

EFFECT OF SAMPLE PREPARATION METHOD ON 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 or fresh bitter orange peel as raw material and CO₂ as solvent. The influence of sample preparing (dried or fresh sample) on the Limonene extraction yield was studied. And also, 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_d=15min and C_{co-solvent}=10% (V/V). In these conditions the yield of limonene extraction and the composition of extracted essential oil are different for fresh and dried sample and drying process may cause an important loss of volatile components.

Key words: Supercritical fluid extraction, bitter orange, sample preparation method, and operation conditions.

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 CO₂ 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 CO₂ 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 CO₂ 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 (25-30°C) avoiding light for 72 hours. Dried samples were ground in a coffee grinder and sieved. For experiences with fresh sample, the mixture of fresh grounded peel and Na₂SO₄ was placed in sample cell.

I-2-CHEMICALS

CO₂ 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-APPARATUES

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⁻¹. The temperature of column increased from 66°C with a temperature gradient equal to 6°Cmin⁻¹ 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 L₉ (3⁴) orthogonal table (table 1) used for the optimisation process.

Run	Pressure (atm)	Temperature (°C)	Dynamic time (min)	C _{methanol} (% 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 L₉ 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 effect of sample dehydration on the quality of extraction by its principal component, Limonene [1, 8].

In the extracts different compounds were identified which are gathered in table 2. 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 in both methods. Then it can be considered as the best deterpenation condition (Fig1).

However our results show some differences in extract composition among runs and dried or fresh samples. The chemical composition of investigated samples (fresh or dried sample) is presented in Table 2. As it can be seen in dried sample the amount of small molecule compounds (C₁₀ – C₁₅) is low compared to fresh sample. Knowing that most of aroma compounds are small and volatile, we can say drying sample may reduce aroma content of extract.

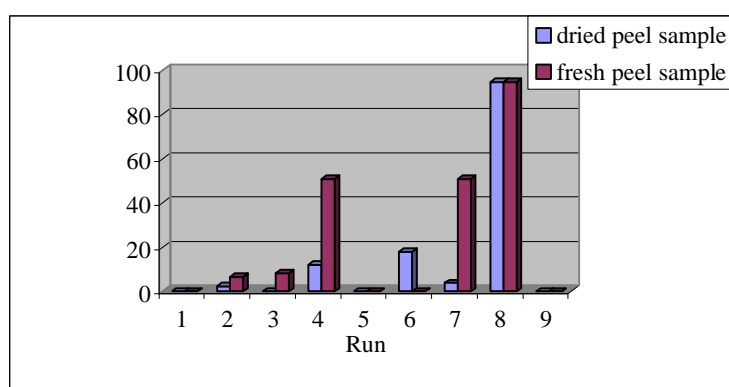


Figure 1- The quality of extraction process based on the amount of extracted limonene.

Constituent	Recovery (%)							
	Limonene	α -pinene	Terpineol	Terpinene	Cresol	Linalool	Linalyl formate	camphen
Run 1 D	0	0	0	0	0	0	0	0
Run 1 F	0	0	1.66	0	0	30.7	0	0
Run 2 D	2.31	0	0	0	2.33	0	0	0
Run 2 F	6.51	0	20.7	0	0	2.56	0	0
Run 3 D	0	22.87	0	7.85	4.88	0	15.9	0
Run 3 F	8.13	0	20.4	2.45	0	4.16	0	0
Run 4 D	12.1	0	0	0	0	0	0	0.57
Run 4 F	50.88	0	31.5	0	0	3.23	0	0
Run 5 D	0	0	0	0	0	0	0	0
Run 5 F	0	12.98	0	0	0	0	0	0
Run 6 D	17.88	0	0	0	0	0	0	0
Run 6 F	0	0	3.48	0	0	15.91	0	0
Run 7 D	3.87	0	0	0	0	0	0	0
Run 7 F	50.93	0	13	0	0	0	0	0
Run 8 D	94.67	0	0	0	0	0	0	5.33
Run 8 F	94.67	0	0	0	0	0	0	5.33
Run 9 D	0	0	0	0	0	0	0	0
Run 9 F	0	0	2.33	2.76	0	23.83	0	0

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

Constituent	Recovery (%)	
	Fresh sample	Dried sample
C10	22.24	17.5
C11	20.19	4.25
C12	28.72	1.90
C13	7.95	1.00
C14	7.22	6.10
C15	1.92	1.04
C16	5.78	12.04
C17	4.75	12.67
C18	1.24	19.89
C19	-	12.72
C20	-	-
C21	-	-
C22	-	52.00
C23	-	10.40

Table 3- Effect of sample preparation on the chemical composition of extracts (average of 9 runs).

The effect of operating conditions were on the solubility of limonene is shown in table 4. The results from table 4 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 4- Average effects of 4 operating parameters.

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 (both fresh and dried sample), 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 aroma compounds to the high temperature, it is shown that drying samples can decrease aroma content of extract.

ACKNOWLEDGEMENTS

The authors wish to thank Zamzam Iran Company for the financial support to carry out these experiments.

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