

# OPTIMIZATION OF SUPERCRITICAL FLUID EXTRACTION FOR ESSENTIAL OILS FROM BITTER ORANGE (VAR.AMARA)

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## ABSTRACT

The supercritical fluid extraction of bitter orange essential oil was studied using dehydrated bitter orange peel as raw material and CO<sub>2</sub> as solvent. The influence of different operation conditions was analysed in the pressure range from 100 to 300 atm at temperatures 35,45 and 55 °C for a static time of 20 minutes. In these experiments, the duration of dynamic time was varied from 15 to 35 minutes and also the concentration of co-solvent (methanol) was increased from 0 to 10% (V/V). Limonene was the principal component extracted, the optimum conditions for limonene extraction were P=300 atm, T=45°C, t<sub>d</sub>=15min and C<sub>co-solvent</sub>=10% (V/V). In these conditions limonene represents more than 94% of the essential oil.

## INTRODUCTION

Essential oils produced from fruit peels are widely used as flavours and fragrances in the food, perfume and cosmetic industries. Peel oils are mainly composed of a volatile fraction consisting of terpene hydrocarbons and their oxygenated derivatives, and of a non-volatile fraction including waxes and pigments. Hydrocarbons have to be removed in order to stabilize the product since they can oxidize, developing unpleasant odours [1]. Distillation techniques are commonly used to remove these components but these techniques employ high temperature, which could degrade aroma components. Therefore, supercritical fluid extraction (SFE) seems to be an attractive alternative to traditional extraction techniques, in order to avoid the use of organic solvents and thermal processes.

In the last two decades, numerous researchers have investigated the fundamentals and process applications of supercritical fluids. SFE has been used for the extraction of organic compounds such as flavour and fragrances from natural products [2-4]. Supercritical fluids as solvents have advantages such as excellent mass transfer and control of solubility by temperature and pressure. Liquid CO<sub>2</sub> seems to be the most interesting solvent for flavour extraction because of its high selectivity towards aroma representative constituents and the low temperature of its critical point. Attempts to extract aroma products from fruits and plant materials with supercritical CO<sub>2</sub> have been reported for apples, pears, different aromatic plants, and also different citrus peel oil such as orange, lemon, lime, bergamot, mandarin [5-9], but there is no reported study on bitter orange peel oil extraction. Therefore, the objectives of this study were to investigate the possibilities of using SFE with CO<sub>2</sub> to extract essential oils from bitter orange and to compare the effect of different process parameters on the extract composition.

## I- MATERIALS AND METHODS

### I-1-PLANT MATERIALS

The bitter oranges used in this work were *citrus aurantium* var. *amara* grown in northern province of Iran and harvested when ripe. The peel of fresh fruits was dried at ambient temperature avoiding light. Dried samples were ground in a coffee grinder and sieved.

### I-2-CHEMICALS

CO<sub>2</sub> with a purity of 99.99% was supplied by Parham Company (Tehran, Iran) and methanol with purity more than 99% (Merck, Germany) was used as a co-solvent. Hexane with purity more than 99% (Merck, Germany) was used as solvent in GC and helium with a purity of 99.99% (used as carrier in GC) was purchased from Parham Company (Tehran, Iran).

### I-3-APPARATUS

The experiments were carried out in a Suprex MPS/225 integrated SFE-SFC multipurpose system. Solubility measurements were accomplished with a 1-ml extraction vessel in the pressure range from 100 to 300 atm at temperatures 35,45 and 55 °C for a static time of 20 minutes. In these experiments, the duration of dynamic time was varied from 15 to 35 minutes and also the concentration of co-solvent (methanol) was increased from 0 to 10% (V/V).

The ground samples (2.5 g) were mixed thoroughly with 8.5 g of glass beads and packed into the extraction cell. This procedure prevents channeling, increase the contact surface between the sample and the supercritical fluid, consequently, reduces the equilibrium time.

Extracts were analysed by gas chromatography (Hp-6890) with an Hp-5Ms (30m×0.25mm×0.25µm) column and also by gas chromatography-mass spectrometry (GC-MS) Hp-5973. The GC conditions were: carrier gas, helium at a flow of 1 mL min<sup>-1</sup>. The temperature of column increased from 66°C with a temperature gradient equal to 6°Cmin<sup>-1</sup> and reached to 220°C after 3 minutes then was held at 220°C for 5 minutes.

### I-4-STATISTICAL METHOD

We used Tagushi's method in order to optimise the extraction conditions [10]. To achieve the goal of optimisation, Taguchi advocated a method of robust design involving the identification of control and environmental factors along with the quality characteristic to be studied. In these experiments we had 4 factors each having 3 levels, then a three-level L<sub>9</sub> (3<sup>4</sup>) orthogonal table (table 1) used for the optimisation process.

Run	Pressure (atm)	Temperature (°C)	Dynamic time (min)	C <sub>methanol</sub> (%V/V)
1	100	35	15	0
2	100	45	25	5
3	100	55	35	10
4	200	35	25	10
5	200	45	35	0
6	200	55	15	5
7	300	35	35	5
8	300	45	15	10
9	300	55	25	0

Table1- The L<sub>9</sub> orthogonal table used for the optimisation of experimental conditions.

## II-RESULTS AND DISCUSSIONS

Limonene, the principal component of the citrus peel oil, is an unsaturated terpene hydrocarbon, which is unstable to heat, light and oxygen. Then, it is possible to represent the behaviour of the bitter orange peel oil by its principal component, Limonene [1,8].

In the extracts different compounds were identified which are gathered in table2. As it can be seen in this table employing different conditions results different extract compositions. Based on limonene behaviour, we can observe that using run 8 gives the highest concentration of limonene. Then it can be considered as the best deterpenation condition (Fig1).

Constituent	Recovery (%)								
	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9
Limonene	0	2.31	0	12.1	0	17.88	3.87	94.67	0
$\alpha$ -pinene	0	0	22.17	0	0	0	0	0	0
Terpinene	0	0	7.85	0	0	0	0	0	0
Terpineol	0	0	0	0	0	0	0	0	0
Cresol	0	2.33	4.88	0	0	0	0	0	0
Linalool	0	0	0	0	0	0	0	0	0
Linalyl formate	0	0	15.9	0	0	0	0	0	0
camphen	0	0	0	0.57	0	0	0	5.33	0

Table 2- Extract composition (GC area %) of bitter orange peel oil at different operating conditions (9 runs).

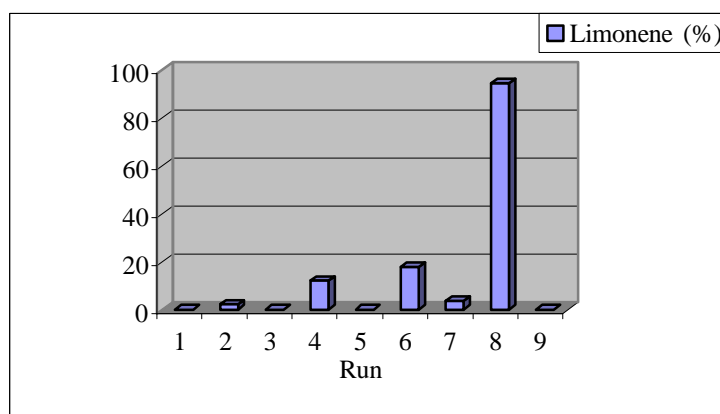


Figure 1- The quality of extraction process based on the amount of extracted limonene.

The effect of operating conditions were on the solubility of limonene is shown in table 3. The results from table 3 show that increasing pressure and co-solvent concentration, the solubility of limonene increases. However, it can be seen that by increasing dynamic time, the solubility of

limonene decreases. Regarding temperature, we can observe that, there is an optimum point (level 2).

Parameter	Level 1	Level 2	Level 3
Pressure	0	9.99	32.85
Temperature	5.23	31.56	5.96
Dynamic Time	37.52	4.03	1.29
Co-solvent	0	7.25	35.59

Table 3- Average effects of 4 operating parameters.

In order to recognize the effect of each parameter (pressure, temperature, dynamic time and co-solvent) on the quality of extraction, Tagushi's statistical method was used (table 4).

Factor	P (%)
Pressure	22.36
Temperature	17.66
Dynamic time	32.08
Co-solvent	27.90
Total	100

Table 4- Individual effect of each parameter on extraction quality obtained by using Taguchi's statistical method.

As it shown in table 4, dynamic time and co-solvent amount respectively with 32.08% and 27.90% have more effects on extraction process than other parameters. It is probably because of the increase of solubility coefficients (k) in presence of co-solvent.

## CONCLUSION

During supercritical fluid extraction of limonene from bitter orange peel oil, the solubility of limonene increases when operating pressure increases from 100 to 300 atm. The use of co-solvent in this extraction has a beneficent effect on the extraction quality. Regarding the sensitivity of limonene to the high temperature, it is preferable to avoid the temperatures more than 50°C.

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