



**HAL**  
open science

# 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-03667282v1>**

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<sup>a</sup>, Martine ESTEBAN-DECLoux<sup>a</sup>, Pierre GIAMPAOLI<sup>a,\*</sup>*

<sup>a</sup> 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](mailto: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.<sup>1</sup> The fruits from bitter orange tree are cultivated for the preparation of marmalade,<sup>1,2</sup> 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.<sup>3</sup> It was also reported that bitter orange peel oil can be used for its antioxidant properties<sup>4,5</sup> and its antimicrobial activity.<sup>6</sup>

Essential oils (EO) are mostly obtained by hydrodistillation.<sup>7</sup> 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, Boelens<sup>8</sup> 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,<sup>9</sup> Lawrence,<sup>10</sup> and Swaine and Swaine<sup>11</sup> due to the misunderstanding of publications originally from Ortiz *et al.*<sup>12</sup> and Maekawa *et al.*<sup>13</sup> 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 system<sup>14</sup> which lists 162 species and the Swingle system<sup>15</sup> which lists only 16 species.<sup>16</sup> 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.<sup>17</sup>

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.<sup>18,19</sup> Obviously, the non-volatile residue is almost absent in oils obtained by hydrodistillation.<sup>19,20</sup>

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.<sup>7</sup> 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 requirements<sup>21</sup> 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:  $\alpha$ -pinene,  $\beta$ -pinene, myrcene, limonene, linalool, linalyl acetate, geranyl acetate,  $\beta$ -caryophyllene, germacrene D, (*E*)- $\beta$ -nerolidol.<sup>21</sup> 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.<sup>21</sup> 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.*<sup>19</sup>

### 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.<sup>22</sup> 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 Chemli<sup>23</sup> and Kale and Adsule.<sup>2</sup> 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,

1  
2  
3 Paraguay, and China.<sup>24,25</sup> The different growing locations of bitter orange EO that were  
4 studied in the literature are: Brazil,<sup>26,27</sup> China,<sup>28</sup> Cuba,<sup>29</sup> Egypt,<sup>30</sup> France,<sup>26,31</sup> Greece,<sup>5,26,32</sup>  
5 Iran,<sup>7,33,34</sup> Italy,<sup>6,35-40</sup> Ivory Coast,<sup>41</sup> Japan,<sup>42-46</sup> Pakistan,<sup>18</sup> Spain,<sup>35,47-49</sup> Tunisia,<sup>4,23,50,51</sup>  
6 Turkey,<sup>52,53</sup> United States,<sup>12</sup> Venezuela,<sup>54</sup> West Indies,<sup>55</sup> and several from unspecified  
7 geographical origins.<sup>23,56-58</sup> Even if Argentina, Haiti, Morocco, Uganda, and Paraguay are in  
8 the top fifteen of the world major producing countries of bitter orange oil,<sup>24,25</sup> to our  
9 knowledge the composition of the bitter orange peel cultivated in those countries has never  
10 been studied in the literature. Actually, the composition of a bitter orange essential oil from  
11 Argentina was reported by De La Torre and Sardi<sup>59</sup>, but as stated by Dugo *et al.*<sup>60</sup> the  
12 composition is too different from a bitter orange oil to be genuine. Abderrezak *et al.*<sup>61</sup>  
13 reported the analysis of a bitter orange peel essential oil from Algeria, but 0.25% limonene is  
14 not compatible with the chemotype of a bitter orange peel oil. Thus, these two studies were  
15 not taken into account in the present review.

16  
17  
18  
19  
20  
21 Some authors tried to compare bitter orange peel oils from different origins using previous  
22 studies of different authors.<sup>41,52</sup> It can be very tricky and conclusions should be taken with  
23 cautiousness because many factors that could influence the bitter orange oil composition are  
24 unknown. As a consequence, when combining results from different authors, the growing  
25 location may not be the only parameter under review.

26  
27  
28 For instance, Dugo<sup>41</sup> compared the composition of bitter orange peel oils from Italy, Spain,  
29 Ivory Coast and Brazil, but the cultivars were not specified for the Italian, the Ivorian, and the  
30 Brazilian EO, whereas Lota *et al.*<sup>31</sup> highlighted that the cultivar can influence a lot the  
31 composition of bitter orange peel oil. Moreover, the differences in the percentages of linalool  
32 and linalyl acetate were attributed to the different growing locations whereas these two  
33 percentages are more likely to be representative of the ripening stage of the bitter oranges  
34 (which are not specified) than of their growing location. Indeed, linalyl acetate and linalool  
35 drastically decrease when citrus are ripening.<sup>62</sup> Finally, designating more precise locations  
36 would be more relevant, as it would be hard to conclude on the influence of a growing  
37 location as big as Brazil.

38  
39  
40  
41 Boussaada *et al.*<sup>26</sup> compared the composition of bitter orange peel oils from Chania (Greece),  
42 Roxani (Greece), Corsica (France), and Brazil. They mentioned being cautious in comparing  
43 the essential oil from trees of approximately the same age, disease-free and submitted to the  
44 same horticultural conditions. Nevertheless, none of the cultivars was specified. And once  
45 more, the name of the location where the trees were grown in Brazil, was not stated.

46  
47  
48  
49 Kirbaslar and Kirbaslar<sup>52</sup> compared the volatile composition of their Turkish EO with the  
50 Spanish and Italian EO of Boelens and Jimenez.<sup>35</sup> They concluded that percentages of  
51 nonanal, tetradecanal, myrtenol, neral, nootkatone and octanol were higher than 0.01% in  
52 Italian and Spanish oils, whereas these compounds were not detected in Turkish oil. Actually,  
53 the fact that fewer compounds were detected could possibly be due to an analysis method with  
54 a more important dilution of the EO. Indeed, Kirbaslar and Kirbaslar<sup>52</sup> did a split injection of  
55 1 µL of Turkish EO diluted 1/20 in hexane (no mention of the split ratio used, neither if the  
56 dilution was by weight or by volume), and they identified between 23 and 27 compounds in  
57 their samples, which is rather low compared to other studies (Tables 4, 5 and 7).

1  
2  
3 Abderrezak et al.<sup>61</sup> claimed that they did a comparative study of the compositions of essential  
4 oils of citrus growing in different countries, but they did not take into account the extraction  
5 method used by the different authors and compared cold-pressed oils with hydrodistilled oils.  
6 Not to mention that the cultivars and the analysis methods were also different between the  
7 EO.  
8  
9

10 Boelens and Jimenez<sup>35</sup> are the only authors who accurately assessed the impact of growing  
11 location on the bitter orange EO composition, using bitter oranges of the same cultivar, the  
12 same year of harvest, with a similar extraction method (cold-pressing) and the same  
13 chromatographic method. Though, there were a few errors in this article in the table listing the  
14 chemical composition of Mediterranean orange oils: (1) octanol and decanol as well as octyl  
15 acetate and decyl acetate were classified in aliphatic aldehydes, hopefully not counted in this  
16 class, but a separate class for aliphatic alcohols and esters should have been created, (2) the  
17 total of aliphatic aldehydes of bitter orange oil from Italy is 0.631, not 0.531, (3) the total of  
18 monoterpenes of bitter orange oil from Spain is 97.406, not 96.85, (4) the total of  
19 monoterpenes of bitter orange oil from Italy is 96.873, not 96.83. The conclusions drawn by  
20 these authors were that  $\alpha$ -pinene,  $\beta$ -pinene, sabinene, aldehydes, and monoterpenoid alcohols  
21 and esters were more concentrated in Sicilian bitter orange oil than in Andalucian oil, whereas  
22 limonene, sesquiterpenes, nerolidol and nootkatone were less concentrated. These conclusions  
23 are based on the analysis of only one sample of each growing location. More studies on the  
24 impact of growing location on the bitter orange EO composition would be needed to be able  
25 to affirm that these conclusions could be generalized to the influence of coastal climate  
26 (Sicilian) and desert-like climate (Andalucian).  
27  
28  
29  
30  
31  
32

33 Thus, not much can be concluded for sure on the influence of the country in which bitter  
34 orange trees are grown on the composition of the essential oil.  
35  
36

## 37 4.2 Cultivars

38  
39 The study by Ortiz *et al.*<sup>12</sup> was meant to compare essential oils obtained by the same  
40 laboratory extraction method, from numerous cultivars of bitter orange harvested in the same  
41 period (June-July), all cultivated in the Citrus Research Centre at Riverside in California.  
42  
43

44 Ortiz *et al.*<sup>12</sup> are not the only authors who rigorously examined the influence of the cultivar on  
45 the volatile composition of the bitter orange peel oil. More recently, Lota *et al.*<sup>31</sup> compared  
46 the composition of 30 different cultivars of bitter orange EO while making sure that the  
47 growing location (Corse, France), the cultural conditions, as well as the laboratory extraction  
48 method were the same, so that the only parameter under review during the study was the  
49 cultivar. They drew the conclusion that the 26 cultivars of *Citrus aurantium* L. that they  
50 studied exhibited a weak chemical variability, except for the Espagne and the Gou Tou  
51 cultivar, and were characterized by limonene as majority component. Actually, limonene was  
52 also the majority component for the Espagne and the Gou Tou cultivar, but to a far less extent  
53 (72.9% peak area for the Espagne cultivar and 84.2% peak area for the Gou Tou cultivar  
54 versus between 87.6% to 95.1% peak area for the 24 other *Citrus aurantium* L. cultivars). The  
55 fact that the peel EO of the Gou Tou cultivar differed from most of the *Citrus aurantium* L. is  
56 probably linked to its supposed natural hybrid origin (possible hybrid between *Citrus*  
57  
58  
59  
60

1  
2  
3 *aurantium* L. and *Citrus grandis*).<sup>63,64</sup> The peel EO of the Tosu cultivar belonging to the  
4 species *Citrus neo-aurantium* Tan. was similar to the Gou Tou cultivar: a lower percentage of  
5 limonene and a higher percentage of  $\beta$ -pinene than the group of 24 cultivars of *Citrus*  
6 *aurantium* L.. As far as *Citrus myrtifolia* Raf. is concerned, the peel EO of the Large Chinotto  
7 cultivar had a profile similar to the 24 cultivars of *Citrus aurantium* L.. The peel EO of the  
8 monovarietal *Citrus natsudaidai* Hay. also had a profile close to the 24 cultivars of *Citrus*  
9 *aurantium* L., despite the fact that it does not contain  $\alpha$ -pinene.  
10  
11  
12

### 13 4.3 Ripening stages

14  
15 Several studies concerning the influence of the ripening stages of bitter oranges on peel oil  
16 composition have been reported in the literature.<sup>7,30,33,39,51,56,65</sup> Citrus are non climacteric,  
17 which means that once fruits are harvested, the maturation process stops and they do not ripen  
18 further.<sup>1</sup> The colour of the peel is an indicator of the maturity of the fruit. When the bitter  
19 orange is immature the peel is dark green. As it ripens, it passes through light green, then  
20 yellow, and finally orange at the mature stage. The evolution of the gas chromatography  
21 percentages of the compounds during the growing and the ripening of bitter oranges are listed  
22 in Table 2. Only the compounds listed in the ISO standard 9844 are taken into account.  
23  
24  
25

26  
27 First of all, one should be careful with the wording chosen by the authors of the studies  
28 dealing with ripening stages, when in need to compare those results. When Ashour and El-  
29 Kebeer<sup>30</sup> referred to immature fruits, they meant fruits which have reached the size of a bitter  
30 orange but were still green in colour, whereas when Rowshan and Najafian<sup>7</sup> referred to  
31 immature fruits, they meant green fruits of the size of a lemon, that is to say smaller than a  
32 bitter orange. Thus, the immature stage of Ashour and El-Kebeer<sup>30</sup> seems to be equivalent to  
33 the semi-mature stage of Rowshan and Najafian<sup>7</sup>. Moreover, the size criteria chosen by  
34 Rowshan and Najafian<sup>7</sup> and by Ashour and El-Kebeer<sup>30</sup> to determine maturity were rather  
35 imprecise since no real measurement was provided. Ashour and El-Kebeer<sup>30</sup> called setting  
36 fruits, green fruits that were really small in size. According to the analysis results of the  
37 setting fruits, it can be assumed that setting fruits were even less mature than the immature  
38 stage stated by Rowshan and Najafian<sup>7</sup>. Azhdarzadeh and Hojjati<sup>33</sup> compared unripe peels and  
39 ripe peels with no further details on the maturity stage (colour, size of the fruits, etc...). From  
40 the analysis results of the unripe peels, one can hypothesize that they were slightly more ripe  
41 than the setting fruits of Ashour and El-Kebeer<sup>30</sup> and less ripe than the immature fruits of  
42 Rowshan and Najafian<sup>7</sup>. Bourgou *et al.*<sup>51</sup> defined the immature, semi-mature and mature fruits  
43 by their colour, just as Dugo *et al.*<sup>39</sup> used the colour to define ripe and unripe fruits. Once  
44 more, neither of them gave any details on the precise colour, which could have been measured  
45 with a colorimeter for instance, nor on the size measurement of the fruit.  
46  
47  
48  
49  
50  
51

52  
53 Boussaada and Chemli<sup>65</sup> found that the percentage of limonene rises at its maximum in the  
54 middle of the productive period when fruits are normally harvested, and then decreases in the  
55 end of the productive period to arrive at a percentage that is approximately the same as in the  
56 beginning (Table 2). Rowshan and Najafian<sup>7</sup> observed that the highest percentage of limonene  
57 was obtained for the semi-mature stage (green colour, size of a bitter orange) and slightly  
58 decrease at the mature stage (orange colour). The lowest percentage of limonene is at the  
59 immature stage (green colour, size of a lemon). Ashour and El-Kebeer<sup>30</sup> found that during the  
60



1  
2  
3 ripening of the fruits (from immature to ripening fruits, no changing in size but changing of  
4 colour), the percentages of limonene slightly decreased from 95.25% to 92.68%. The different  
5 authors, except Bourgou *et al.*<sup>51</sup>, agree on the fact that limonene is formed during the growing  
6 of the bitter orange and slightly decreases during the ripening of the bitter orange. Actually,  
7 the analysis results of the semi-mature bitter orange peel obtained by Bourgou *et al.*<sup>51</sup> seem  
8 suspect: limonene percentage of only 67.90% and products of hydration and oxidation  
9 percentages (1,8-cineole, (*Z*)-sabinene hydrate, octanol, (*Z*)-linalool oxide, terpinen-4-ol)  
10 higher than at the immature and the mature stage.  
11  
12  
13

14 The compositions of unripe and ripe bitter orange peel obtained by Dugo *et al.*<sup>39</sup> are very  
15 close to one another, apart from linalyl acetate, (*E*)- $\beta$ -ocimene, (*E*) and (*Z*)-linalool oxide  
16 which are higher in unripe peel.  
17  
18

19 In Boussaada and Chemli<sup>65</sup> study, the linalool percentage gradually decreases along the  
20 productive period. Rowshan and Najafian<sup>7</sup> noticed that linalool percentage was maximum at  
21 the immature stage and deeply decreases as the fruit grows and ripens. It is suggested that  
22 limonene is formed during growing by decomposition of the linalool and the linalyl  
23 acetate.<sup>47,65</sup>  
24  
25

26 Ashour and El-Kebeer<sup>30</sup> noticed that during growing of bitter oranges (from setting fruits,  
27 which are really small in size, to immature fruits, which reached the size of the bitter orange  
28 but are still dark green in colour)  $\beta$ -pinene, ocimene, linalool, linalyl acetate, and  $\alpha$ -terpineol  
29 decreased, while limonene increased from 46.19% to 95.25%. Ocimene and linalool were not  
30 present any more in the ripening fruit.<sup>30</sup>  
31  
32

33 As far as  $\beta$ -pinene is concerned, Rowshan and Najafian<sup>7</sup> came to the same conclusion as  
34 Ashour and El-Kebeer<sup>30</sup>:  $\beta$ -pinene decreased during growing. But contrary to Ashour and El-  
35 Kebeer<sup>30</sup>, Rowshan and Najafian<sup>7</sup> found that  $\alpha$ -pinene and myrcene decreased during  
36 growing.  
37  
38

39 Boussaada and Chemli<sup>65</sup> concluded that  $\beta$ -pinene and  $\alpha$ -terpinolene percentages showed a  
40 slight increase throughout the productive season. But, is this slight increase (from 0.2% to  
41 0.3% and from 0.4% to 0.6% respectively) sufficient to be significant? The percentages of the  
42 other monoterpenes were considered as being constant from unripe to ripe citrus oil.  
43  $\alpha$ -terpineol, geraniol, nerol and geranyl acetate percentages were said to be fairly constant in  
44 all the oils from unripe and ripe fruits. Actually, geraniol varies from 0.1% to 0.2% and  
45 geranyl acetate from 0.2% to 0.3%. Boelens and Jimenez<sup>56</sup> also came to the conclusion that  
46  $\alpha$ -terpineol, geraniol, and nerol were fairly constant during the ripening of bitter oranges,  
47 whereas geranyl acetate and neryl acetate increased from 0.06% to 0.13% and from 0.02% to  
48 0.04% respectively.  
49  
50  
51

52 As far as octanal and decanal are concerned, Azhdarzadeh and Hojjati<sup>33</sup>, Ashour and El-  
53 Kebeer<sup>30</sup>, Boelens and Jimenez<sup>56</sup>, Rowshan and Najafian<sup>7</sup>, as well as Dugo *et al.*<sup>39</sup> to a lesser  
54 extent, all agree that these two aldehydes increase during the ripening of bitter oranges.  
55  
56

57 Boelens and Jimenez<sup>56</sup> pointed out that the oxygen-containing sesquiterpenes (nerolidol,  
58 farnesol, nootkatone and  $\alpha$ -selinenone) appear to be formed during the ripening.  
59  
60

1  
2  
3 All those authors drew conclusions on the evolution of the peel oil composition during  
4 ripening based on comparisons of relative percentages of peak area. None of them quantified  
5 using external or internal standard, or standard additions.  
6  
7

#### 8 4.4 Storage conditions 9

10 Boelens and Jimenez<sup>56</sup> studied the impact of one to two weeks storage on picked ripe fruits.  
11 They observed that linalool, linalyl acetate, nerolidol, nootkatone and  $\alpha$ -selinenone kept on  
12 increasing after picking up the fruits, and attributed the formation of these compounds to  
13 enzymatic reactions. In plants, endogenous glycosidases are involved in enzymatic hydrolysis  
14 of the glycosidic bond of glycosidic aroma precursors, releasing free aroma compounds.<sup>66</sup>  
15  
16

17 Njoroge *et al.*<sup>43</sup> studied the impact of storage conditions (temperature and time of storage) on  
18 the volatile composition of Daidai (*Citrus aurantium* L. var. *cyathifera* Y. Tanaka)  
19 cold-pressed peel oil (Table 3). Artefact compounds may be formed during storage of  
20 essential oils upon oxidation, heat or chemical interactions between the compounds that are  
21 initially present in the oil.<sup>43</sup> No changes were found over time when cold-pressed Daidai oil  
22 was stored at -21°C. Changes occurred in the Daidai oil stored several months at 5°C and  
23 20°C. The longer the oil was stored, the more important the changes in the composition were.  
24 Limonene, myrcene,  $\gamma$ -terpinene and linalyl acetate percentages decreased when the  
25 temperature and the time of storage increased. The decreases of myrcene and  $\gamma$ -terpinene were  
26 observed when stored at 20°C or when stored 12 months at 5°C (Table 3). Under the same  
27 conditions, terpinolene, *p*-cymene,  $\alpha$ -terpineol, thujol and octyl acetate slightly increased,  
28 while (*Z*)-carveol, limonene-10-yl acetate, (*E*)-*p*-mentha-2,8-dien-1-ol, linalool, nonyl acetate,  
29 decyl acetate,  $\beta$ -caryophyllene, (*E*)- $\beta$ -farnesene,  $\alpha$ -humulene sharply increased. Germacrene  
30 D decreased until disappearing. Some compounds that did not exist in the fresh oil were  
31 formed upon storage: carvone, sabinene hydrate, (*Z,E*)-farnesol, and several oxygen  
32 derivatives of the farnesene group. Their relative percentages increased with time and  
33 temperature. Surprisingly neither linalool oxides nor limonene oxides were identified in the  
34 oils after long storage periods.  
35  
36  
37  
38  
39  
40  
41

#### 42 4.5 Extraction methods 43

44 In the literature, the extraction methods used to obtain peel oil from citrus are: cold pressing,  
45 hydrodistillation, supercritical carbon dioxide<sup>57</sup> or solvent<sup>67</sup> extraction. The most common  
46 methods are cold pressing and hydrodistillation, and the result of extraction is not called  
47 essential oil when using supercritical carbon dioxide or solvent extraction.  
48  
49

##### 50 4.5.1 Cold pressing 51

52 On an industrial scale, bitter orange essential oils are obtained by cold pressing. All the  
53 different mechanical systems for cold extraction of citrus oils are based on the following  
54 steps.<sup>68,69</sup> First, mechanical action on fruits or on peels breaks essential oil glands (utricle)  
55 and releases the EO. Then, the citrus oil is dispersed in water which acts as a vehicle agent. In  
56 most cases, the aqueous phase is continuously recycled to minimize the loss of oxygenated  
57 compounds. Finally, the emulsion is centrifuged to obtain pure EO. The water-soluble  
58 compounds are lost. The different methods are Sfumatrice, Torchi, Pelatrice, Brown Oil  
59  
60

1  
2  
3 Extractor and FMC (Food Machinery Corporation, now called JBT).<sup>70</sup> Sfumatrice machines  
4 work on peels without pulp; now these are not widely used and are replaced by Torchi, which  
5 work on the entire fruits. Pelatrice machines work on the entire fruit, and the EO is obtained  
6 by rolling the fruit over spikes. The Brown Oil Extractor is quite similar to the Pelatrice  
7 method.<sup>71</sup> FMC machines also process the entire fruit: the extraction of juice and EO are  
8 performed simultaneously. The aqueous phase that carries the EO is easily recycled when oil  
9 is produced using the Pelatrice and FMC processes. The ability to recycle the aqueous phase  
10 is more difficult for Sfumatrice and Torchi machines because of the rapid increase of pectins  
11 in the aqueous phase.<sup>41,72</sup>  
12  
13  
14

15 On a laboratory scale, three different types of cold extraction were found in the bibliography.  
16 (1) Lota<sup>73</sup> used a semi-automatic method (« Roze-Schwob ») consisting of two jaws that  
17 rotate the bitter orange and a sharp point which breaks the oils glands.<sup>3</sup> The dispersion that  
18 was released (mix of EO and aqueous phase) was then centrifuged to obtain pure EO. (2)  
19 Kirbaslar and Kirbaslar,<sup>52</sup> Njoroge *et al.*,<sup>42,43</sup> and Sawamura<sup>45</sup> applied manual pressure on the  
20 flavedo to release the oil. Njoroge *et al.*<sup>42,43</sup> added brine to the oil. Then, the oil was  
21 centrifuged and dried over anhydrous sodium sulphate. (3) Boelens and Jimenez<sup>35,56</sup> extracted  
22 the peel oil by needle puncturing followed by centrifugation.  
23  
24  
25

26 Tables 4 and 5 compare the volatile composition of bitter orange essential oils obtained by  
27 cold pressing on an industrial scale and a laboratory scale respectively. The compositions  
28 reported in table 4 and 5 are not exhaustive since some authors found more than 50 volatiles,  
29 but focused on the compounds that are found in bitter orange EO according to the ISO  
30 standard.  
31  
32

33 Among all the gas chromatographic analysis results of cold-pressed bitter orange oil that can  
34 be found in the literature, the only ones which are in accordance with the ISO standard 9844  
35 are Dugo *et al.*<sup>19,36</sup> and Mondello *et al.*<sup>37,40</sup> Veriotti and Sacks<sup>58</sup> are the ones who identified  
36 the lowest number of compounds in cold-pressed bitter orange oil. The chromatographic  
37 method used is to blame: the lowest volume injected and the highest split of all the studies on  
38 bitter orange, when specified (Table 4).  
39  
40  
41

#### 42 43 4.5.2 Hydrodistillation

44 Hydrodistillation is used to extract bitter orange essential oil on a laboratory scale for analysis  
45 and quantification of oil contained in the peels. To our knowledge, all the bitter orange  
46 hydrodistilled oils were obtained using a Clevenger type apparatus. The Table 6 gives  
47 exhaustive details on the different methods used to obtain bitter orange essential oil with a  
48 Clevenger apparatus.<sup>5-7,18,23,26,32,50,54,65</sup>  
49  
50  
51

52 In general, authors give very few information on the methods employed for peel  
53 hydrodistillation. Gorgorcena and Ortiz<sup>49</sup> also used hydrodistillation to extract essential oil  
54 from bitter orange but they were not recorded in table 6 because no details are given on the  
55 method. The study by Gonzalez de *et al.*<sup>54</sup> is quite terse. The methods used are not detailed  
56 and only seven compounds were identified in this Venezuelan oil, but this can probably be  
57 attributed to the analysis method not being adapted since very few compounds were also  
58 found in the other *Citrus* species of this study. The essential oils can be obtained by  
59  
60

1  
2  
3 hydrodistillation using dried or fresh peels. The mass of bitter orange peel used for one  
4 extraction varies quite a lot: from 35 g of fresh peels to 100 g of dried peels. The time of  
5 hydrodistillation varies from 1.5 hours<sup>4</sup> to 6 hours.<sup>18</sup> The only article which specifies the  
6 distillate flow rate (3 to 3.5 mL/min) is Sarrou *et al.*<sup>5</sup> It can be assumed that the extractions  
7 by Protopapadakis and Papanikolaou<sup>32</sup> and Rowshan and Najafian<sup>7</sup> were done with a distillate  
8 flow rate of 2 to 3 mL/min, since these authors claim that they followed the method described  
9 in the European Pharmacopoeia<sup>74</sup>. And, when specified, after extraction the essential oil is  
10 dried over Na<sub>2</sub>SO<sub>4</sub> anhydrous and stored in sealed vials between -20°C and 6°C.

11  
12  
13  
14 To compare the yields expressed in different units in the articles, they were all converted in  
15 g/100g dry matter using values found in the literature. Dried bitter orange peels contain 90%  
16 dry matter and fresh bitter orange peels contain 30% dry matter. According to the ISO  
17 standard 9844, the bitter orange essential oil density ranges from 0.84 to 0.86 g/mL, so the  
18 density of the different oils obtained was considered as being 0.85 g/mL. The yields obtained  
19 vary from 0.21 to 1.12 g/100g dry matter (Table 6). These important differences in yield  
20 obtained could be due to different factors: (1) part of the essential oil may be lost during  
21 drying of peel, (2) the mass of peel from which the oil is extracted is too small to scale up  
22 (concerning Sarrou *et al.*<sup>5</sup>), (3) the time of hydrodistillation is too short to extract the whole  
23 quantity of essential oil contained in the peels, (4) the cultivars of bitter oranges are different,  
24 (5) the ripening stage may be different, (6) the growing location is different.

25  
26  
27  
28  
29  
30 Concerning Azhdarzadeh and Hojjati<sup>33</sup>, Boussaada and Chemli<sup>23,65</sup>, Boussaada *et al.*<sup>26</sup> and  
31 Bourgou *et al.*<sup>51</sup>, the yields given in the article were expressed for 100 g of fresh peel but it  
32 was not specified if the figures given were in mL or in g. Thus, they could not be converted in  
33 g/100g dry matter.

34  
35  
36  
37 The extraction method influences not only the yields but also the composition of the EO.  
38 Indeed, the hydrodistillation requires heating during several hours and also the citrus peels,  
39 when in water, form an aqueous acidic solution. According to Boelens,<sup>8</sup> the pH of bitter  
40 orange peels is between 2.5 and 4. Temperature, time and pH are the three main factors that  
41 cause degradation of terpenes, elimination, rearrangement and cyclization of alcohols and  
42 esters.

43  
44  
45 Table 7 compares the volatile composition of bitter orange essential oils obtained by  
46 hydrodistillation to the ISO standard obtained by cold pressing. The compositions of  
47 hydrodistilled oils reported in table 7 are not exhaustive, but focused on the 12 compounds  
48 that are mentioned in the ISO standard. The germacrene D percentage in hydrodistilled oils  
49 appears to be always lower than the ISO standard. The linalyl acetate percentage is always  
50 lower than the ISO standard (Table 7). This can be imputed to the decomposition of the linalyl  
51 acetate present in peels during hydrodistillation due to low pH and heating. Except for Hosni  
52 *et al.*,<sup>50</sup> the linalool percentage in hydrodistilled oils is always higher than the ISO standard.  
53 This can be attributed to the formation of linalool during hydrodistillation. Thus, it can be  
54 assumed that linalyl acetate is converted partially in linalool during hydrodistillation. Indeed,  
55 linalyl acetate undergoes thermal degradation that generates linalyl carbocation. In aqueous  
56 acid solution, the linalyl carbocation is hydrated to give linalool.<sup>75</sup>

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

Adapted from the literature,<sup>3,47,75-90</sup> 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.<sup>75</sup> 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).<sup>87</sup> Baxter *et al.*<sup>84</sup> 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.*<sup>89</sup> 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.<sup>88</sup> 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 Richard<sup>75</sup> 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%),  $\alpha$ -terpinene (coeluted with 1,4-cineole 13.1%), limonene (9.3%),  $\gamma$ -terpinene (7.2%)... Many alcohols were also produced in smaller quantities, among which:  $\alpha$ -terpineol (3.9%), (*Z*)- $\beta$ -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.<sup>56</sup> 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  $\alpha$ -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.*<sup>91</sup> stated that linalool could form limonene during growing, but it is not the only precursor<sup>62</sup> 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.<sup>92</sup> 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

1  
2  
3 and DMAPP can be formed through two different pathways which are located in two different  
4 cellular compartments: the cytosolic mevalonate pathway and the plastic methylerythritol  
5 phosphate pathway. GPP is the precursor of monoterpenes such as ocimene and limonene.  
6 Monoterpene synthase genes were characterized in citrus<sup>93–95</sup>. A high expression of those  
7 genes appears to have an important role in the formation of citrus essential oils.<sup>92</sup> Indeed,  
8 terpenes synthases are responsible for the synthesis of monoterpenes.  
9  
10

11  
12 When submitting  $\beta$ -pinene to hydrodistillation in harsh acid conditions during 3 hours using a  
13 Clevenger apparatus, Haro-Guzmán and Huet obtained important quantities of  $\alpha$ -terpineol,  
14 terpinolene and 1,4-cineole, as well as lesser quantities of 1,8-cineole and *p*-cymene.<sup>96</sup>  
15 Muneyuki *et al.* reviewed the acid catalysed rearrangements of  $\alpha$ -pinene and  $\beta$ -pinene.<sup>97</sup>  
16 Under acidic conditions, the degradation of pinenes mainly gave  $\alpha$ -terpineol, and smaller  
17 amounts of  $\alpha$ -fenchol and borneol. When pH is even lower, some hydrocarbons such as  
18 limonene, terpinolene, camphene and fenchene can be formed as well.  
19  
20  
21

22  
23 The major hydration products of acid-catalysed reaction of sabinene and  $\alpha$ -thujene are:  
24 terpinen-4-ol,  $\gamma$ -terpinene,  $\alpha$ -terpinene, terpinolene, sabinene hydrate and 1,4-terpin.<sup>82</sup>  
25  
26

27  
28 In plants, not all aroma compounds are found in a free state. Some are glycosidically bound  
29 aroma compounds: they are called aroma precursors since they are able to release free  
30 odorous aroma compounds when the glycosidic bond is hydrolysed,<sup>98</sup> but they are not  
31 odorous as such, when glycosidically bound. Glycosidic aroma precursors are preferential  
32 storage and transport forms probably due to the toxicity of the free form in the cells above a  
33 certain concentration.<sup>66</sup> Deglycosylation occurs by enzymatic or acid hydrolysis of the  
34 glycosidic bond<sup>99</sup> during fruit maturation, spontaneously when the pH is acid enough or  
35 because of heat treatment. Thus, it can be assumed that due to the acidity of bitter orange  
36 peels in aqueous solution,<sup>8</sup> hydrodistillation of peels induces deglycosylation and release of  
37 aroma precursors, but no literature was found on this point.  
38  
39

40  
41 Oxidations of the terpenes and the sesquiterpenes can happen in the presence of dioxygen.  
42 Boelens and Sindreu<sup>47</sup> stated that nerolidol can form various sesquiterpenes such as  
43 germacrene D,  $\alpha$ -humulene, caryophyllene,  $\alpha$ -selinene that can oxidise in  $\alpha$ -selinenone, and  
44 valencene that can oxidise in nootkatone.  
45

46  
47 Ikeda *et al.*<sup>76</sup> were the first authors to assume that when  $\gamma$ -terpinene is exposed to air at room  
48 temperature it could possibly form *p*-cymene as  $\gamma$ -terpinene concentration decreases while *p*-  
49 cymene concentration increases. Kimura *et al.*<sup>77</sup> studied the impact of storage on lemon oil  
50 and on different compounds: citral, limonene,  $\beta$ -pinene, and  $\gamma$ -terpinene, in acidic conditions  
51 at 38°C. They found out that citral and  $\beta$ -pinene concentrations significantly decreased after  
52 60 days of storage at these temperature and pH conditions, while  $\alpha$ -terpineol and *p*-cymene  
53 which are more stable increased, due to low degradation of limonene and  $\gamma$ -terpinene  
54 respectively. As the storage time increases, the concentration of *p*-cymene increases.<sup>100</sup> In  
55 2014, Lorjaroenphon *et al.*<sup>85</sup> studied the degradation products of citral when stored three days  
56 in citric acid solution. They proposed rearrangement pathways for citral in aqueous acidic  
57  
58  
59  
60

1  
2  
3 solution very similar to the ones by Clark *et al.*<sup>87</sup> dating from 1977. These reaction schemes  
4 are more detailed than the ones presented by Kimura *et al.*<sup>77</sup>  
5

## 6. CONCLUSION

7  
8  
9 More than 50 studies have been published on the composition of the bitter orange essential  
10 oils. Many factors can influence its composition. Up until now, the following factors have  
11 been discussed in the literature: growing location, cultivars, ripening stage, storage conditions,  
12 and extraction methods.  
13

14  
15 First of all, it may be underlined that only one article did a true quantification of the  
16 compounds of a sample of bitter orange essential oil using internal standards.<sup>55</sup> All the studies  
17 that compared different samples of bitter orange essential oils draw conclusions based on raw  
18 GC percentages. It ensues from the comparison of the number of compounds identified in  
19 each study, that some authors should have injected a volume of essential oil more important  
20 or have lowered the split, in order to identify more compounds. When trying to identify more  
21 compounds, authors should be cautious when it comes to the saturation of limonene, or inject  
22 the limonene separately for its quantification.  
23

24  
25  
26 It results from the examination of the compositions of the bitter orange peel oils published in  
27 the literature, that 50 out of the 53 cold-pressed peel oils extracted from *Citrus aurantium* L.  
28 reported in this review would not be identified as bitter orange peel oil according to the ISO  
29 standard 9844, because they do not comply with the normative ranges of values for the 10  
30 compounds of the standard. The only three results of analysis of cold-pressed oils which are  
31 compliant with the ISO standard 9844 are Dugo *et al.*,<sup>19,36</sup> the Maroc cultivar from Lota *et*  
32 *al.*,<sup>31</sup> and Mondello *et al.*<sup>37,40</sup> Moreover, the means of the percentages of limonene, linalyl  
33 acetate, and germacrene D of the 24 cultivars of *Citrus aurantium* L. which have a similar  
34 chemotype according to Lota *et al.*<sup>31</sup> are not compliant with the standard. Hence, the ISO  
35 standard 9844, may consider broadening some of its normative ranges of values.  
36  
37

38  
39  
40 Bitter orange peel essential oil coming from some of the major producing countries such as  
41 Argentina, Haiti, Morocco, Uganda, and Paraguay have not been studied yet in the literature.  
42 Boelens and Jimenez<sup>35</sup> tried to assess the influence of the growing location, but more  
43 experiments are needed to draw firm conclusions.  
44

45  
46 Only half of the authors mentioned the name of the cultivar they used for their study, whereas  
47 it has been established that some cultivars such as the Espagne and the Gou Tou cultivar  
48 differ a lot from the other cultivars of *Citrus aurantium* L..<sup>31</sup>  
49

50  
51 All the authors agree on the fact that limonene is the majority compound of the bitter orange  
52 peel oil by far. Seven studies were published on the influence of the growing and the ripening  
53 stage of the bitter orange on the composition of its essential oil. The limonene percentage  
54 increases during growing of the fruit by cyclisation of geranyl pyrophosphate. The linalool  
55 percentage dramatically decreases during growing as the limonene increases, so it could be  
56 possible that the limonene is also partly formed by the degradation of the linalool. The  
57 question remains if two pathways can coexist as far as the formation of limonene is  
58 concerned. If so, the predominance of one of them would probably be determined by the gene  
59  
60

1  
2  
3 pool and the environment of the bitter orange. The cultivars being examples of genetic  
4 variability among bitter oranges, and the growing location (climate, soil quality...) a factor of  
5 the environment.  $\beta$ -pinene seems to decrease during growing.  $\alpha$ -terpineol, geraniol, and nerol  
6 appear to be fairly constant during the ripening of bitter oranges. Aldehydes (mainly octanal  
7 and decanal) which are known to have an important odorant impact<sup>55</sup> increase during the  
8 ripening of bitter oranges.  
9

10  
11 Boelens and Jimenez<sup>56</sup> are the only authors who studied the impact of storage of bitter  
12 oranges on the composition of its oil. Unfortunately, only one analysis was done at the end of  
13 a short storage time (one to two weeks). According to this analysis compared with the  
14 analysis of the ripe fruit right after picking up, they concluded that linalool, linalyl acetate,  
15 nerolidol, nootkatone and  $\alpha$ -selenenone increased after picking up the fruits. Enzymatic  
16 reactions were accounted for the formation of these compounds.  
17  
18

19  
20 Njoroge *et al.*<sup>43</sup> are the only authors who assessed the impact of storage of bitter orange  
21 essential oil on its composition. It can be concluded from this study that bitter orange EO  
22 should be stored at -21°C to prevent any degradation caused by long storage period. The main  
23 changes in composition are observed when stored at 20°C or stored at 5°C during at least 12  
24 months. Structural rearrangements of monoterpenes such as limonene and  $\gamma$ -terpinene due to  
25 temperature and oxidation form *p*-cymene via terpinolene when the temperature and the time  
26 of storage increase. The oxidation of limonene explains the sharp increases of (*Z*)-carveol,  
27 carvone, limonene-10-yl acetate, and (*E*)-*p*-mentha-2,8-dien-1-ol, and the slight increase of  $\alpha$ -  
28 terpineol. The increase of linalool was probably due to the decrease of linalyl acetate and  
29 maybe as well to the autoxidation of myrcene.<sup>83</sup> It can be hypothesized that both thujol and  
30 sabinene hydrate came from sabinene, by structural rearrangement followed by hydration and  
31 simply by hydration respectively.  
32  
33

34  
35  
36 The main extraction methods used to obtain oil from bitter orange peel are cold pressing and  
37 hydrodistillation. Cold pressing consists in applying mechanical pressure on the peels to  
38 release the essential oil. Then, water is added to the EO. The mixture is centrifuged and  
39 finally the aqueous phase is discarded which means that soluble compounds such as  
40 glycosidic aroma compounds are thrown away. Actually, the presence of glycosidic aroma  
41 compounds in bitter orange peels has never been proved yet in the literature, but their  
42 presence has been reported in several citrus species (three cultivars of mandarin, one cultivar  
43 of sweet orange, one cultivar of pink grapefruit and one cultivar of white grapefruit).<sup>98</sup>  
44 Hydrodistillation is exclusively used to extract bitter orange peel oil on laboratory scale. The  
45 use of a Clevenger apparatus provides the advantage of measuring directly the volume of oil  
46 extracted per mass of peels involved. Despite the use of a Clevenger, the different parameters  
47 of the hydrodistillation method appear to vary a lot (mass of peel involved, time of  
48 hydrodistillation, distillation rate), and so does the quantity of essential oil extracted per mass  
49 of peel. During hydrodistillation, the aroma compounds of the bitter orange peels are  
50 submitted to acid-catalysed reactions, due to the acidity of the bitter orange peels which lower  
51 the pH of the aqueous solution. At acid pH, linalyl acetate is decomposed in many terpene  
52 derivatives, which explains that the percentages of linalyl acetate are much lower in  
53 hydrodistilled oils than in cold pressed oils. The main differences between cold pressed bitter  
54 orange oils and hydrodistilled oils are due to the acid-catalysed reactions during  
55  
56  
57  
58  
59  
60



1  
2  
3 hydrodistillation and possibly to the release of aroma compounds by deglycosylation of the  
4 glycosidic bound.  
5

6 Understanding all these reactions is essential because the odour profile of the bitter orange  
7 peel oil can be impacted a lot. Monoterpenes do not play a major role in the flavour of bitter  
8 orange peel oil,<sup>101</sup> but many of their reaction products formed by acid-catalysed  
9 rearrangement followed by hydration are extremely important to the flavour of the essential  
10 oil. For instance, the hydration of a relatively weak flavorant such as limonene leads to a  
11 temporary increase in flavour until the terpineols are converted to terpins.<sup>88</sup>  
12  
13  
14

## 15 REFERENCES

- 16 1. Ortiz JM. Botany: taxonomy, morphology and physiology of fruits, leaves and flowers.  
17 In: Dugo G, Di Giacomo A, eds. *Citrus: The Genus Citrus*. Boca Raton, FL: CRC  
18 Press; 2002:16-35.
- 19 2. Kale PN, Adsule PG. Citrus. In: Salunkhe DK, Kadam SS, eds. *Handbook of Fruit*  
20 *Science and Technology: Production, Composition, Storage and Processing*. New  
21 York, NY: Marcel Dekker; 1995:39-66. doi:10.1201/9781482273458
- 22 3. Huet R. Les huiles essentielles d'agrumes. *Fruits*. 1991;46(4):501-513.
- 23 4. Jabri Karoui I, Marzouk B. Characterization of bioactive compounds in Tunisian bitter  
24 orange (*Citrus aurantium* L.) peel and juice and determination of their antioxidant  
25 activities. *Biomed Res Int*. 2013:1-12.
- 26 5. Sarrou E, Chatzopoulou P, Dimassi-Theriou K, Therios I. Volatile constituents and  
27 antioxidant activity of peel, flowers and leaf oils of *Citrus aurantium* L. growing in  
28 Greece. *Molecules*. 2013;18(9):10639-10647. doi:10.3390/molecules180910639
- 29 6. Caccioni DRL, Guizzardi M, Biondi DM, Renda A, Ruberto G. Relationship between  
30 volatile components of citrus fruit essential oils and antimicrobial action on *Penicillium*  
31 *digitatum* and *Penicillium italicum*. *Int J Food Microbiol*. 1998;43(1-2):73-79.  
32 doi:10.1016/S0168-1605(98)00099-3
- 33 7. Rowshan V, Najafian S. Changes of peel essential oil composition of *Citrus aurantium*  
34 L. during fruit maturation in Iran. *J Essent Oil-Bearing Plants*. 2015;18(4):1006-1012.  
35 doi:10.1080/0972060X.2014.977564
- 36 8. Boelens MH. A critical review on the chemical composition of Citrus oils. *Perfum*  
37 *Flavorist*. 1991;16:17-34.
- 38 9. Shaw PE. Review of quantitative analyses of Citrus essential oils. *J Agric Food Chem*.  
39 1979;27(2):246-257. doi:10.1021/jf60222a032
- 40 10. Lawrence BM. Progress in essential oils. *Perfum Flavorist*. 1982;7(5):43-48.
- 41 11. Swaine RL, Swaine RLJ. Citrus oils: Processing, technology, and applications. *Perfum*  
42 *Flavorist*. 1988;13:1-20.
- 43 12. Ortiz JM, Kumamoto J, Scora RW. Possible relationship among sour oranges by  
44 analysis of their essential oils. *Int flavours food Addit*. 1978;9:224-226.
- 45 13. Maekawa K, Kodama M, Kushii M, Mitamura M. Essential oils of some orange peels.  
46 *Agric Biol Chem*. 1967;31(3):373-377. doi:10.1080/00021369.1967.10858818
- 47 14. Tanaka T. Fundamental discussion of Citrus classification. *Stud Citrologia*. 1977;14:1-6.
- 48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

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.

- 1
- 2
- 3 32. Protopapadakis E, Papanikolaou X. Characterization of *Citrus aurantium* and *Citrus taiwanica* rootstocks by isoenzyme and essential oil analysis. *J Horticult Sci Biotechnol.* 1998;73(1):81-85. doi:10.1080/14620316.1998.11510947
- 4
- 5
- 6
- 7 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
- 8
- 9
- 10
- 11 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.
- 12
- 13
- 14
- 15
- 16 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
- 17
- 18
- 19 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.
- 20
- 21
- 22
- 23
- 24 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
- 25
- 26
- 27
- 28 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.
- 29
- 30
- 31
- 32
- 33
- 34 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
- 35
- 36
- 37
- 38 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
- 39
- 40
- 41
- 42 41. Dugo G. The composition of the volatile fraction of the Italian Citrus essential oils. *Perfum Flavorists.* 1994;19(6):29-51.
- 43
- 44
- 45 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
- 46
- 47
- 48
- 49 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
- 50
- 51
- 52
- 53 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
- 54
- 55
- 56
- 57 45. Sawamura M. Volatile components of essential oils of the citrus genus. *Recent Res Dev Agric & Food Chem.* 2000;4(1):131-164.
- 58
- 59
- 60 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 Hort.* 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

- 1  
2  
3 study of the percentage variations in peel and leaf oil terpenes during one season.  
4 *Phytochemistry*. 1967;6:25-32.  
5
63. Siragusa M, De Pasquale F, Abbate L, Tusa N. Identification of sour orange accessions  
7 and evaluation of their genetic variability by molecular marker analyses. *HortScience*.  
8 2006;41(1):84-89.  
9
64. Castle WS, Baldwin JC, Grosser JW. Performance of “washington” navel orange trees  
10 in rootstock trials located in lake and st. lucie counties. *Proc Florida State Hort Soc*.  
11 2000;113:106-111.  
12
65. Boussaada O, Chemli R. Seasonal variation of essential oil composition of *Citrus*  
13 *aurantium* L. var. *amara*. *J Essent Oil-Bearing Plants*. 2007;10(2):109-120.  
14 doi:10.1080/0972060X.2007.10643528  
15
66. Vasserot Y, Arnaud A, Galzy P. Monoterpenol glycosides in plants and their  
16 biotechnological transformation. *Acta Biotechnol*. 1995;15(1):77-95.  
17 doi:10.1002/abio.370150110  
18
67. Kusunose H, Sawamura M. Aroma constituents of some sour *Citrus* oils. *Nippon*  
19 *shokuhin kogyo gakkaishi*. 1980;27(10):517-521. doi:10.3136/nskkk1962.27.10\_517  
20
68. Tranchida PQ, Bonaccorsi I, Dugo P, Mondello L, Dugo G. Analysis of *Citrus* essential  
21 oils: State of the art and future perspectives. A review. *Flavour Fragr J*.  
22 2012;27(2):98-123. doi:10.1002/ffj.2089  
23
69. Dugo G, Cotroneo A, Bonaccorsi I, Allessandra T. Composition of the volatile fraction  
24 of citrus peel oils. In: Dugo G, Mondello L, eds. *Citrus Oils: Composition, Advanced*  
25 *Analytical Techniques, Contaminants, and Biological Activity*. Boca Raton, FL: CRC  
26 Press; 2010:1-162.  
27
70. Porat R, Deterre S, Giampaoli P, Plotto A. The flavor of citrus fruit. In: Havkin-Frenkel  
28 D, Dudai N, eds. *Biotechnology in Flavor Production*. Second. John Wiley & Sons;  
29 2016:1-31. doi:10.1002/9781118354056.ch1  
30
71. Schmidt E. Production of essential oils. In: Baser KHC, Buchbauer G, eds. *Handbook*  
31 *of Essential Oils: Science, Technology and Applications*. Boca Raton, FL: CRC Press;  
32 2010:83-119.  
33
72. Ruberto G. Analysis of volatile components of *Citrus* fruit essential oils. In: Jackson  
34 JF, Linskens HF, eds. *Analysis of Taste and Aroma*. Springer Berlin Heidelberg;  
35 2002:123-157.  
36
73. Lota M. Les huiles essentielles d'agrumes: caractérisation par RMN du carbone-13  
37 CPG-IK et CPG/SM, PhD Thesis. 1999.  
38
74. European Pharmacopoeia. Determination of Essential Oils in Herbal Drugs. In: Council  
39 of Europe (EDQM), ed. *European Pharmacopoeia*. 9th ed. Strasbourg, FR; 2017:285-  
40 286.  
41
75. Morin P, Richard H. Thermal degradation of linalyl acetate during steam distillation.  
42 *Prog Flavour Res*. 1985:563-576.  
43
76. Ikeda RM, Stanley WL, Vannier SH, Rolle LA. Deterioration of lemon oil. Formation  
44 of p-cymene from  $\gamma$ -terpinene. *Food Technol*. 1961;15:379-380.  
45
77. Kimura K, Nishimura H, Iwata I, Mizutani J. Identification and formation mechanism  
46 of components responsible for off-odor of deteriorated lemons. *Nippon shokuhin kogyo*  
47 *gakkaishi*. 1984;31(12):761-764.  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

- 1
- 2
- 3 78. Mazza G. Studio sulle ossidazioni dei monoterpeni negli oli essenziali. *Essenze Deriv*
- 4 *Agrum*. 1987;57:5-18.
- 5
- 6 79. Mazza G. Etude sur la composition aromatique de l'huile essentielle de bergamotte
- 7 (Citrus aurantium subsp. Bergamia Risso et Poiteau Engler) par chromatographie
- 8 gazeuse et spectrometrie de masse. *J Chromatogr*. 1986;362:87-99.
- 9
- 10 80. Mitzner BM, Lemberg S, Theimer ET. Preparation of cis- and trans-ocimene from
- 11 catalytic pyrolysis of linalyl acetate. *Can J Chem*. 1966;44:1090-1092.
- 12
- 13 81. Williams CM, Whittaker D. Rearrangements of pinane derivatives. Part II. Products of
- 14 acid-catalysed rearrangement of  $\alpha$ -pinene and  $\beta$ -pinene in acetic acid. *J Chem Soc B*
- 15 *Phys Org*. 1971;(672):672-677. doi:10.1039/J29710000672
- 16
- 17 82. Cooper MA, Holden CM, Loftus P, Whittaker D. The acid catalysed hydration of
- 18 sabinene and  $\alpha$ -thujene. *J Chem Soc Perkin Trans 2*. 1973;(5):665-667.
- 19
- 20 83. Dieckmann RH, Palamand SR. Autoxidation of some constituents of hops. I. The
- 21 monoterpene hydrocarbon, myrcene. *J Agric Food Chem*. 1974;22(3):498-503.
- 22 doi:10.1021/jf60193a033
- 23
- 24 84. Baxter RL, Laurie WA, Mchale D. Transformations of monoterpenoids in aqueous
- 25 acids. The reactions of linalool, geraniol, nerol and their acetates in aqueous citric acid.
- 26 *Tetrahedron*. 1978;34(14):2195-2199. doi:10.1016/0040-4020(78)89026-7
- 27
- 28 85. Lorjaroenphon Y, Hausch BJ, Cadwallader KR. *Formation and Stability of 2,3-*
- 29 *Dehydro-1,8-Cineol in a Model Carbonated Beverage System*. Elsevier Inc.; 2014.
- 30 doi:10.1016/b978-0-12-398549-1.00044-1
- 31
- 32 86. Ziegler M, Brandauer H, Ziegler E, Ziegler G. A different aging model for orange oil:
- 33 Deterioration products. *J Essent Oil Res*. 1991;3(4):209-220.
- 34 doi:10.1080/10412905.1991.9697931
- 35
- 36 87. Clark BC, Powell CC, Radford T. The acid catalyzed cyclization of citral.
- 37 *Tetrahedron*. 1977;33(17):2187-2191.
- 38
- 39 88. Clark BC, Chamblee TS. Acid-Catalyzed Reactions of Citrus Oils and Other Terpene-
- 40 Containing Flavors. In: Charalambous G, ed. *Off-Flavors in Foods and Beverages*.
- 41 Amsterdam, NL: Elsevier B.V.; 1992:229-285. doi:https://doi.org/10.1016/B978-0-
- 42 444-88558-6.50015-5
- 43
- 44 89. Cori O, Chayet L, Perez LM, Bunton CA, Hachey D. Rearrangement of Linalool,
- 45 Geraniol, and Nerol and Their Derivatives. *J Org Chem*. 1986;51(8):1310-1316.
- 46 doi:10.1021/jo00358a028
- 47
- 48 90. Degenhardt A, Liebig M, Kohlenberg B, et al. *Studies on Stability of Citrus Flavors*
- 49 *and Insights into Degradation Pathways of Key Aroma Compounds*. Elsevier Inc.;
- 50 2014. doi:10.1016/b978-0-12-398549-1.00045-3
- 51
- 52 91. Attaway JA, Pieringer AP, Barabas LJ. The origin of citrus flavor components - I. The
- 53 analysis of citrus leaf oils using gas-liquid chromatography, thin-layer chromatography
- 54 and mass spectrometry. *Phytochemistry*. 1966;5:141-151.
- 55
- 56 92. Takita MA, Berger IJ, Basilio-Palmieri AC, Borges KM, Souza de JM, Targon MLNP.
- 57 Terpene production in the peel of sweet orange fruits. *Genet Mol Biol*. 2007;30(3
- 58 (suppl)):841-847.
- 59
- 60 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*.

- 1  
2  
3 2005;168(4):987-995. doi:10.1016/j.plantsci.2004.11.012  
4  
5 94. Dornelas MC, Mazzafera P. A genomic approach to characterization of the Citrus  
6 terpene synthase gene family. *Genet Mol Biol.* 2007;30(3 (suppl.)):832-840.  
7  
8 95. Rodríguez A, Andrés VS, Cervera M, et al. Terpene down-regulation in orange reveals  
9 the role of fruit aromas in mediating interactions with insect herbivores and pathogens.  
10 *Plant Physiol.* 2011;156(2):793-802. doi:10.1104/pp.111.176545  
11  
12 96. Haro-Guzman L, Huet R. L'huile essentielle de lime au mexique (*Citrus aurantifolia*  
13 Swingle). *Fruits.* 1970;25(12):887-899.  
14  
15 97. Muneyuki R, Yoshimura Y, Tori K, Terui Y, Shoolery JN. Acid-catalyzed  
16 rearrangements of the pinyl system. *J Org Chem.* 1988;53:358-366.  
17  
18 98. Ren JN, Tai YN, Dong M, et al. Characterisation of free and bound volatile compounds  
19 from six different varieties of Citrus fruits. *Food Chem.* 2015;185:25-32.  
20 doi:10.1016/j.foodchem.2015.03.142  
21  
22 99. Praet T, Van Opstaele F, Jaskula-Goiris B, Aerts G, De Cooman L. Biotransformations  
23 of hop-derived aroma compounds by *Saccharomyces cerevisiae* upon fermentation.  
24 *Cerevisia.* 2012;36(4):125-132. doi:10.1016/j.cervis.2011.12.005  
25  
26 100. Wiley RC, Louie MK, Sheu MJ. Studies on turpentine-like off-odor in Cola. *J Food*  
27 *Sci.* 1984;49:485-488.  
28  
29 101. Schieberle P, Grosch W. Identification of potent flavor compounds formed in an  
30 aqueous lemon oil/citric acid emulsion. *J Agric Food Chem.* 1988;36(4):797-800.  
31 doi:10.1021/jf00082a031  
32  
33  
34  
35  
36  
37  
38  
39  
40  
41  
42  
43  
44  
45  
46  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

## 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,<sup>33</sup> Ashour and El-Kebeer,<sup>30</sup> Boelens and Jimenez,<sup>31</sup> Bourgou *et al.*,<sup>51</sup> Boussaada and Chemli,<sup>63</sup> Dugo *et al.*<sup>39</sup> and Rowshan and Najafian<sup>7</sup> (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 9844<sup>43</sup> (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.*,<sup>83</sup> Boelens and Sindreu,<sup>47</sup> Clark *et al.*,<sup>86</sup> Clark and Chamblee,<sup>87</sup> Cooper *et al.*,<sup>82</sup> Cori *et al.*,<sup>88</sup> Degenhardt *et al.*,<sup>89</sup> Dieckmann and Palamand,<sup>90</sup> Huet,<sup>3</sup> Ikeda *et al.*,<sup>76</sup> Kimura *et al.*,<sup>77</sup> Lorjaroenphon *et al.*,<sup>84</sup> Mazza,<sup>78,79</sup> Mitzner *et al.*,<sup>80</sup> Morin and Richard,<sup>75</sup> Williams and Whittaker,<sup>81</sup> Ziegler *et al.*<sup>86</sup>



TABLE 1

<b>Family</b>	Rutaceae			
<b>Genus</b>	Citrus			
	Bitter orange / Sour Orange / Bigarade			
<b>Species</b>	<i>Citrus aurantium</i> L.	<i>Citrus myrtifolia</i> Raf.	<i>Citrus natsudaidai</i> Hay.	<i>Citrus neo-aurantium</i> Tan.
<b>Cultivars</b>	Country of origin	Cultivars examples		
	Algeria	Algerian, Trabou		
	Brazil	Azeda, Brazilian, Jaboticaba		
	China	Gou Tou, Huahong, Kabusu		
	Cuba	Cajel, Cuban		
	France	Cardosi, De Grasse, Donnet, Filippi, Luisi, Menton, Santucci	Large chinotto	Tosu
	Italy	Bizzarrio, Gallesio, Palermo,	Small chinotto	Natsudaidai
	Jamaica	Jacmel		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 <sup>21</sup>	Ashour and El-Kebeer <sup>30</sup>	Azhdarzadeh and Hojjati <sup>33</sup>		Boelens and Jimenez <sup>56</sup>				
Extraction method	Cold-pressed	Steam distillation		Hydrodistillation		Laboratory cold-pressed			
Cultivar		Egypt			Iran (Khuzestan province)				
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	$\alpha$ -pinene	0.30-0.65	0.38	0.43	0.55	2.56*	1.96*	0.60	0.65
	$\beta$ -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
	$\beta$ -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
	(E)- $\beta$ -nerolidol	0.06-0.18	-*	-*	-*	0*	0.09	0.03*	0.10
<b>Total volatile compounds identified</b>		14	12	8	21	39	44		

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

TABLE 2 Continued

	ISO standard 9844 <sup>21</sup>	Bourgou <i>et al.</i> <sup>51</sup>			Boussaada and Chemli <sup>65</sup>				Dugo <i>et al.</i> <sup>39</sup>		Rowshan and Najafian <sup>7</sup>		
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)
<b><math>\alpha</math>-pinene</b>	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
<b><math>\beta</math>-pinene</b>	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*
<b>myrcene</b>	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
<b>octanal</b>	X	-	-	-	-	-	-	-	0.05	0.07	1.38	0.40	0.68
<b>limonene</b>	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*
<b>linalool</b>	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*
<b>decanal</b>	X	-	-	-	-	-	-	-	0.08	0.09	0.34	0.15	0.20
<b>linalyl acetate</b>	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*
<b>geranyl acetate</b>	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
<b><math>\beta</math>-caryophyllene</b>	0.05-0.15	-*	-*	-*	tr*	tr*	tr*	tr*	0.04*	0.04*	0.02*	0.03*	0*
<b>germacrene-D</b>	0.08-0.18	0.10	0.25*	0.04*	-*	-*	-*	-*	0.02*	0.03*	0.07*	0*	0*
<b>(E)-<math>\beta</math>-nerolidol</b>	0.06-0.18	-*	-*	-*	tr*	tr*	tr*	0.1	0.04*	0.03*	-*	-*	-*
<b>Total volatile compounds identified</b>		31			26				56	58	31	22	24

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

TABLE 3

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

\*percentages of peak area out of the ISO range

TABLE 4

References	Extraction method	Industrial cold-pressed extraction										
	Cold-pressed	ISO standard 9844 <sup>21</sup>	Boelens and Jimenez <sup>35</sup>	Boelens and Oporto <sup>48</sup>	Dugo <i>et al.</i> <sup>19,36</sup> Dugo <sup>41</sup>	Dugo <i>et al.</i> <sup>39</sup>	Koketsu <i>et al.</i> <sup>27</sup>	Mondello <i>et al.</i> <sup>37,40</sup>	Pino and Rosado <sup>29</sup>	Veriotti and Sacks <sup>58</sup>		
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												
ISO standard 9844 compounds	<i>α</i> -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*
	<i>β</i> -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 ( <i>α</i> -phellandrene)	-*	0.03
	limonene	92.95-95.00	94.338	92.430*	93.770	95.363 ( <i>p</i> -cymene, <i>β</i> -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
	<i>β</i> -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> )- <i>β</i> -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 $\mu$ L	-	-	1 $\mu$ L	1 $\mu$ L	-	1 $\mu$ L	-	0.2 $\mu$ 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 <sup>21</sup>	Boelens and Jimenez <sup>56</sup>	Gionfriddo <i>et al.</i> <sup>38</sup>	Huang and Wu <sup>28</sup>		Kirbaslar and Kirbaslar <sup>52</sup>			
Cultivar				Morocco	Bangan	Daidai				
Growing location	ripe fruits			Italy (Calabria) 2001	China (Chongqing)		Turkey (Antalya) 1999	Turkey (Antalya) 2000	Turkey (Marmaris) 2000	
Ripening stage										
ISO standard 9844 compounds	$\alpha$ -pinene	0.30-0.65	0.65	0.367	0.52	0.32	0.51	0.42	0.45	0.39
	$\beta$ -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*
	$\beta$ -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> )- $\beta$ -nerolidol	0.06-0.18	0.10	0.050*	0.11	0.32*	0.10	0.07	0.05*	0.03*
	<b>Total volatile compounds identified</b>		44	46	41	42	43	23	26	27
<b>Dilution EO</b>		-	pure		-		1/20 in hexane			
<b>Volume injected</b>		-	0.3 $\mu$ L		-		1 $\mu$ L diluted			
<b>Split</b>		-	1:60		-		split			

\* percentages of peak area out of the ISO range

TABLE 5 Continued

Extraction method	Cold-pressed			Laboratory cold-pressed extraction				
	References	ISO standard 9844 <sup>21</sup>	Lota <i>et al.</i> <sup>31</sup>					
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	$\alpha$ -pinene	0.30-0.65	0.4	0.9*	0.4 ± 0.1	0.8*	0.5	0*
	$\beta$ -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
	$\beta$ -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> )- $\beta$ -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 ± standard deviation

tr: traces

TABLE 5 Continued

Extraction method	Cold-pressed		Laboratory cold-pressed extraction									
	References	ISO standard 9844 <sup>21</sup>	Njoroge <i>et al.</i> <sup>42</sup>	Njoroge <i>et al.</i> <sup>43</sup>	Sawamura <i>et al.</i> <sup>44</sup>			Sawamura <sup>45</sup>		Song <i>et al.</i> <sup>46</sup>		
Cultivar			Daidai**	Daidai**	Daidai**	Kabusu	Bittersweet	Daidai**	Kabusu	Figaradia Hooker	Tokosu	Daidai**
Growing location			Japan (Kochi)	Japan (Kochi)	Japan	Japan	Italy	Japan	Japan	Japan	Japan	Japan (Kochi)
Ripening stage			ripe fruits	ripe fruits								ripe fruits
ISO standard 9844 compounds	$\alpha$ -pinene	0.30-0.65	0.470	0.5	0.5	0.6	0.28*	0.51	0.54	0.57	0.47	0.6
	$\beta$ -pinene	0.30-1.15	0.684	0.7	0.7	0.9	0.03*	0.18*	0.91	1.05	0.69	0.4
	myrcene	1.60-1.95	1.565*	1.6	1.6	1.7	1.73	1.73	1.65	1.68	1.64	2.2*
	octanal	X	0.103	0.1	0.1	0.2	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	92.5*
	linalool	0.10-0.35	0.149	0.2	0.2	1.1*	0.34	0.18	1.04*	0.62*	0.15	0.2
	decanal	X	0.090	0.1	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*	0*	1.4*
	geranyl acetate	0.10-0.25	0.085*	-*	0.1	0.1	tr*	0.06*	0.09*	0.13	0.01*	0.2
	$\beta$ -caryophyllene	0.05-0.15	0.042*	<0.05*	-*	-*	tr*	0.02*	0.01*	0.02*	0.18*	tr*
	germacrene-D	0.08-0.18	0.090	0.1	-*	-*	0.01*	0.04*	0.04*	0.09	0.47*	0.1
	(E)- $\beta$ -nerolidol	0.06-0.18	0.049*	-*	-*	-*	0*	0.03*	0.04*	0.08	0*	0.1
	<b>Total volatile compounds identified</b>			62	18	16	12	48	45	44	47	55
<b>Dilution EO</b>			pure	pure	pure			pure				-
<b>Volume injected</b>			1 $\mu$ L	0.2 $\mu$ L	0.1 $\mu$ L			1 $\mu$ L				-
<b>Split</b>			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 <i>et al.</i> <sup>18</sup>	<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 <sup>33</sup>	<i>C. aurantium</i>	Iran (Khouzestan province)	fresh	100	3	-	dried over Na <sub>2</sub> SO <sub>4</sub> anhydrous passed through membrane filter (Whatman filter No.1) stored at 4°C	unripe: 0.66% ripe: 0.86%	
Bourgou <i>et al.</i> <sup>51</sup>	<i>C. aurantium</i>	Tunisia (Menzel Bouzelfa)	fresh	100	2	-	dried over Na <sub>2</sub> SO <sub>4</sub> anhydrous stored at 20°C in darkness	Immature: 0.23% Semi-mature: 0.12% Mature: 0.46%	
Boussaada and Chemli <sup>23</sup>	<i>C. aurantium</i> L. ssp. <i>amara</i>	Tunisia (Nabeul)						February: 0.7%	
		Greece (Chania)						0.8%	
Boussaada <i>et al.</i> <sup>26</sup>	<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 <sub>2</sub> SO <sub>4</sub> anhydrous stored in sealed glass vials in a refrigerator at 4-5°C	0.8%	
		Greece (Roxani)						1.1%	
Boussaada and Chemli <sup>65</sup>	<i>C. aurantium</i> L. ssp. <i>amara</i>	-						November: 1.0% December: 1.2% January: 1.3% February: 0.8%	
Caccioni <i>et al.</i> <sup>6</sup>	<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 <sub>2</sub> SO <sub>4</sub> anhydrous stored under N <sub>2</sub> 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)
<b>Gonzalez de et al.</b> <sup>54</sup>	<i>C. aurantium</i> L. ssp. <i>amara</i>	Venezuela (Tachira)	fresh	-	-	-	-	-	-
<b>Hosni et al.</b> <sup>50</sup>	<i>C. aurantium</i> L.	Tunisia (Zaghouan)	air dried ground	100	3	-	dried over Na <sub>2</sub> SO <sub>4</sub> anhydrous stored in amber and air-tight sealed vials at 0°C	1.24 (g/100g dry peel)	1.12
<b>Jabri Karoui and Marzouk</b> <sup>4</sup>	<i>C. aurantium</i> L.	Tunisia (Cape Bon)	fresh	100	1.5	-	collected in diethyl ether using liquid-liquid isolation	-	-
<b>Protopapadakis and Papanikolaou</b> <sup>32</sup>	<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		before					1.09 (mL/100g dry peel)	0.83
<b>Rowshan and Najafian</b> <sup>7</sup>	<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 <sub>2</sub> SO <sub>4</sub> anhydrous and stored in sealed vials at 4°C	-	-
<b>Sanei-Dehkordi et al.</b> <sup>34</sup>	<i>C. aurantium</i>	Iran (Babol)	fresh	50	3	-	dried over Na <sub>2</sub> SO <sub>4</sub> anhydrous and stored into an air-tight amber coloured vial at 4°C	0.7 ± 0.12 (g/100g fresh peel)	0.21
<b>Sarrou et al.</b> <sup>5</sup>	<i>C. aurantium</i> L.	Greece	fresh	35	3	3 to 3.5	dried over Na <sub>2</sub> SO <sub>4</sub> 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 <sup>21</sup>	Ali <i>et al.</i> <sup>18</sup>	Azhdarzadeh and Hojjati <sup>33</sup>	Bourgou <i>et al.</i> <sup>51</sup>	Boussaada and Chemli <sup>23</sup>	Boussaada <i>et al.</i> <sup>26</sup>			Boussaada and Chemli <sup>65</sup>					
Cultivar	Larange													
Growing location	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		
Ripening stage														
Harvest date														
ISO standard 9844 compounds	$\alpha$ -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	
	$\beta$ -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	1.4*
	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	87*
	linalool	0.10-0.35	1.78*	2.19*	0.1*	1.5*	2*	0.8*	1.4*	1*	1.3*	1*	0.8*	0.6*
	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*	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	0.3*
	$\beta$ -caryophyllene	0.05-0.15	0.05	0.22*	-*	tr*	tr*	tr*	tr*	tr*	tr*	tr*	tr*	tr*
	germacrene-D	0.08-0.18	0.06*	0.22*	0.04*	-*	-*	-*	-*	-*	-*	-*	-*	-*
	( <i>E</i> )- $\beta$ -nerolidol	0.06-0.18	0.05*	0.09	-*	0.1	0.1	0.1	0.1	0.1	tr*	tr*	tr*	0.1
	<b>Total volatile compounds identified</b>		25	39	31	26		26			26			
<b>Dilution EO</b>		pure	pure	-	1% solution (diluted in pentane)		-			1% solution (diluted in pentane)				
<b>Volume injected</b>		1 $\mu$ L	0.5 $\mu$ L	-	0.1 $\mu$ L		-			0.1 $\mu$ L				
<b>Split</b>		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 <sup>21</sup>	Caccioni <i>et al.</i> <sup>6</sup>	Gonzalez de <i>et al.</i> <sup>54</sup>	Hosni <i>et al.</i> <sup>50</sup>	Jabri Karoui and Marzouk <sup>4</sup>	Protopapadakis and Papanikolaou <sup>32</sup>				Rowshan and Najafian <sup>7</sup>	Sanei-Dehkordi <i>et al.</i> <sup>34</sup>	Sarrou <i>et al.</i> <sup>5</sup>	
Cultivar						Chania	Brazilian	Keen	Bittersweet				
Growing location						Italy (Lentiny)	Venezuela (Tachira)	Tunisia (Zaghouan)	Tunisia (Cape Bon)	Greece (La Canée)	Iran mature: orange	Iran (Babol)	Greece
Ripening stage													
Harvest date													
ISO standard 9844 compounds	$\alpha$ -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
	$\beta$ -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*
	$\beta$ -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)- $\beta$ -nerolidol	0.06-0.18	0.07	-*	0*	0.35*	-*	-*	-*	-*	-*	0.06	0*
	<b>Total volatile compounds identified</b>		36	7	13	27			21		24	21	12
	<b>Dilution EO</b>		-	-	-	-			1:9 in pentane		-	-	-
<b>Volume injected</b>		-	-	-	-			1 $\mu$ L		-	-	-	
<b>Split</b>		-	-	-	60:1			-		1:50	-	1:30	

\* percentages of peak area out of the ISO range

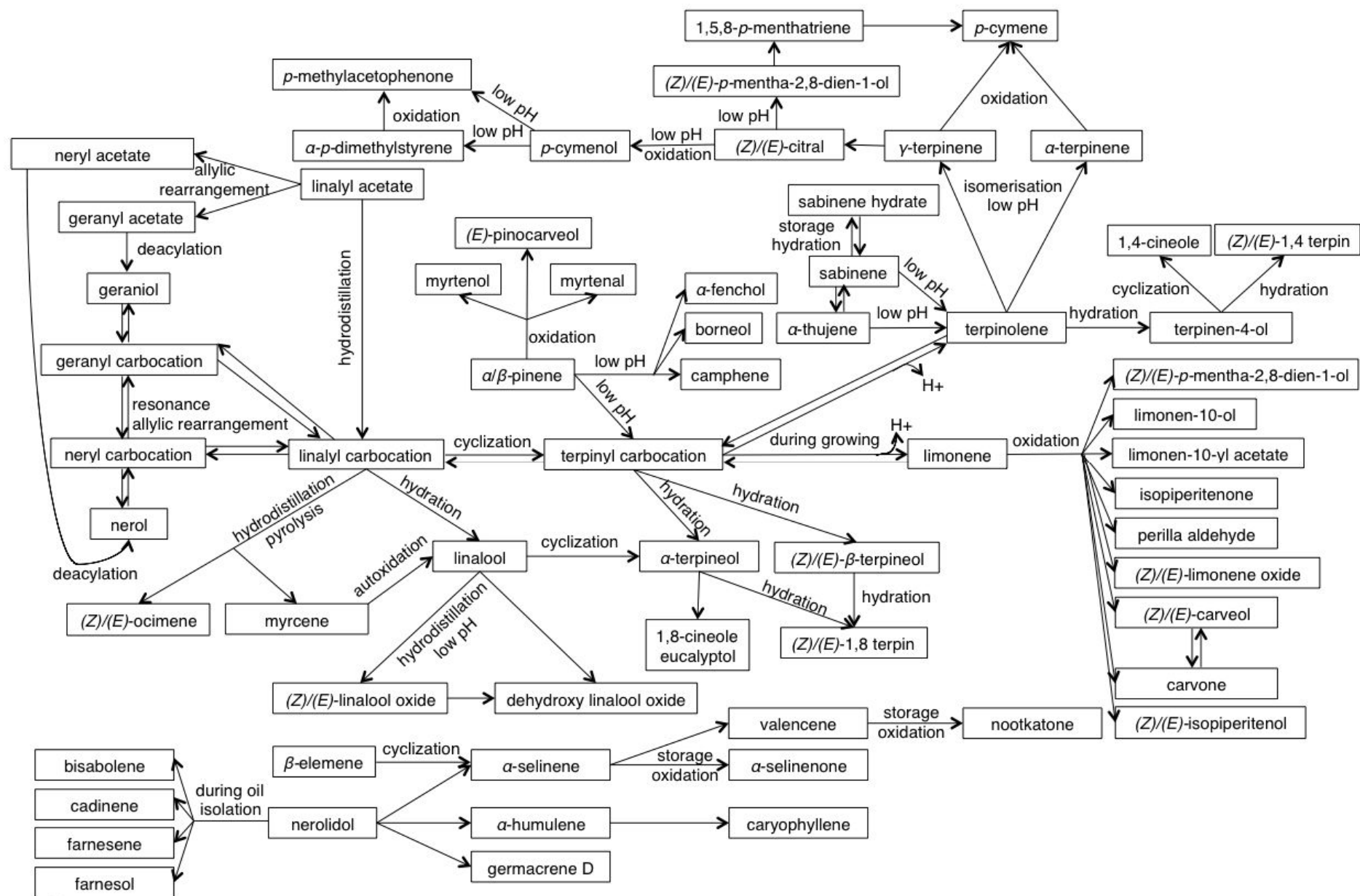
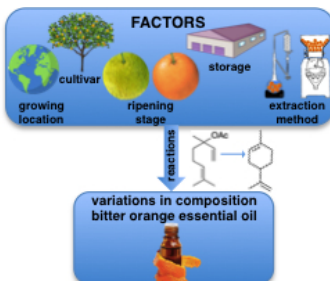


FIGURE 1

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  
47  
48  
49  
50  
51  
52  
53  
54  
55  
56  
57  
58  
59  
60

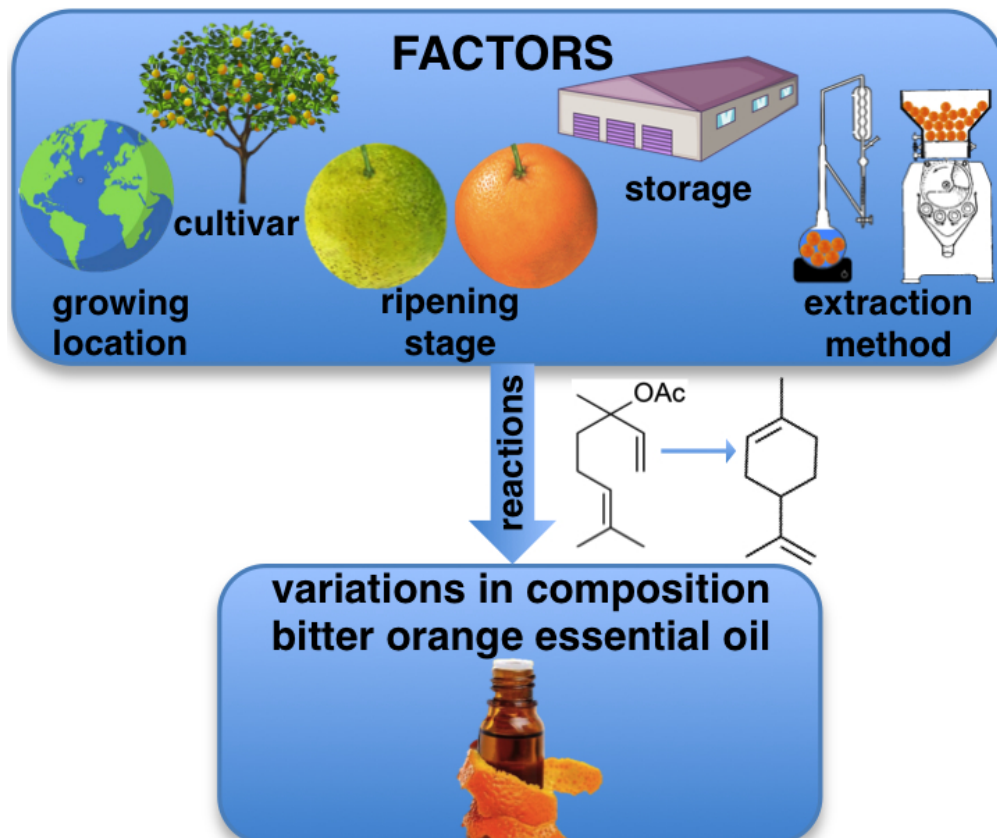
The influence of the growing location, the cultivar, the ripening stage, the storage and the extraction method on the composition of bitter orange peel essential oil is critically reviewed. The chemical reactions underlying the changes in composition due to these factors are presented.



**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)