the literature. Boelens and Jimenez³⁵ tried to assess the influence of the growing location, but more experiments are needed to draw firm conclusions.

Only half of the authors mentioned the name of the cultivar they used for their study, whereas it has been established that some cultivars such as the Espagne and the Gou Tou cultivar differ a lot from the other cultivars of *Citrus aurantium* L..³¹

All the authors agree on the fact that limonene is the majority compound of the bitter orange peel oil by far. Seven studies were published on the influence of the growing and the ripening stage of the bitter orange on the composition of its essential oil. The limonene percentage increases during growing of the fruit by cyclisation of geranyl pyrophosphate. The linalool percentage dramatically decreases during growing as the limonene increases, so it could be possible that the limonene is also partly formed by the degradation of the linalool. The question remains if two pathways can coexist as far as the formation of limonene is concerned. If so, the predominance of one of them would probably be determined by the gene

pool and the environment of the bitter orange. The cultivars being examples of genetic variability among bitter oranges, and the growing location (climate, soil quality...) a factor of the environment. β -pinene seems to decrease during growing. α -terpineol, geraniol, and nerol appear to be fairly constant during the ripening of bitter oranges. Aldehydes (mainly octanal and decanal) which are known to have an important odorant impact⁵⁵ increase during the ripening of bitter oranges.

Boelens and Jimenez⁵⁶ are the only authors who studied the impact of storage of bitter oranges on the composition of its oil. Unfortunately, only one analysis was done at the end of a short storage time (one to two weeks). According to this analysis compared with the analysis of the ripe fruit right after picking up, they concluded that linalool, linalyl acetate, nerolidol, nootkatone and α -selinenone increased after picking up the fruits. Enzymatic reactions were accounted for the formation of these compounds.

Njoroge *et al.*⁴³ are the only authors who assessed the impact of storage of bitter orange essential oil on its composition. It can be concluded from this study that bitter orange EO should be stored at -21°C to prevent any degradation caused by long storage period. The main changes in composition are observed when stored at 20°C or stored at 5°C during at least 12 months. Structural rearrangements of monoterpenes such as limonene and γ -terpinene due to temperature and oxidation form *p*-cymene via terpinolene when the temperature and the time of storage increase. The oxidation of limonene explains the sharp increases of (*Z*)-carveol, carvone, limonene-10-yl acetate, and (*E*)-*p*-mentha-2,8-dien-1-ol, and the slight increase of α -terpineol. The increase of linalool was probably due to the decrease of linalyl acetate and maybe as well to the autooxidation of myrcene.⁸³ It can be hypothesized that both thujol and sabinene hydrate came from sabinene, by structural rearrangement followed by hydration and simply by hydration respectively.

The main extraction methods used to obtain oil from bitter orange peel are cold pressing and hydrodistillation. Cold pressing consists in applying mechanical pressure on the peels to release the essential oil. Then, water is added to the EO. The mixture is centrifuged and finally the aqueous phase is discarded which means that soluble compounds such as glycosidic aroma compounds are thrown away. Actually, the presence of glycosidic aroma compounds in bitter orange peels has never been proved yet in the literature, but their presence has been reported in several citrus species (three cultivars of mandarin, one cultivar of sweet orange, one cultivar of pink grapefruit and one cultivar of white grapefruit).⁹⁸ Hydrodistillation is exclusively used to extract bitter orange peel oil on laboratory scale. The use of a Clevenger apparatus provides the advantage of measuring directly the volume of oil extracted per mass of peels involved. Despite the use of a Clevenger, the different parameters of the hydrodistillation method appear to vary a lot (mass of peel involved, time of hydrodistillation, distillation rate), and so does the quantity of essential oil extracted per mass of peel. During hydrodistillation, the aroma compounds of the bitter orange peels are submitted to acid-catalysed reactions, due to the acidity of the bitter orange peels which lower the pH of the aqueous solution. At acid pH, linalyl acetate is decomposed in many terpene derivatives, which explains that the percentages of linalyl acetate are much lower in hydrodistilled oils than in cold pressed oils. The main differences between cold pressed bitter orange oils and hydrodistilled oils are due to the acid-catalysed reactions during

hydrodistillation and possibly to the release of aroma compounds by deglycosylation of the glycosidic bound.

Understanding all these reactions is essential because the odour profile of the bitter orange peel oil can be impacted a lot. Monoterpenes do not play a major role in the flavour of bitter orange peel oil,¹⁰¹ but many of their reaction products formed by acid-catalysed rearrangement followed by hydration are extremely important to the flavour of the essential oil. For instance, the hydration of a relatively weak flavorant such as limonene leads to a temporary increase in flavour until the terpineols are converted to terpins.⁸⁸

REFERENCES

1. Ortiz JM. Botany: taxonomy, morphology and physiology of fruits, leaves and flowers. In: Dugo G, Di Giacomo A, eds. *Citrus : The Genus Citrus*. Boca Raton, FL: CRC Press; 2002:16-35.
2. Kale PN, Adsule PG. Citrus. In: Salunkhe DK, Kadam SS, eds. *Handbook of Fruit Science and Technology: Production, Composition, Storage and Processing*. New York, NY: Marcel Dekker; 1995:39-66. doi:10.1201/9781482273458
3. Huet R. Les huiles essentielles d'agrumes. *Fruits*. 1991;46(4):501-513.
4. Jabri Karoui I, Marzouk B. Characterization of bioactive compounds in Tunisian bitter orange (*Citrus aurantium* L.) peel and juice and determination of their antioxidant activities. *Biomed Res Int*. 2013:1-12.
5. Sarrou E, Chatzopoulou P, Dimassi-Therios K, Therios I. Volatile constituents and antioxidant activity of peel, flowers and leaf oils of *Citrus aurantium* L. growing in Greece. *Molecules*. 2013;18(9):10639-10647. doi:10.3390/molecules180910639
6. Caccioni DRL, Guizzardi M, Biondi DM, Renda A, Ruberto G. Relationship between volatile components of citrus fruit essential oils and antimicrobial action on *Penicillium digitatum* and *Penicillium italicum*. *Int J Food Microbiol*. 1998;43(1-2):73-79. doi:10.1016/S0168-1605(98)00099-3
7. Rowshan V, Najafian S. Changes of peel essential oil composition of *Citrus aurantium* L. during fruit maturation in Iran. *J Essent Oil-Bearing Plants*. 2015;18(4):1006-1012. doi:10.1080/0972060X.2014.977564
8. Boelens MH. A critical review on the chemical composition of Citrus oils. *Perfum Flavorist*. 1991;16:17-34.
9. Shaw PE. Review of quantitative analyses of Citrus essential oils. *J Agric Food Chem*. 1979;27(2):246-257. doi:10.1021/jf60222a032
10. Lawrence BM. Progress in essential oils. *Perfum Flavorist*. 1982;7(5):43-48.
11. Swaine RL, Swaine RLJ. Citrus oils: Processing, technology, and applications. *Perfum Flavorist*. 1988;13:1-20.
12. Ortiz JM, Kumamoto J, Scora RW. Possible relationship among sour oranges by analysis of their essential oils. *Int flavours food Addit*. 1978;9:224-226.
13. Maekawa K, Kodama M, Kushii M, Mitamura M. Essential oils of some orange peels. *Agric Biol Chem*. 1967;31(3):373-377. doi:10.1080/00021369.1967.10858818
14. Tanaka T. Fundamental discussion of Citrus classification. *Stud Citrologia*. 1977;14:1-6.

15. Swingle WT. The botany of Citrus and its relatives in the orange subfamily. In: Webber HJ, Batchelor LD, eds. *The Citrus Industry I*. University of California Press; 1943:190-430.
16. Handa T, Ishizawa Y, Oogaki C. Phylogenetic study of Fraction I protein in the genus Citrus and its close related genera. *Japanese J Genet.* 2008;61(1):15-24. doi:10.1266/jjg.61.15
17. Luro F, Curk F, Froelicher Y, Patrick O. Recent insights on Citrus diversity and phylogeny. In: Zech-Matterne V, Fiorentino G, eds. *AGRUMED: Archaeology and History of Citrus Fruit in the Mediterranean: Acclimatization, Diversifications, Uses*. Naples, Italy: Publications du Centre Jean Bérard; 2017.
18. Ali J, Khan A, Ullah N, et al. Essential oil chemical composition and antimicrobial activity of sour oranges (*Citrus aurantium*) peels. *J Pharm Res.* 2012;5(3):1690-1693. <http://jpronline.info/index.php/jpr/article/view/12311/6294>.
19. Dugo G, Bartle KD, Bonaccorsi I, et al. Advanced analytical techniques for the analysis of Citrus essential oils, part 1: volatile fraction: HRGC/MS Analysis. *Essenze Deriv Agrum.* 1999;69:79-111.
20. McHale D, Sheridan JB. Detection of adulteration of cold-pressed lemon oil. *Flavour Fragr J.* 1988;3(July):127-133.
21. ISO (International Organization for Standardization). Huile essentielle d'orange amère (*Citrus aurantium* L.). *ISO.* 2006.
22. Lota ML, De Rocca Serra D, Tomi F, Casanova J. Chemical variability of peel and leaf essential oils of mandarins from *Citrus reticulata* Blanco. *Biochem Syst Ecol.* 2000;28(1):61-78. doi:10.1016/S0305-1978(99)00036-8
23. Boussaada O, Chemli R. Chemical composition of essential oils from flowers, leaves and peel of *Citrus aurantium* L. var. amara from Tunisia. *J Essent Oil Bear Plants.* 2006;9(2):133-139. doi:10.1080/0972060X.2006.10643484
24. Di Giacomo A. The market of Citrus oils around the world. In: Dugo G, Di Giacomo A, eds. *Citrus : The Genus Citrus*. Boca Raton, FL: CRC Press; 2002:532-538.
25. Bovill H. Trade of essential oils. In: Baser KHC, Buchbauer G, eds. *Handbook of Essential Oils*. Boca Raton, FL: Taylor & Francis; 2010:895-902.
26. Boussaada O, Skoula M, Kokkalou E, Chemli R. Chemical variability of flowers, leaves, and peels oils of four sour orange provenances. *J Essent Oil-Bearing Plants.* 2007;10(6):453-464. doi:10.1080/0972060X.2007.10643579
27. Koketsu M, Magalhães MT, Wilnerg VC, Donalisio MGR. Óleos essenciais de frutos cítricos cultivados no Brasil. *Embrapa Agroindústria Aliment.* 1983.
28. Huang Y, Wu Y. The chemical components of essential oils from peels of twenty-five citrus species and cultivars. *Nat Prod Res Dev.* 1998;10(4):48-54.
29. Pino JA, Rosado A. Composition of cold-pressed bitter orange oil from Cuba. *J Essent Oil Res.* 2000;12(6):675-676. doi:10.1080/10412905.2000.9712187
30. Ashour FM, El-Kebeer ME. Studies on physicochemical properties and chemical composition of bitter orange peel oil. *9th Int Essent Oil Congr Singapore.* 1983.
31. Lota M, De Rocca Serra D, Jacquemond C, Tomi F, Casanova J. Chemical variability of peel and leaf essential oils of sour orange. *Flavour Fragr J.* 2001:89-96.

32. Protopapadakis E, Papanikolaou X. Characterization of *Citrus aurantium* and *Citrus taiwanica* rootstocks by isoenzyme and essential oil analysis. *J Hortic Sci Biotechnol*. 1998;73(1):81-85. doi:10.1080/14620316.1998.11510947
33. Azhdarzadeh F, Hojjati M. Chemical Composition and Antimicrobial Activity of Leaf, Ripe and Unripe Peel of Bitter Orange (*Citrus aurantium*) Essential Oils. *Nutr Food Sci Res*. 2016;3(1):43-50. doi:10.18869/acadpub.nfsr.3.1.43
34. Sanei-Dehkordi A, Sedaghat MM, Vatandoost H, Abai MR. Chemical compositions of the peel essential oil of *Citrus aurantium* and its natural larvicidal activity against the malaria vector *Anopheles stephensi* (Diptera: Culicidae) in comparison with *Citrus paradisi*. *J Arthropod Borne Dis*. 2016;10(4):577-585.
35. Boelens MH, Jimenez R. The chemical composition of some mediterranean Citrus oils. *J Essent Oil Res*. 1989;1(4):151-159. doi:10.1080/10412905.1989.9697776
36. Dugo G, Verzera A, Stagno d'Alcontres I, Cotroneo A, Ficarra R. On the genuineness of Citrus essential oils. Part XLI. Italian bitter orange essential oil: composition and detection of contamination and additions of oils and terpenes of sweet orange and of lemon. *Flavour Fragr J*. 1993;8:25-33.
37. Mondello L, Casilli A, Tranchida PQ, Cicero L, Dugo P, Dugo G. Comparison of fast and conventional GC analysis for citrus essential oils. *J Agric Food Chem*. 2003;51(19):5602-5606. doi:10.1021/jf0341971
38. Gionfriddo F, Catalfamo M, Siano F, Mangiola C, Cautela D, Castaldo D. Determinazione delle caratteristiche analitiche e della composizione enantiomerica di oli essenziali agrumari ai fini dell'accertamento della purezza e della qualità. Nota I - Essenze di arancia amara, arancia dolce e bergamotto.pdf. *Essenze Deriv Agrum*. 2003;73(1):29-39.
39. Dugo G, Bonaccorsi I, Sciarrone D, et al. Characterization of oils from the fruits, leaves and flowers of the bitter orange tree. *J Essent Oil Res*. 2011;23(2):45-59. doi:10.1080/10412905.2011.9700446
40. Mondello L, Casilli A, Tranchida PQ, Costa R, Dugo P, Dugo G. Fast GC for the analysis of citrus oils. *J Chromatogr Sci*. 2004;42(8):410-416. doi:10.1093/chromsci/42.8.410
41. Dugo G. The composition of the volatile fraction of the Italian Citrus essential oils. *Perfum Flavorists*. 1994;19(6):29-51.
42. Njoroge SM, Ukeda H, Kusunose H, Sawamura M. Volatile components of the essential oils from Kabosu, Daidai, and Yuko, Japanese sour Citrus fruits. *Flavour Fragr J*. 1994;9(6):289-297. doi:10.1002/ffj.2730090603
43. Njoroge SM, Ukeda H, Sawamura M. Changes of the volatile profile and artifact formation in daidai (*Citrus aurantium*) cold-pressed peel oil on storage. *J Agric Food Chem*. 2003;51(14):4029-4035. doi:10.1021/jf021215q
44. Sawamura M, Sun SH, Ozaki K, Ishikawa J, Ukeda H. Inhibitory effects of citrus essential oils and their components on the formation of N-nitrosodimethylamine. *J Agric Food Chem*. 1999;47(12):4868-4872. doi:10.1021/jf9903206
45. Sawamura M. Volatile components of essential oils of the citrus genus. *Recent Res Dev Agric & Food Chem*. 2000;4(1):131-164.
46. Song HS, Sawamura M, Ito T, Ido A, Ukeda H. Quantitative determination and

- characteristic flavour of daidai (*Citrus aurantium* L. var *cyathifera* Y. Tanaka) peel oil. *Flavour Fragr J.* 2000;15:323-328.
47. Boelens MH, Jimenez Sindreu R. Essential oils from Seville bitter orange (*Citrus aurantium* L. ssp. *amara* L.). In: *Flavors and Fragrances: A World Perspective Proceedings of the 10th International Congress of Essential Oils, Fragrances and Flavors, Washington, DC, U.S.A., 16-20 November 1986*. Elsevier B.V.; 1988:551-565.
 48. Boelens MH, Oporto A. Natural isolates from Seville bitter orange tree. *Perfum Flavorists*. 1991;16(6):2-7.
 49. Gogorcena Y, Ortiz JM. Characterisation of sour orange (*Citrus aurantium*) cultivars. *J Sci Food Agric*. 1989;48(3):275-284. doi:10.1002/jsfa.2740480304
 50. Hosni K, Zahed N, Chrif R, et al. Composition of peel essential oils from four selected Tunisian Citrus species: Evidence for the genotypic influence. *Food Chem*. 2010;123(4):1098-1104. doi:10.1016/j.foodchem.2010.05.068
 51. Bourgou S, Rahali FZ, Ourghemmi I, Tounsi, MS. Changes of peel essential oil composition of four tunisian Citrus during fruit maturation. *Sci World J*. 2012:1-10. doi:10.1100/2012/528593
 52. Kirbaslar G, Kirbaslar II. Composition of cold-pressed bitter orange peel oil from Turkey. *J Essent Oil Res*. 2003;15(1):6-9. doi:10.1080/10412905.2003.9712247
 53. Ziegler H, Feger W. Citrus oils. In: Ziegler H, ed. *Flavourings: Production, Composition, Applications, Regulations*. Second. Wiley-VCH; 2007:187-213.
 54. González De CN, Sánchez F, Quintero A. Chemotaxonomic value of essential oil compounds in Citrus species. *Acta Horti*. 2002;576:49-51.
 55. Deterre S, Rega B, Delarue J, Decloux M, Lebrun M, Giampaoli P. Identification of key aroma compounds from bitter orange (*Citrus aurantium* L.) products: Essential oil and macerate-distillate extract. *Flavour Fragr J*. 2012;27(1):77-88. doi:10.1002/ffj.2087
 56. Boelens MH, Jimenez R. The chemical composition of the peel oils from unripe and ripe fruits of bitter orange, *Citrus aurantium* L. ssp. *amara* engl. *Flavour Fragr J*. 1989;4(3):139-142. doi:10.1002/ffj.2730040313
 57. Chouchi D, Barth D, Reverchon E, Della Porta G. Bigarade peel oil fractionation by supercritical carbon dioxide desorption. *J Agric Food Chem*. 1996;44(4):1100-1104.
 58. Veriotti T, Sacks R. High-speed characterization and analysis of orange oils with tandem-column stop-flow GC and time-of-flight MS. *Anal Chem*. 2002;74(21):5635-5640. doi:10.1021/ac0202482
 59. De La Torre PC, Sardi TC. Study on bitter orange (*Citrus aurantium*) essential oil. *Arch Bioquim Quim Farm Tucuman*. 1977;20:69-72.
 60. Dugo G, Cotroneo A, Verzera A, Bonaccorsi I. Composition of the volatile fraction of cold-pressed citrus peel oils. In: Dugo G, Di Giacomo A, eds. *Citrus: The Genus Citrus*. London, UK: Taylor & Francis; 2002:201-317.
 61. Abderrezak MK, Abaza I, Aburjai T, Kabouche A, Kabouche Z. Comparative compositions of essential oils of *Citrus aurantium* growing in different soils. 2014;5(6):1913-1918.
 62. Attaway JA, Pieringer AP, Barabas LJ. The origin of citrus flavor components - III. A

- study of the percentage variations in peel and leaf oil terpenes during one season. *Phytochemistry*. 1967;6:25-32.
63. Siragusa M, De Pasquale F, Abbate L, Tusa N. Identification of sour orange accessions and evaluation of their genetic variability by molecular marker analyses. *HortScience*. 2006;41(1):84-89.
 64. Castle WS, Baldwin JC, Grosser JW. Performance of “washington” navel orange trees in rootstock trials located in lake and st. lucie counties. *Proc Florida State Hort Soc*. 2000;113:106-111.
 65. Boussaada O, Chemli R. Seasonal variation of essential oil composition of *Citrus aurantium* L. var. *amara*. *J Essent Oil-Bearing Plants*. 2007;10(2):109-120. doi:10.1080/0972060X.2007.10643528
 66. Vasserot Y, Arnaud A, Galzy P. Monoterpenol glycosides in plants and their biotechnological transformation. *Acta Biotechnol*. 1995;15(1):77-95. doi:10.1002/abio.370150110
 67. Kusunose H, Sawamura M. Aroma constituents of some sour Citrus oils. *Nippon shokuhin kogyo gakkaiishi*. 1980;27(10):517-521. doi:10.3136/nskkk1962.27.10_517
 68. Tranchida PQ, Bonaccorsi I, Dugo P, Mondello L, Dugo G. Analysis of Citrus essential oils: State of the art and future perspectives. A review. *Flavour Fragr J*. 2012;27(2):98-123. doi:10.1002/ffj.2089
 69. Dugo G, Cotroneo A, Bonaccorsi I, Allessandra T. Composition of the volatile fraction of citrus peel oils. In: Dugo G, Mondello L, eds. *Citrus Oils: Composition, Advanced Analytical Techniques, Contaminants, and Biological Activity*. Boca Raton, FL: CRC Press; 2010:1-162.
 70. Porat R, Deterre S, Giampaoli P, Plotto A. The flavor of citrus fruit. In: Havkin-Frenkel D, Dudai N, eds. *Biotechnology in Flavor Production*. Second. John Wiley & Sons; 2016:1-31. doi:10.1002/9781118354056.ch1
 71. Schmidt E. Production of essential oils. In: Baser KHC, Buchbauer G, eds. *Handbook of Essential Oils: Science, Technology and Applications*. Boca Raton, FL: CRC Press; 2010:83-119.
 72. Ruberto G. Analysis of volatile components of Citrus fruit essential oils. In: Jackson JF, Linskens HF, eds. *Analysis of Taste and Aroma*. Springer Berlin Heidelberg; 2002:123-157.
 73. Lota M. Les huiles essentielles d'agrumes: caractérisation par RMN du carbone-13 CPG-IK et CPG/SM, PhD Thesis. 1999.
 74. European Pharmacopoeia. Determination of Essential Oils in Herbal Drugs. In: Council of Europe (EDQM), ed. *European Pharmacopoeia*. 9th ed. Strasbourg, FR; 2017:285-286.
 75. Morin P, Richard H. Thermal degradation of linalyl acetate during steam distillation. *Prog Flavour Res*. 1985:563-576.
 76. Ikeda RM, Stanley WL, Vannier SH, Rolle LA. Deterioration of lemon oil. Formation of p-cymene from γ -terpinene. *Food Technol*. 1961;15:379-380.
 77. Kimura K, Nishimura H, Iwata I, Mizutani J. Identification and formation mechanism of components responsible for off-odor of deteriorated lemons. *Nippon shokuhin kogyo gakkaiishi*. 1984;31(12):761-764.

78. Mazza G. Studio sulle ossidazioni dei monoterpeni negli oli essenziali. *Essenze Deriv Agrum.* 1987;57:5-18.
79. Mazza G. Etude sur la composition aromatique de l'huile essentielle de bergamotte (*Citrus aurantium* subsp. *Bergamia* Risso et Poiteau Engler) par chromatographie gazeuse et spectrometrie de masse. *J Chromatogr.* 1986;362:87-99.
80. Mitzner BM, Lemberg S, Theimer ET. Preparation of cis- and trans-ocimene from catalytic pyrolysis of linalyl acetate. *Can J Chem.* 1966;44:1090-1092.
81. Williams CM, Whittaker D. Rearrangements of pinane derivatives. Part II. Products of acid-catalysed rearrangement of α -pinene and β -pinene in acetic acid. *J Chem Soc B Phys Org.* 1971;(672):672-677. doi:10.1039/J29710000672
82. Cooper MA, Holden CM, Loftus P, Whittaker D. The acid catalysed hydration of sabinene and α -thujene. *J Chem Soc Perkin Trans 2.* 1973;(5):665-667.
83. Dieckmann RH, Palamand SR. Autoxidation of some constituents of hops. I. The monoterpene hydrocarbon, myrcene. *J Agric Food Chem.* 1974;22(3):498-503. doi:10.1021/jf60193a033
84. Baxter RL, Laurie WA, Mchale D. Transformations of monoterpenoids in aqueous acids. The reactions of linalool, geraniol, nerol and their acetates in aqueous citric acid. *Tetrahedron.* 1978;34(14):2195-2199. doi:10.1016/0040-4020(78)89026-7
85. Lorjaroenphon Y, Hausch BJ, Cadwallader KR. *Formation and Stability of 2,3-Dehydro-1,8-Cineol in a Model Carbonated Beverage System.* Elsevier Inc.; 2014. doi:10.1016/b978-0-12-398549-1.00044-1
86. Ziegler M, Brandauer H, Ziegler E, Ziegler G. A different aging model for orange oil: Deterioration products. *J Essent Oil Res.* 1991;3(4):209-220. doi:10.1080/10412905.1991.9697931
87. Clark BC, Powell CC, Radford T. The acid catalyzed cyclization of citral. *Tetrahedron.* 1977;33(17):2187-2191.
88. Clark BC, Chamblee TS. Acid-Catalyzed Reactions of Citrus Oils and Other Terpene-Containing Flavors. In: Charalambous G, ed. *Off-Flavors in Foods and Beverages.* Amsterdam, NL: Elsevier B.V.; 1992:229-285. doi:https://doi.org/10.1016/B978-0-444-88558-6.50015-5
89. Cori O, Chayet L, Perez LM, Bunton CA, Hachey D. Rearrangement of Linalool, Geraniol, and Nerol and Their Derivatives. *J Org Chem.* 1986;51(8):1310-1316. doi:10.1021/jo00358a028
90. Degenhardt A, Liebig M, Kohlenberg B, et al. *Studies on Stability of Citrus Flavors and Insights into Degradation Pathways of Key Aroma Compounds.* Elsevier Inc.; 2014. doi:10.1016/b978-0-12-398549-1.00045-3
91. Attaway JA, Pieringer AP, Barabas LJ. The origin of citrus flavor components - I. The analysis of citrus leaf oils using gas-liquid chromatography, thin-layer chromatography and mass spectrometry. *Phytochemistry.* 1966;5:141-151.
92. Takita MA, Berger IJ, Basilio-Palmieri AC, Borges KM, Souza de JM, Targon MLNP. Terpene production in the peel of sweet orange fruits. *Genet Mol Biol.* 2007;30(3 (suppl)):841-847.
93. Shimada T, Endo T, Fujii H, Hara M, Omura M. Isolation and characterization of (*E*)-beta-ocimene and 1,8 cineole synthases in *Citrus unshiu* Marc. *Plant Sci.*

- 2005;168(4):987-995. doi:10.1016/j.plantsci.2004.11.012
94. Dornelas MC, Mazzafera P. A genomic approach to characterization of the Citrus terpene synthase gene family. *Genet Mol Biol.* 2007;30(3 (suppl.)):832-840.
95. Rodríguez A, Andrés VS, Cervera M, et al. Terpene down-regulation in orange reveals the role of fruit aromas in mediating interactions with insect herbivores and pathogens. *Plant Physiol.* 2011;156(2):793-802. doi:10.1104/pp.111.176545
96. Haro-Guzman L, Huet R. L'huile essentielle de lime au mexique (*Citrus aurantifolia* Swingle). *Fruits.* 1970;25(12):887-899.
97. Muneyuki R, Yoshimura Y, Tori K, Terui Y, Shoolery JN. Acid-catalyzed rearrangements of the pinyl system. *J Org Chem.* 1988;53:358-366.
98. Ren JN, Tai YN, Dong M, et al. Characterisation of free and bound volatile compounds from six different varieties of Citrus fruits. *Food Chem.* 2015;185:25-32. doi:10.1016/j.foodchem.2015.03.142
99. Praet T, Van Opstaele F, Jaskula-Goiris B, Aerts G, De Cooman L. Biotransformations of hop-derived aroma compounds by *Saccharomyces cerevisiae* upon fermentation. *Cerevisia.* 2012;36(4):125-132. doi:10.1016/j.cervis.2011.12.005
100. Wiley RC, Louie MK, Sheu MJ. Studies on turpentine-like off-odor in Cola. *J Food Sci.* 1984;49:485-488.
101. Schieberle P, Grosch W. Identification of potent flavor compounds formed in an aqueous lemon oil/citric acid emulsion. *J Agric Food Chem.* 1988;36(4):797-800. doi:10.1021/jf00082a031

LIST OF TABLES

TABLE 1 Botanical cultivars belonging to bitter orange of Rutaceae family, citrus genus

TABLE 2 Evolution of composition of bitter orange oil during growing and ripening according to Azhdarzadeh and Hojjati,³³ Ashour and El-Kebeer,³⁰ Boelens and Jimenez,³¹ Bourgou *et al.*,⁵¹ Boussaada and Chemli,⁶³ Dugo *et al.*³⁹ and Rowshan and Najafian⁷ (expressed as relative percentages of peak area)

TABLE 3 Effect of storage on ripe bitter oranges and on cold-pressed oil extracted from ripe bitter oranges compared to the composition recorded in ISO standard 9844⁴³ (relative percentages of peak area)

TABLE 4 Comparison of percentages of peak area of volatile compounds listed in the ISO standard 9844 for industrial cold-pressed bitter orange peel oils from the literature

TABLE 5 Comparison of percentages of peak area of volatile compounds listed in the ISO standard 9844 for laboratory cold-pressed bitter orange peel oils from the literature

TABLE 6 Comparison of the hydrodistillation (HD) methods used and the yields of essential oil obtained from bitter orange peels with a Clevenger apparatus

TABLE 7 Comparison of percentages of peak area of volatile compounds listed in the ISO standard 9844 for hydrodistilled oils from the literature

LIST OF FIGURES

FIGURE 1 Review of the chemical reactions susceptible to occur in bitter orange essential oils during growing, ripening, hydrodistillation and storage: hydrations of double bonds, dehydrations, rearrangements, cyclizations, and pyrolysis. Adapted from Baxter *et al.*,⁸³ Boelens and Sindreu,⁴⁷ Clark *et al.*,⁸⁶ Clark and Chamblee,⁸⁷ Cooper *et al.*,⁸² Cori *et al.*,⁸⁸ Degenhardt *et al.*,⁸⁹ Dieckmann and Palamand,⁹⁰ Huet,³ Ikeda *et al.*,⁷⁶ Kimura *et al.*,⁷⁷ Lorjaroenphon *et al.*,⁸⁴ Mazza,^{78,79} Mitzner *et al.*,⁸⁰ Morin and Richard,⁷⁵ Williams and Whittaker,⁸¹ Ziegler *et al.*⁸⁶

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35
36
37
38
39
40
41
42
43
44
45
46

TABLE 1

Family		Rutaceae			
Genus		Citrus			
		Bitter orange / Sour Orange / Bigarade			
Species		<i>Citrus</i>	<i>Citrus</i>	<i>Citrus</i>	<i>Citrus</i>
		<i>aurantium</i>	<i>myrtifolia</i>	<i>natsudaidai</i>	<i>neo-aurantium</i>
		L.	Raf.	Hay.	Tan.
Cultivars	Country of origin	Cultivars examples			
	Algeria	Algerian, Trabout			
	Brazil	Azeda, Brazilian, Jaboticaba			
	China	Gou Tou, Huahong, Kabusu			
	Cuba	Cajel, Cuban			
	France	Cardosi, De Grasse, Donnet, Filippi, Luisi, Menton, Santucci	Large chinotto	Natsudaidai	Tosu
	Italy	Bizzarrio, Gallesio, Palermo,			
	Jamaica	Jacmel	Small chinotto	Konejime	
	Japan	Daidai, Du Japon, Shuto	Crispifolia		
	Spain	Bouquetier de Nice à fleurs doubles, Espagne, Sevillano (Seville)			
	Tunisia	Alibert, Petit Pierre, Tunisian			
	United States	Bittersweet, Keen, Standard			
			

TABLE 2

		ISO standard 9844 ²¹	Ashour and El-Kebeer ³⁰		Azhdarzadeh and Hojjati ³³		Boelens and Jimenez ⁵⁶		
Extraction method		Cold-pressed	Steam distillation		Hydrodistillation		Laboratory cold-pressed		
Cultivar									
Growing location		Egypt			Iran (Khuzestan province)				
Growing / Ripening			Setting fruits (very small in size)	Immature fruits (size of a bitter orange)	Ripening fruits	Unripe fruits	Ripe fruits	Unripe fruits on tree	Ripe fruits on tree
ISO standard 9844 compounds	<i>α</i> -pinene	0.30-0.65	0.38	0.43	0.55	2.56*	1.96*	0.60	0.65
	<i>β</i> -pinene	0.30-1.15	2.38*	0.14*	0.11*	0.51	0.56	0.13*	0.14*
	myrcene	1.60-1.95	1.69	1.82	2.25*	3.93*	5.74*	2.12*	1.95
	octanal	X	-	-	-	0.39	0.83	0.23	0.30
	limonene	92.95-95.00	46.19*	95.45*	92.68*	59.9*	81.6*	94.50	93.20
	linalool	0.10-0.35	25.23*	0.38*	0*	1.33*	2.19*	0.20	0.24
	decanal	X	0	0	1.63	-	-	0.14	0.20
	linalyl acetate	0.6-1.02	4.50*	0.03*	0.12*	0.52*	0*	0.23*	0.28*
	geranyl acetate	0.10-0.25	1.33*	0.13	0*	2.09*	0.33*	0.06*	0.13
	<i>β</i> -caryophyllene	0.05-0.15	-*	-*	-*	0*	0.22*	0.07	0.06
	germacrene-D	0.08-0.18	-*	-*	-*	0*	0.22*	0.09	0.08
	(<i>E</i>)- <i>β</i> -nerolidol	0.06-0.18	-*	-*	-*	0*	0.09	0.03*	0.10
Total volatile compounds identified			14	12	8	21	39	44	

*percentages of peak area out of the ISO range tr: traces

TABLE 2 Continued

		ISO standard 9844 ²¹	Bourgou <i>et al.</i> ⁵¹			Boussaada and Chemli ⁶⁵				Dugo <i>et al.</i> ³⁹		Rowshan and Najafian ⁷							
Extraction method		Cold-pressed	Hydrodistillation			Hydrodistillation				Cold-pressed		Hydrodistillation							
Cultivar		Larange																	
Growing location		Tunisia (Menzel Bouzelfa)					Egypt					Iran							
Growing / Ripening		Immature green colour	Semi-mature yellow colour	Mature orange colour	November	December	January	February	Unripe green colour	Ripe orange colour	Immature (green - size of a lemon)	Semi-mature (green - size of a bitter orange)	Ripe (orange)						
ISO standard 9844 compounds	α -pinene	0.30-0.65	0.13*	0.03*	0.36	0.4	0.4	0.4	0.3	0.33	0.34	1.17*	0.44	0.43					
	β -pinene	0.30-1.15	0.11*	0.07*	0.38	0.2*	0.3	0.3	0.3	0.15*	0.10*	0.58	0.07*	0.1*					
	myrcene	1.60-1.95	0.09*	0.48*	0.07*	1.7	1.6	1.6	1.4*	1.44*	1.56*	3.43*	1.87	1.78					
	octanal	X	-	-	-	-	-	-	-	0.05	0.07	1.38	0.40	0.68					
	limonene	92.95-95.00	80.54*	67.90*	90.95*	87.7*	88*	92.2	87*	94.54	96.52*	78.14*	94.72*	93.47*					
	linalool	0.10-0.35	0.03*	tr*	0.1*	1.3*	1*	0.8*	0.6*	0.06*	0.06*	8.19*	0.91*	1.25*					
	decanal	X	-	-	-	-	-	-	-	0.08	0.09	0.34	0.15	0.20					
	linalyl acetate	0.6-1.02	0.07*	0.09*	0.01*	0.1*	0.2*	0.2*	0.2*	0.12*	0.08*	0.54*	0.16*	0.22*					
	geranyl acetate	0.10-0.25	tr*	0.11	0.02*	0.2	0.2	0.2	0.3*	0.08*	0.08*	0.16	0.07*	0.13					
	β -caryophyllene	0.05-0.15	-*	-*	-*	tr*	tr*	tr*	tr*	0.04*	0.04*	0.02*	0.03*	0*					
	germacrene-D	0.08-0.18	0.10	0.25*	0.04*	-*	-*	-*	-*	0.02*	0.03*	0.07*	0*	0*					
	(E)- β -nerolidol	0.06-0.18	-*	-*	-*	tr*	tr*	tr*	0.1	0.04*	0.03*	-*	-*	-*					
Total volatile compounds identified			31			26				56	58	31	22	24					

*percentages of peak area out of the ISO range tr: traces

TABLE 3

		ISO standard 9844 ²¹	Boelens and Jimenez ⁵⁶			Njoroge <i>et al.</i> ⁴³			
Extraction method		Cold-pressed	Laboratory cold-pressed			Laboratory cold-pressed			
Cultivar		Daidai							
Growing location		Japan							
Storage			ripe fruits on tree	ripe fruits stored 1-2 weeks	fresh oil	5°C during 3 months	20°C during 12 months	during 3 months	during 12 months
ISO standard 9844 compounds	<i>α</i> -pinene	0.30-0.65	0.65	0.55	0.5	0.6	0.5	0.6	0.5
	<i>β</i> -pinene	0.30-1.15	0.14*	0.20*	0.7	1.0	0.6	0.7	0.5
	myrcene	1.60-1.95	1.95	2.00*	1.6	1.6	0.5*	0.8*	0.1*
	octanal	X	0.30	0.12	0.1	0.1	<0.05	0.1	0.1
	limonene	92.95-95.00	93.20	92.20*	94.7	92.1*	85.8*	88.5*	65.1*
	linalool	0.10-0.35	0.24	0.34	0.2	0.2	0.4*	1.3*	1.0*
	decanal	X	0.20	0.14	0.1	0.1	0.1	0.2	0.1
	linalyl acetate	0.6-1.02	0.28*	0.75	1.1*	1.6*	0.7	0.9	0.5*
	geranyl acetate	0.10-0.25	0.13	0.15	_*	_*	_*	_*	_*
	<i>β</i> -caryophyllene	0.05-0.15	0.06	0.13	<0.05*	0.1	0.5*	0.4*	1.1*
	germacrene-D	0.08-0.18	0.08	0.10	0.1	<0.05*	0.1	<0.05*	0*
(<i>E</i>)- <i>β</i> -nerolidol	0.06-0.18	0.10	0.23*	0*	0*	<0.05*	<0.05*	0.1	
Total volatile compounds identified			44		18	41	58	55	58

*percentages of peak area out of the ISO range

TABLE 4

ISO standard 9844 compounds	Extraction method	Cold-pressed			Industrial cold-pressed extraction							
	References	ISO standard 9844 ²¹	Boelens and Jimenez ³⁵	Boelens and Oporto ⁴⁸	Dugo <i>et al.</i> ^{19,36} Dugo ⁴¹	Dugo <i>et al.</i> ³⁹	Koketsu <i>et al.</i> ²⁷	Mondello <i>et al.</i> ^{37,40}	Pino and Rosado ²⁹	Veriotti and Sacks ⁵⁸		
	Cultivar		Seville	Seville								
	Growing location		Spain (Andalucia) 1987	Italy (Sicily) 1987	Spain	Italy	Italy	Egypt ripe fruits extraction by Pelatrice	Brazil extraction by FMC	Italy	Cuba	
	Ripening stage											
	α -pinene	0.30-0.65	0.447	0.592	0.383	0.563	0.38	0.34	0.89*	0.62	0.9*	0.94*
	β -pinene	0.30-1.15	0.295*	0.900	0.440	0.940	0.59	0.10*	1.17*	1.01	0.6	0.91
	myrcene	1.60-1.95	1.806	2.073*	1.733	1.789	1.38*	1.56*	3.10*	1.90	4.9*	2.05*
	octanal	X	0.130	0.239	0.065	0.135	0.07	0.07	-*	0.20 (α -phellandrene)	-*	0.03
	limonene	92.95-95.00	94.338	92.430*	93.770	95.363 (<i>p</i> -cymene, β -phellandrene)	93.43	96.52*	92.29*	93.26 (<i>p</i> -cymene)	86.2*	93.42
linalool	0.10-0.35	0.152	0.371*	0.220	0.270	0.30	0.06*	0.21	0.32	0.3	0.10	
decanal	X	0.137	0.193	0.130	0.123	0.07	0.09	0.12	0.13	<0.1	-	
linalyl acetate	0.6-1.02	0.284*	0.775	0.426*	0.894	0.81	0.08*	0.37*	1.04*	-*	0.16*	
geranyl acetate	0.10-0.25	0.133	0.094*	0.142	0.098	0.07*	0.08*	tr	0.12	0.5*	0.11	
β -caryophyllene	0.05-0.15	-*	-*	0.103	0.052	0.04*	0.04*	0.10	0.06	-*	0.05	
germacrene-D	0.08-0.18	-*	-*	0.135	0.108	0.06*	0.03*	-*	0.12	0.2*	-*	
(<i>E</i>)- β -nerolidol	0.06-0.18	-*	-*	0.112	0.062	0.06	0.03*	-*	0.09	-*	-*	
Total volatile compounds identified			44	58	64	60	58	37	54	30	12	
Dilution EO			-	-	1:10 (v/v) in hexane	1:10 (v/v) in hexane		-	1:10 (v/v) in hexane	-	-	
Volume injected			0.2 μ L	-	1 μ L	1 μ L		-	1 μ L	-	0.2 μ L	
Split			-	-	1:25	1:50		-	1:26	-	150:1	
*percentages of peak area out of the ISO range				tr: traces								

TABLE 5

Extraction method		Cold-pressed		Laboratory cold-pressed extraction						
References		ISO standard 9844 ²¹	Boelens and Jimenez ⁵⁶	Gionfriddo <i>et al.</i> ³⁸	Huang and Wu ²⁸		Kirbaslar and Kirbaslar ⁵²			
Cultivar					Morocco	Bangan	Daidai			
Growing location Ripening stage			ripe fruits	Italy (Calabria) 2001	China (Chongqing)		Turkey (Antalya) 1999	Turkey (Antalya) 2000	Turkey (Marmaris) 2000	
ISO standard 9844 compounds	<i>α</i> -pinene	0.30-0.65	0.65	0.367	0.52	0.32	0.51	0.42	0.45	0.39
	<i>β</i> -pinene	0.30-1.15	0.14*	0.749	0.78	0.24*	0.51	0.40	0.42	0.57
	myrcene	1.60-1.95	1.95	1.498*	1.93	1.90	1.89	1.81	1.86	1.73
	octanal	X	0.30	0.146	-	-	-	0.08	0.10	0.16
	limonene	92.95-95.00	93.20	91.522*	92.96	92.53*	94.29	94.25	94.32	93.68
	linalool	0.10-0.35	0.24	0.378*	0.35	0.37*	0.16	0.38*	0.33	0.46*
	decanal	X	0.20	0.137	0	0	0	0.22	0.16	0.21
	linalyl acetate	0.6-1.02	0.28*	0.960	0.73	0.32*	0.32*	1.32*	1.27*	1.17*
	geranyl acetate	0.10-0.25	0.13	0.091*	0.20	0.34*	0.16	0.13	0.08*	0.04*
	<i>β</i> -caryophyllene	0.05-0.15	0.06	0.065	0.07	0.12	0.08	0.09	0.06	0.16*
	germacrene-D	0.08-0.18	0.08	0.094	_*	_*	_*	_*	_*	_*
	(<i>E</i>)- <i>β</i> -nerolidol	0.06-0.18	0.10	0.050*	0.11	0.32*	0.10	0.07	0.05*	0.03*
Total volatile compounds identified			44	46	41	42	43	23	26	27
Dilution EO			-	pure	-		1/20 in hexane			
Volume injected			-	0.3 μL	-		1 μL diluted			
Split			-	1:60	-		split			

* percentages of peak area out of the ISO range

TABLE 5 Continued

Extraction method		Laboratory cold-pressed extraction						
References		Lota <i>et al.</i> ³¹						
Species		<i>C. aurantium</i> L.			<i>C. neo-aurantium</i> Tan.	<i>C. myrtifolia</i> Raf.	<i>C. natsudaidai</i> Hay.	
Cultivar		Espagne	Gou Tou	24 other cultivars of <i>C. aurantium</i> L.**	Tosu	Large Chinotto	Natsudaidai	
Growing location		France (Corsica)						
Ripening stage								
ISO standard 9844 compounds	<i>α</i> -pinene	0.30-0.65	0.4	0.9*	0.4 ± 0.1	0.8*	0.5	0*
	<i>β</i> -pinene	0.30-1.15	1.2*	4.0*	1.0 ± 0.5	5.2*	1.0	0.7
	myrcene	1.60-1.95	1.0*	1.6	1.6 ± 0.1	1.5*	1.8	1.4*
	octanal	X	0	0.1	0.1 ± 0.1	0.1	tr	0.1
	limonene	92.95-95.00	72.9*	84.2*	92.7* ± 1.9	82.2*	94.9	93.5
	linalool	0.10-0.35	5.2*	0.8*	0.3 ± 0.3	1.1*	0.1	0.1
	decanal	X	0	0	0.1 ± 0.1	0.1	0.1	0
	linalyl acetate	0.6-1.02	5.0*	0*	1.2* ± 0.9	0*	0.2*	0.6
	geranyl acetate	0.10-0.25	0.6*	0.1	0.2 ± 0.1	0.1	0.1	0.2
	<i>β</i> -caryophyllene	0.05-0.15	0.9*	0.2*	0.1 ± 0.1	0.1	tr*	0.2*
	germacrene-D	0.08-0.18	2.1*	0.8*	0.2* ± 0.1	1.0*	tr*	0*
	(<i>E</i>)- <i>β</i> -nerolidol	0.06-0.18	3.2*	0.1	0.2 ± 0.1	0.1	0.1	0.4*
Total volatile compounds identified		19	19		27	22	19	
Dilution EO		-						
Volume injected		-						
Split		-						

* percentages of peak area out of the ISO range ** mean \pm standard deviation tr: traces

TABLE 5 Continued

Extraction method		Laboratory cold-pressed extraction									
References	Cold-pressed										
	ISO standard 9844 ²¹	Njoroge <i>et al.</i> ⁴²	Njoroge <i>et al.</i> ⁴³	Sawamura <i>et al.</i> ⁴⁴			Sawamura ⁴⁵			Song <i>et al.</i> ⁴⁶	
Cultivar		Daidai**	Daidai**	Daidai**	Kabusu	Bittersweet	Daidai**	Kabusu	Figaradia Hooker	Tokosu	Daidai**
Growing location		Japan (Kochi) ripe fruits	Japan (Kochi) ripe fruits	Japan	Japan	Italy	Japan	Japan	Japan	Japan	Japan (Kochi) ripe fruits
Ripening stage											
ISO standard 9844 compounds	α -pinene	0.30-0.65	0.470	0.5	0.6	0.28*	0.51	0.54	0.57	0.47	0.6
	β -pinene	0.30-1.15	0.684	0.7	0.7	0.03*	0.18*	0.91	1.05	0.69	0.4
	myrcene	1.60-1.95	1.565*	1.6	1.6	1.73	1.73	1.65	1.68	1.64	2.2*
	octanal	X	0.103	0.1	0.1	0.10	0.09	0.14	0.16	tr	0.6
	limonene	92.95-95.00	94.682	94.7	94.7	92.0*	96.28*	95.85*	92.03*	92.99	94.36
	linalool	0.10-0.35	0.149	0.2	0.2	1.1*	0.34	0.18	1.04*	0.62*	0.15
	decanal	X	0.090	0.1	0.1	0.10	0.07	0.07	0.09	0.01	0.2
	linalyl acetate	0.6-1.02	1.111*	1.1*	0*	0*	0*	0*	0*	0*	1.4*
	geranyl acetate	0.10-0.25	0.085*	-*	0.1	0.1	tr*	0.06*	0.09*	0.13	0.01*
	β -caryophyllene	0.05-0.15	0.042*	<0.05*	-*	-*	tr*	0.02*	0.01*	0.02*	0.18*
	germacrene-D	0.08-0.18	0.090	0.1	-*	-*	0.01*	0.04*	0.04*	0.09	0.47*
	(E)- β -nerolidol	0.06-0.18	0.049*	-*	-*	-*	0*	0.03*	0.04*	0.08	0*
Total volatile compounds identified		62	18	16	12	48	45	44	47	55	54
Dilution EO			pure	pure	pure			pure			-
Volume injected			1 μ L	0.2 μ L	0.1 μ L			1 μ L			-
Split			50:1	32:1	32:1			1:34			1:34

*percentages of peak area out of the ISO range

** *Citrus aurantium* L. var. *cyathifera* Y. Tanaka tr: traces

TABLE 6

	Species and cultivar if indicated	Growing location	Peels state	Mass (g)	Time of HD (hours)	Distillation rate (mL/min)	Details after extraction and before analysis	Yield (as stated in the article)	Yield (g/100g dry matter)
Ali et al. ¹⁸	<i>C. aurantium</i>	Pakistan	fresh	-	6	-	stored in a sealed vial at 4°C	1.0 (mL/100g fresh peel)	0.26
Azhdarzadeh and Hojjati ³³	<i>C. aurantium</i>	Iran (Khouzestan province)	fresh	100	3	-	dried over Na ₂ SO ₄ anhydrous passed through membrane filter (Whatman filter No.1) stored at 4°C	unripe: 0.66% ripe: 0.86%	
Bourgou et al. ⁵¹	<i>C. aurantium</i>	Tunisia (Menzel Bouzelfa)	fresh	100	2	-	dried over Na ₂ SO ₄ anhydrous stored at - 20°C in darkness	Immature: 0.23% Semi-mature: 0.12% Mature: 0.46%	
Boussaada and Chemli ²³	<i>C. aurantium</i> L. ssp. <i>amara</i>	Tunisia (Nabeul)						February: 0.7%	
		Greece (Chania)						0.8%	
Boussaada et al. ²⁶	<i>C. aurantium</i> L. ssp. <i>amara</i>	Brazil						0.8%	
		France (Corsica)	fresh	(peel of ten fresh ripe fruits)	3	-	dried over Na ₂ SO ₄ anhydrous stored in sealed glass vials in a refrigerator at 4-5°C	0.8%	
		Greece (Roxani)						1.1%	
Boussaada and Chemli ⁶⁵	<i>C. aurantium</i> L. ssp. <i>amara</i>	-						November: 1.0% December: 1.2% January: 1.3% February: 0.8%	
Caccioni et al. ⁶	<i>C. aurantium</i>	Italy (Lentiny)	fresh at ripening stage	500 to 900	until there was no significant increase in the volume of oil collected	-	dried over Na ₂ SO ₄ anhydrous stored under N ₂ in sealed vials	-	

TABLE 6 Continued

	Species and cultivar if indicated	Growing location	Peels state	Mass (g)	Time of HD (hours)	Distillation rate (mL/min)	Details after extraction and before analysis	Yield (as stated in the article)	Yield (g/100g dry matter)
Gonzalez de et al.⁵⁴	<i>C. aurantium</i> L. ssp. <i>amara</i>	Venezuela (Tachira)	fresh	-	-	-	-	-	-
Hosni et al.⁵⁰	<i>C. aurantium</i> L.	Tunisia (Zaghuan)	air dried ground	100	3	-	dried over Na ₂ SO ₄ anhydrous stored in amber and air-tight sealed vials at 0°C	1.24 (g/100g dry peel)	1.12
Jabri Karoui and Marzouk⁴	<i>C. aurantium</i> L.	Tunisia (Cape Bon)	fresh	100	1.5	-	collected in diethyl ether using liquid-liquid isolation	-	-
Protopapadakis and Papanikolaou³²	<i>C. aurantium</i> L. Chania	Greece (La Canée)	dried cut into small pieces	-	-	2 to 3	method of the European Pharmacopoeia	0.78 (mL/100g dry peel)	0.60
	<i>C. aurantium</i> L. Brazilian		immersed in water for 2 hours					0.48 (mL/100g dry peel)	0.37
	<i>C. aurantium</i> L. Keen		before					0.78 (mL/100g dry peel)	0.60
	<i>C. aurantium</i> L. Bittersweet							1.09 (mL/100g dry peel)	0.83
Rowshan and Najafian⁷	<i>C. aurantium</i> L.	Iran	air-dried at ambient temperature in the shade until semi-dried	100	3	2 to 3	method of the European Pharmacopoeia. dried over Na ₂ SO ₄ anhydrous and stored in sealed vials at 4°C	-	-
Sanei-Dehkordi et al.³⁴	<i>C. aurantium</i>	Iran (Babol)	fresh	50	3	-	dried over Na ₂ SO ₄ anhydrous and stored into an air-tight amber coloured vial at 4°C	0.7 ± 0.12 (g/100g fresh peel)	0.21
Sarrou et al.⁵	<i>C. aurantium</i> L.	Greece	fresh	35	3	3 to 3.5	dried over Na ₂ SO ₄ anhydrous and stored at 4-6°C	1.67 (mL/100g fresh peels)	0.43

TABLE 7

	Extraction method	cold-pressed	hydrodistilled oils											
		ISO standard 9844 ²¹	Ali <i>et al.</i> ¹⁸	Azhdarzadeh and Hojjati ³³	Bourgou <i>et al.</i> ⁵¹	Boussaada and Chemli ²³	Boussaada <i>et al.</i> ²⁶			Boussaada and Chemli ⁶⁵				
	Cultivar	Larange												
	Growing location Ripening stage Harvest date	Pakistan	Iran (Khouzestan province) ripe peel	Tunisia (Menzel Bouzelfa) ripe peel	Tunisia (Nabeul)	Greece (Chania Crete)	Brazil	France (Corsica)	Greece (Roxani Crete)	November	December	January	February	
ISO standard 9844 compounds	α -pinene	0.30-0.65	0.6	1.96*	0.36	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.3	
	β -pinene	0.30-1.15	0.21*	0.56*	0.38	0.3	0.5	0.2*	0.5	0.3	0.2*	0.3	0.3	
	myrcene	1.60-1.95	2.57*	5.74*	0.07*	1.6	1.5*	1.6	1.6	1.6	1.7	1.6	1.6	
	octanal	X	0.14	0.83	-	-	-	-	-	-	-	-	-	
	limonene	92.95-95.00	91.98*	81.6*	90.95*	90.6*	89.6*	93	91.8	92*	87.7*	88*	92.2	
	linalool	0.10-0.35	1.78*	2.19*	0.1*	1.5*	2*	0.8*	1.4*	1*	1.3*	1*	0.8*	
	decanal	X	0.08	-	-	-	-	-	-	-	-	-	-	
	linalyl acetate	0.6-1.02	-*	0*	0.01*	0.2*	0.5*	0.2*	0.3*	0.2*	0.1*	0.2*	0.2*	
	geranyl acetate	0.10-0.25	0.08*	0.33*	0.02*	0.2	0.3*	0.2	0.3*	0.2	0.2	0.2	0.2	
	β -caryophyllene	0.05-0.15	0.05	0.22*	-*	tr*	tr*	tr*	tr*	tr*	tr*	tr*	tr*	
	germacrene-D	0.08-0.18	0.06*	0.22*	0.04*	-*	-*	-*	-*	-*	-*	-*	-*	
	(E)- β -nerolidol	0.06-0.18	0.05*	0.09	-*	0.1	0.1	0.1	0.1	0.1	tr*	tr*	tr*	
	Total volatile compounds identified		25	39	31	26		26			26			
	Dilution EO	pure	pure	-	1% solution (diluted in pentane)		-			1% solution (diluted in pentane)				
	Volume injected	1 μ L	0.5 μ L	-	0.1 μ L		-			0.1 μ L				
	Split	1:50	-	60:1	1:50		-			1:50				

* percentages of peak area out of the ISO range

TABLE 7 Continued

Extraction method	cold-pressed					hydrodistilled oils							
	ISO standard 9844 ²¹	Caccioni <i>et al.</i> ⁶	Gonzalez de <i>et al.</i> ⁵⁴	Hosni <i>et al.</i> ⁵⁰	Jabri Karoui and Marzouk ⁴	Protopapadakis and Papanikolaou ³²				Rowshan and Najafian ⁷	Sanei-Dehkordi <i>et al.</i> ³⁴	Sarrou <i>et al.</i> ⁵	
Cultivar						Chania	Brazilian	Keen	Bittersweet				
Growing location		Italy (Lentiny)	Venezuela (Tachira)	Tunisia (Zaghuan)	Tunisia (Cape Bon)		Greece (La Canée)			Iran mature: orange	Iran (Babol)	Greece	
Ripening stage													
Harvest date													
ISO standard 9844 compounds	α -pinene	0.30-0.65	0.40	0.64	0.27*	0.55	0.38	0.38	0.39	0.37	0.43	0.30	0.53
	β -pinene	0.30-1.15	0.33	3.25*	1.37*	0.44	0.51	0.23*	0.48	0.33	0.1*	0.65	0.62
	myrcene	1.60-1.95	1.88	1.74	0*	0.04*	1.55*	1.62	1.61	1.60	1.78	1.00*	2.00*
	octanal	X	0.08	0	-	-	-	-	-	-	0.68	-	0.24
	limonene	92.95-95.00	94.27	90.04*	96.9*	90.25*	89.95*	93.00	91.80*	92.20*	93.47*	94.81	94.67
	linalool	0.10-0.35	0.8*	2.87*	0.17	1.56*	1.97*	0.82*	1.44*	1.05*	1.25*	-*	0.76*
	decanal	X	0.11	-	-	-	-	-	-	-	0.20	-	0.16
	linalyl acetate	0.6-1.02	-*	-*	0*	0.29*	0.47*	0.19*	0.28*	0.21*	0.22*	0.32*	0.18*
	geranyl acetate	0.10-0.25	0*	-*	0*	0.11	0.26*	0.23	0.25	0.22	0.13	0.13	0*
	β -caryophyllene	0.05-0.15	0.07	-*	-*	0.05	-*	-*	-*	-*	0*	0.03*	0*
	germacrene-D	0.08-0.18	-*	-*	tr*	-*	-*	-*	-*	-*	0*	0.08	0*
	(E)- β -nerolidol	0.06-0.18	0.07	-*	0*	0.35*	-*	-*	-*	-*	-*	0.06	0*
	Total volatile compounds identified		36	7	13	27	21				24	21	12
Dilution EO		-	-	-	-	1:9 in pentane				-	-	-	
Volume injected		-	-	-	-	1 μ L				-	-	-	
Split		-	-	-	60:1	-				1:50	-	1:30	

* percentages of peak area out of the ISO range

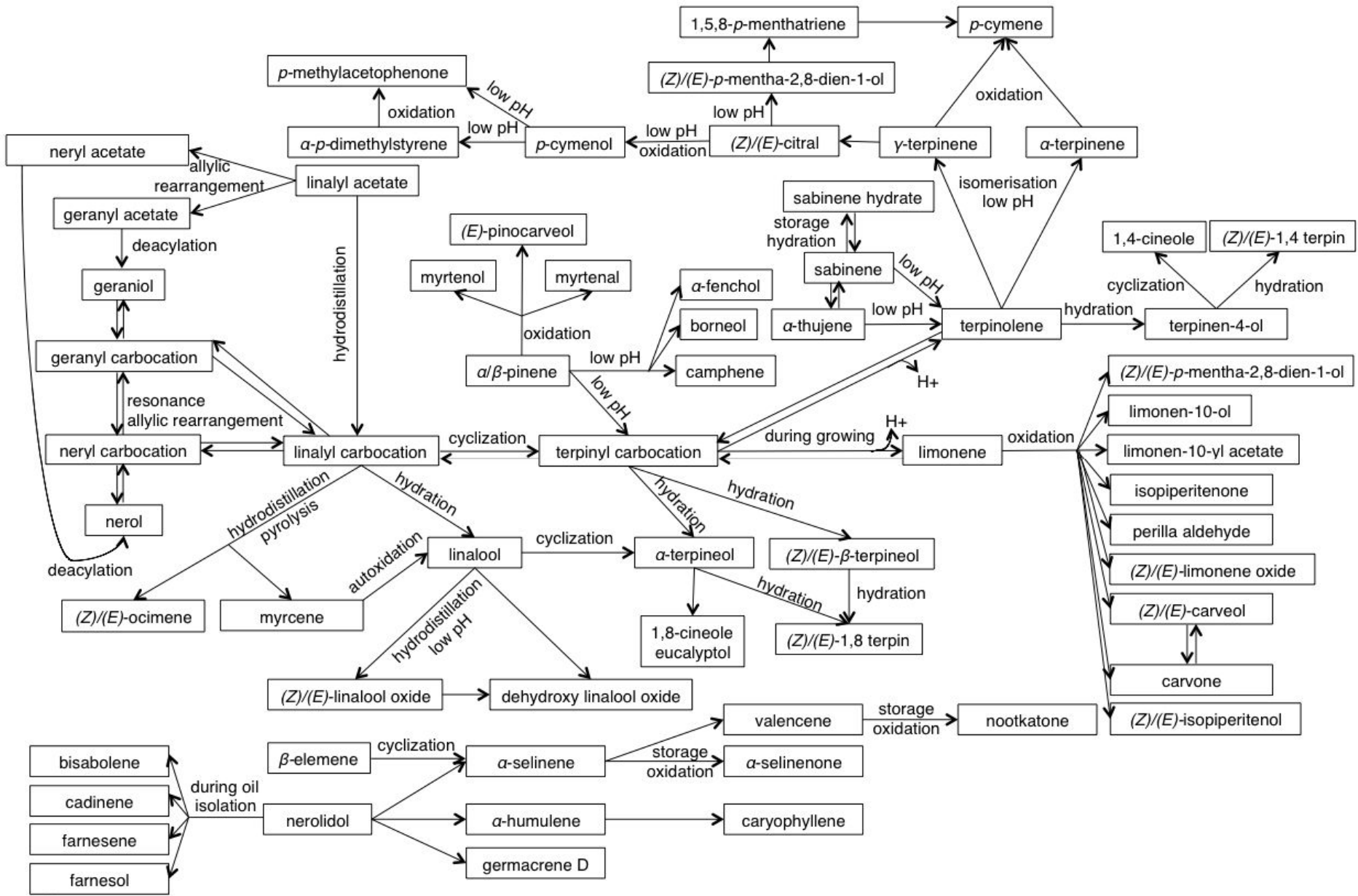
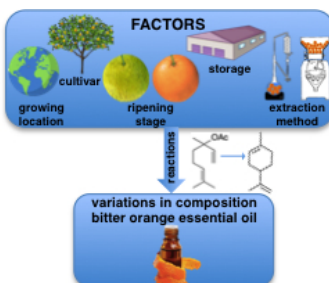


FIGURE 1

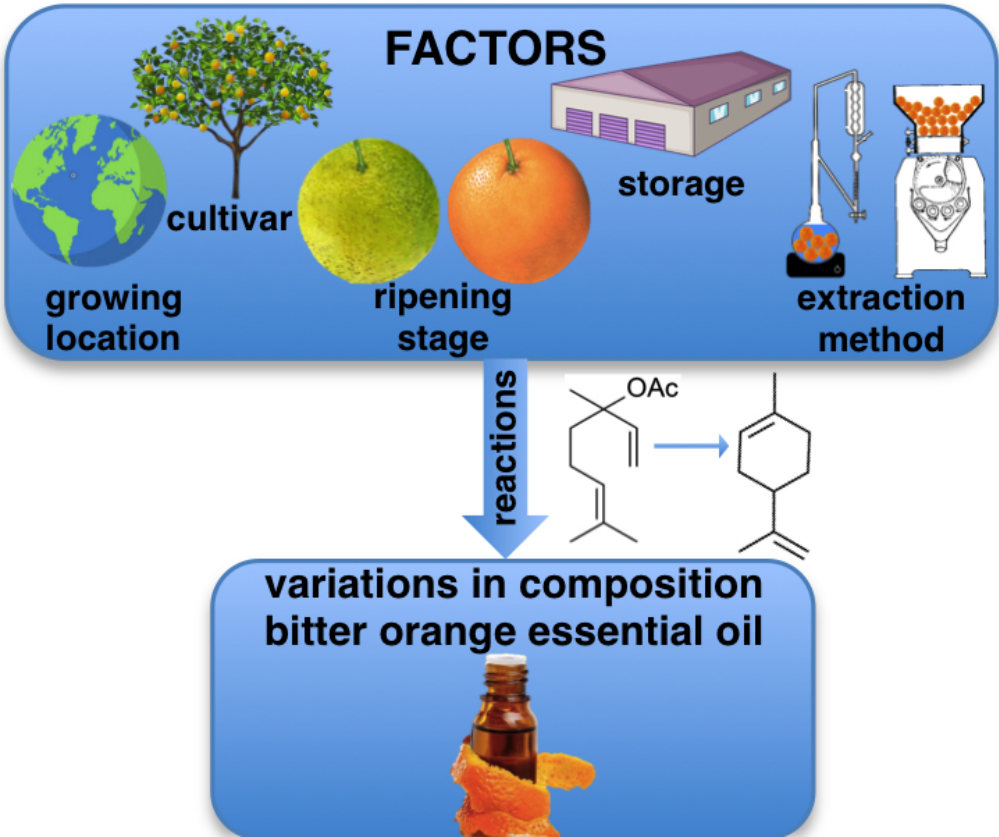
The influence of the growing location, the cultivar, the ripening stage, the storage and the extraction method on the composition of bitter orange peel essential oil is critically reviewed. The chemical reactions underlying the changes in composition due to these factors are presented.



Bitter orange peel essential oil: a review of the different factors and chemical reactions influencing its composition

Marion Gaff, Martine Esteban-Decloux and Pierre Giampaoli

254x70mm (72 x 72 DPI)



253x211mm (72 x 72 DPI)