

## GC-MASS ANALYSIS OF BITTER ORANGE PETITGRAIN BIGARADE OIL PRODUCED IN EGYPT AND STORED UNDER DIFFERENT TEMPERATURES

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### ABSTRACT

Identification of bitter orange petitgrain bigarade oil produced in Egypt was studied by GC/MS on carbowax – 20M column. It was found that linalyl acetate, which represented more than 25% of the whole oil was considered to be the major component of ester fraction; while linalool, which ranged from (30-33.7%) was the main alcohol components. On the other hand, limonene, (E)- $\beta$ -ocimene, myrcene and  $\beta$ -pinene were the highest monoterpene hydrocarbons. Room temperature (about 20°C), 4 and -18°C were used for storing the samples for 6 months. No pronounced effect was noticed on the composition of petitgrain oil with the exception of slight effect on the color, acid number and solubility (v/v 70% alcohol) when samples stored at 20°C. Statistical analyses proved that 4 °C could be considered the best fit temperature at which no significant changes occur in both the major chemical analyses and the main identified volatile constituents.

**Key Words:** *Citrus aurantium*, Petitgrain bigarade oil composition, Automated GC/MS, Linalyl acetate, Linalool.

### INTRODUCTION

*Citrus aurantium* swingle is commonly known as the bitter, sour or Seville orange. Bitter orange is a source of several important products, although they are not usually produced from the same subspecies. The most wide spread products are classified by **Boelens and Oporto, (1991)** as follows: leaf oils chiefly petitgrain, flower oils mainly neroli, peel and fruit oils. Leaf oil is

considered to be a secondary product from bitter orange trees grown to produce the oil and so, being in second order after flower oil. The most important constituent of leaf oil that defines its quality is its ester content which usually calculated as linalyl acetate; and the oil is sold commercially and marketly categorized on such a base; (**Boelens, 1991**).

Petitgrain bigarade is produced mainly in Europe and North Africa, while

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petitgrain from other localities is usually designated by the country of origin; i.e petitgrain Paraguay and Haiti (**Lawrence, 1993**).

Petitgrain bigarade oils are usually obtained by steam-distillation of leaves, twigs and small unripe fruits of true bitter orange. Such oil is pale to mid-yellow but crude oil is almost black. The odor of the same oil is pleasant being of floral-sweet and orange-like characteristics. The taste like orange and bitter-sweet, are related to the high linalyl acetate content being also considered responsible for the strong sweetness. This oil is extensively used in perfumery and flavoring to enhance the frequently harsh synthetic compounds used in some food stuffs, sweets, soft drinks and cordials. North African oils are generally conform to this quality; since most plantations were established from French material (**Weiss, 1997**). The chemical composition of petitgrain oils is not well defined like that of citrus peel oils. This is probably due to the main following reasons: It is difficult to identify and completely quantify components of natural complex mixture such as petitgrain oils, and the samples analyzed may have different origins (**Mondello and Dugo, 1996**).

The aim of this study is to take a brief knowledge of the effect of different storage temperature 20, 4 and -18 C° on the composition of petitgrain oil produced in Egypt, to be used as reference for quality and genuineness control. To reach such goal, storage period was planned to be for 6 months and the analyses were

carried out by GC/MS, using high resolution GC columns and a mass library equipped with Linear Retention Indices, which can be used interactively with MS data for component identification.

## MATERIAL AND METHODS

### Materials

The bitter orange petitgrain bigarade oil was provided from the Sugar and Integrated Industries Company, Perfumes and Essences Factories, Hawamdia, Giza, Egypt. In February 2004. The oil was packed in special dark vials to prevent any undesirable effect of light.

Petitgrain oils were stored at three different temperatures as follows:

- \*\* At room temperature (about 20°C).
- \*\* Under cooling condition (4°C).
- \*\* Under frozen condition (-18°C).

Samples were analyzed at zero time, after one, 3 and 6 months for identifying the main properties of the tested oil. And the gas chromatography mass spectrum was applied for analyzing the fresh sample (zero time) as well as those stored under the aforementioned conditions.

### Methods

The main properties in terms of color, specific gravity (15°C), optical rotation (20°C), refractive index (20°C) were determined by the methods given in the **AOAC. (1995)**. Solubility (v/v 70% alcohol), acid number and ester number

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were determined by the methods described in FCC. (1996).

GC/MS analysis, was performed using HP5890 Gas Chromatograph with a flame ionization detector (FID) was used. The column was HP 20M (carbowax-20M) (25m X 0.2mm X 0.2 $\mu$ m film thickness). The column temperature was isothermal at 45°C for 3min. and then programmed to 220°C at a rate of 3°C min<sup>-1</sup> for 50 min. The temperature of the injection part was 200°C and that of the detector was 250°C. The column head pressure was adjusted to 10 psi and the relative concentration of the components were based on the peak areas obtained with a Shimadzu CR-18 Chromatopac. (Kirbaslar and Ismail Kirbaslar, 2004).

Statistical computer program was applied to calculate correlation coefficient and principal component analysis were also performed using the same program (SAS, 1996)

## RESULTS AND DISCUSSION

The main constituents of the investigated petitgrain bigarade oils that

stored at different temperature for 6 months are shown in Tables (1, 2 and 3). With respect to the petitgrain oil stored at room temperature (about 20°C), its color that was yellow before storage become slightly brown yellow after 6 months of storage as seen in Table (1). On the other hand, the color of petitgrain oils stored at 4°C and -18°C turned very slightly brown yellow and pale yellow after the same storage period (6 months), as seen in Tables (2 and 3). These findings were found to be agree with the opinion that the oil is quickly darkens and its odor deteriorates unless kept in well-sealed containers in a cool dark store place (Lota *et al* 2001).

Concerning the specific gravity and refractive index of the same investigated samples, the obtained data indicted a level of stability under any given storage temperature and storage time. On the other hand, optical rotation recorded a value of +0.8° at zero time and +1° for all petitgrain oil samples stored at the three different storage temperatures (Tables 1, 2 and 3).

Table 1. Some main constituents of Petitgrain bigarade oils produced in Egypt and stored for 6 months at room temperature (about 20°C)

Tested Parameters	Storage periods / months			
	zero	1	3	6
Color (O.D)	Yellow	Yellow	Yellow	Very s.b.y*
	0.561	0.555	0.597	0.764
Specific gravity (15°C)	0.8853	0.8867	0.8841	0.8857
Optical rotation (20°C)	+0.8°	+0.9°	+1°	+1°
Refractive index (20°C)	1.4652	1.4651	1.4655	1.4652

	1:3	1:2.9	1:2.3	1:2.3
Solubility (v/v 70% alcohol)	more clear	more clear	Hazy	more hazy
Acid number	0.50	0.90	1.10	1.20
Ester number % (as linaly acetate)	43.90	44.10	46.6	41.30

\* Very slightly brown yellow

Table 2. Main constituents of Petitgrain bigarade oils produced in Egypt and stored under cooling condition 4°C for 6 months.

Tested Parameters	Storage periods / months			
	zero	1	3	6
Color (O.D)	yellow	yellow	yellow	Very s.b.y.*
	0.561	0.549	0.526	0.600
Specific gravity (15°C)	0.8853	0.8866	0.8834	0.8866
Optical rotation (20°C)	+0.8°	+0.9°	+1°	+1°
Refractive index (20°C)	1.4652	1.4650	1.4654	1.4652
Solubility (v/v 70% alcohol)	1:3 more clear	1:2.8 more clear	1:2.5 more clear	1:2.2 more clear
Acid number	0.5	0.75	1.00	1.20
Ester number % (as linaly acetate)	43.90	43.05	46.6	42.7

\* Very slightly brown yellow

Table 3. Main constituents of Petitgrain bigarade oils produced in Egypt and stored under frozen condition (-18°C) for 6 months

Tested Parameters	Storage periods / months			
	zero	1	3	6
Color (O.D)	Yellow	Yellow	Yellow	Pale yellow

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## GC-Mass analysis of bitter orange petitgrain bigarade oil

Refractive index (20°C)	1.4652	1.4651	1.4645	1.4647
Solubility (v/v 70% alcohol)	1:3 more clear	1:2.6 more clear	1:2.7 more clear	1:2.3 more clear
Acid number	0.50	0.70	0.80	1.00
Ester number % (as linaly acetate)	43.90	44.90	46.90	41.20

The available data indicate a relation between acid number and storage temperatures as given in Tables (1, 2 and 3). For examples; acid number that was 0.50 at zero time increased to 1.20, 1.20 and 1.00 after 6 months of storage at 20°, 4° and -18°C, respectively. However, ester number in terms of linalyl acetate showed upward and downward trends when the tested samples stored at 20°C; values obtained were 43.90% at zero time and reached to 46.60% at the third month of storage and dropped to 41.30% when storage extend to 6 months. These results are matched with **Dugo, (1994)** who reported that the ester number of a similar oil sample was ranged from 25 to 68% and based on origin of the sample. On the other hand, solubility in 70% alcohol was greatly affected by storage condition where the solution was clear in both samples at zero time and those stored for 6 months under either cooling (4°C) or frozen conditions (-18°C). On contrary, samples stored at 20°C were found to be more hazy after 6 months storage (**Baaliouamer and Meklati, 1986**).

The GC/MS chromatograms of bitter orange petitgrain oil produced in Egypt and stored at different temperatures namely fresh (zero time) as well as 20°C, 4°C and -18°C respectively are given in Fig. (1). The identified compounds of the analyzed samples are reported in Table (4). As can be seen from the same table and the figures, sixty-four components were identified, (representing over than 99.5%) of the whole oil ; from which 31 components were highly and exactly well

identified and confirmed according to the available standards.

From Table (4), it is clear that linalyl acetate, the percentage of which was never less than 25%, is considered to be the major component of the ester fraction, followed by geranyl acetate (4.2-5.6%) and citronellyl acetate (0.054–0.073%). On the other hand, Linalool was the main alcohol, varying from (30 – 33.7%), followed by  $\alpha$ -terpinol (2.5%). Limonene, (E)- $\beta$ -ocimene, myrcene and  $\beta$ -pinene were the main components among the monoterpene hydrocarbons, constituting 2.8 to 6.9% (**Huang and Pu, 2000**). Tricyclene, 6-methyl-5-hepten-2-one and p-mentha-(2,4)8-diene have been identified in bitter orange petitgrain oil. Similar findings were given by **Mosandl and Juchelka, (1997)**. Using Italian Citrus petitgrain oil.

Statistical analysis in terms of principle components (factor 1 and factor 2) is given in Table (5). Concerning “factor 1”, data proved the suitability of storing the tested samples at about 20°C since the factor 1 is 0.9654 which is approximately going with that of the fresh sample that scored a value of 0.9672. On the other hand, when “factor 2” was considered, values obtained namely 0.9844 and 0.9864 were noticed for samples stored at 4 °C and -18 °C, respectively. In other words; statistical analysis (principle component) confirmed the data given in Table (4) that indicates higher stability of the petitgrain bigarade oils during storage for 6 months at 4 or at -18 °C. In terms of cost; it is

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## GC-Mass analysis of bitter orange petitgrain bigarade oil

recommended to store the tested samples at 4 °C since there is no significant changes between 4 and -18 °C as indicated by analysis of variance given in the Table (5). This simply means that, properties of petitgrain bigarade oil stored

at 4 °C for 6 months were too close to the un-stored sample (fresh sample).

Many components that identified in this oil were not previously detected. Moreover, results of the analyses of the

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Table 4. GC /MS analysis of bitter orange petitgrain bigarade oils obtained by. GC column HP-20M. (Values are given as area %).

Identified components	Zero time	Storage under different temperature		
		20°C	4°C	-18°C
Tricyclene	0.816	1.743	0.424	0.640
$\alpha$ - Pinene	0.172	0.157	0.175	0.145
$\beta$ - Pinene	0.984	0.871	0.973	0.963
Sabinene	0.019	0.016	0.022	0.021
$\delta$ -3- Carene	2.013	1.822	2.029	2.020
Myrcene	3.017	2.76	3.017	2.886
$\gamma$ - Terpinene	2.010	1.820	2.030	2.020
Limonene	6.712	6.124	6.905	6.835
$\beta$ - Phellandrene	0.177	0.152	0.174	0.161
(Z)- $\beta$ - Ocimene	1.153	1.014	1.053	1.078
(E)- $\beta$ - Ocimene	3.078	2.771	2.832	2.899
Terpinolene	0.750	0.670	0.658	0.692
6-Methyl-5-hepten-2-one	0.013	0.012	Traces	Traces
Nonanal	0.173	0.150	0.171	0.170
P-cymenene	0.050	0.027	0.038	0.042
Citronellal	0.118	0.105	0.187	0.235
Linalool	32.95	30.70	33.73	32.68
Linalyl acetate	27.72	25.19	25.23	28.34
Trans- $\alpha$ -bergamotene	0.562	0.510	0.569	0.566
(Z)- $\beta$ - famesene	0.283	0.264	0.293	0.274
Neral	0.061	0.047	0.070	0.067
$\alpha$ -humulene	4.348	3.999	4.454	4.376
$\alpha$ - terpineol	2.527	2.335	2.570	2.552
Bicyclogermacrene	Traces	Traces	Traces	Traces
Geranyl acetate	5.580	4.207	4.680	4.614
Nerol	1.063	0.967	1.050	1.062
Geraniol	2.273	2.111	2.292	2.258
(E)-nerolidol	0.475	0.401	0.536	0.423
Citronellyl acetate	0.073	0.055	0.054	0.054
P-cymene	0.110	0.087	0.124	0.101
P-mentha-(2,4)-8-diene	0.055	0.042	0.045	0.045

## GC-Mass analysis of bitter orange petitgrain bigarade oil

Table 5. Principle components analysis of the identified constituents of petitgrain oil.

Factor analysis	principle components		Extraction of principle components	
	Factor 1	Factor 2	From factor 1	From factor 2
Zero time	0.967219	0.153234	0.669415	0.958994
Room temp.	0.965461	0.152678	0.666678	0.955426
Temp. 4 °C	0.148811	0.984453	0.597539	0.991292
Temp. -18 °C	0.140673	0.986454	0.590345	0.992881
Experimental Variance.	2.099759	1.989550	-	-

## Analysis of Variance

Source	DF	SS	MS	F	P
Factor	3	1.2	0.4	0.01	0.999
Error	124	6423.7	51.8		
Total	127	6424.9			

Individual 95% CIs For Mean  
Based on Pooled StDev

Level	N	Mean	StDev	
Zero	32	3.103	7.372	(-----+-----+-----+-----)
Room	32	2.849	6.785	(-----*-----)
4 °C	32	3.027	7.215	(-----*-----)
-18 °C	32	3.070	7.402	(-----*-----)
Pooled StDev = 7.197				1.5 3.0 4.5

four samples are in good agreement showing the robustness of the procedure used and the selection of raw material to be distilled. As a consequence, the results of this study can be thought as being representative of the composition of the Egyptian industrial bitter orange petitgrain oil.

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795-805، 2005، 13(3) قره اقل، سمشن ي ع ة عم ا ح ة ي ع ار ز ل ن ل و ح بل ل ا س ار د ق ل ي ب ر ع ل ت ا ع م ا ج ل د ا ح ت ا ق ل ح م

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