Supercritical CO_2 Extraction of Essential Oil from Clove Bud: Effect of Operation Conditions on the Selective Isolation of Eugenol and Eugenyl Acetate

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The supercritical fluid extraction (SFE) of clove bud essential oil was studied using CO_2 as solvent. The effect of operation conditions was analyzed in a series of experiments at temperatures between 325 and 416 K and pressures between 110 and 190 bar. The collected extracts were analyzed and the relative composition of the essential oil was determined. The optimum condition was found in a temperature of 353 K and at a pressure of 190 bar, minimizing the number of extracts to two compounds (eugenol and eugenyl acetate). The extract obtained from clove bud by using supercritical fluid extraction was compared with the essential oil obtained by steam distillation and microwave-assisted extraction by considering both quantity and quality of the product. The oil yield was higher in steam distillation and microwave oven extraction. In contrast, oil extracted by using SFE contained higher amount of eugenol and eugenyl acetate.

Key words: Clove Bud, Eugenol, Extraction

Introduction

Identification, extraction and preparation of clove bud oil are gaining interest as its applications are becoming widespread in different sectors like food, medicinal, pharmaceutical and cosmetic industries [1].

Different extraction methods are used for the recovery of essential oils from plant material. Supercritical fluid extraction (SFE) has gained increasing attention over the traditional techniques, like steam distillation and solvent extraction, in the recovery of edible and essential oils, as the use of a non-toxic and volatile solvent, such as CO_2, protects extracts from thermal degradation and solvent contamination [2].

In recent years considerable effort has been devoted to researching these processes and increasing the number of applications for them [2 – 7]. Many of these applications were to oil extraction [7 – 9].

Carbon dioxide is the most commonly used supercritical fluid in food industry because of its low critical temperature and pressure (T_c = 31.1 °C; P_c = 72.8 atm), its non-toxic and non-flammable properties and its availability in high purity with low cost. It is an inert gas which does not react with the food constituents. In addition, it is easily removable from the extract following decompression [10, 11].

The high pressure is used for processes where total extraction of a target compound is desired, since most extractable compounds exhibit their maximum solubility in a supercritical solvent at higher pressures. The selective extraction of the materials having low to medium volatility is possible by suitable choice and subsequent manipulation of the operating conditions [12].

The SFE of essential oil from clove bud has been previously studied. Most of the studies in the literature were focused on the analytical aspects of the problem such as the composition of the extracts at various extraction conditions. The feasibility of extracting of essential oil from clove bud by using SC-CO_2 (100 – 250 bar, 20 – 40 °C) has been studied [13]. Based on the literature, the SFE product has been found to be superior to the one produced by steam distillation [14 – 16]. Moreover, the extraction was studied under designed CO_2 flow rates under various condition at ranges 80 – 200 bar at 50 °C with at most 21% total yield [8, 17]. In all reports, the SFE of essential oil from clove bud has been carried out fewer than 50 °C to avoid the possible extraction of high molecular weight compounds.

In this work, the effect of higher temperature (325 – 416 K) was investigated on the extraction of essential

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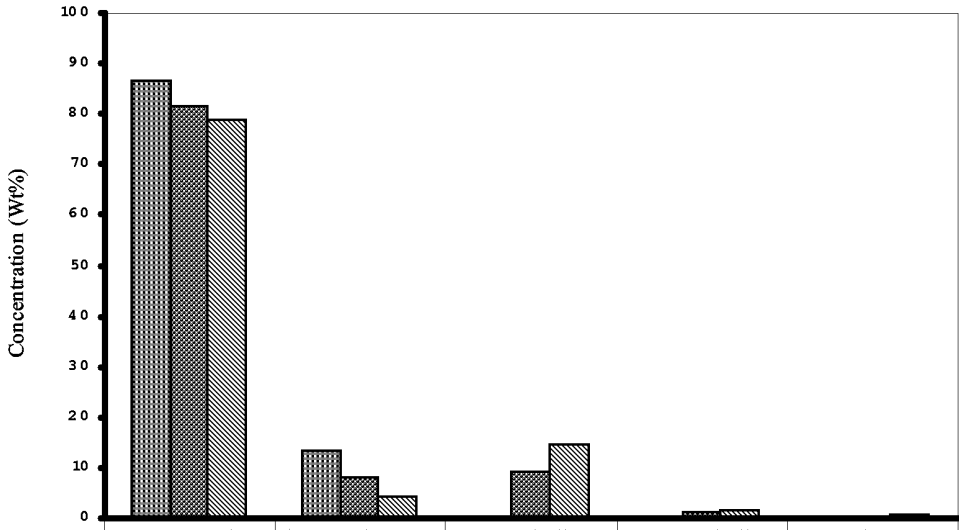

	Eugenol	Eugenylacetate	Caryophyllene	a-Caryophyllene	Others
SFE	86.70	13.30			
MAE	81.47	8.11	9.10	1.32	
SD	79.08	4.13	14.51	1.67	0.62

Scheme 1. Comparison of main components of clove bud under various extraction conditions.

Table 1. Effects of SFE parameters on clove bud oil yield and composition.

Entry	T (K)	P (bar)	Eugenol (%)	Eugenyl acetate (%)	Caryo-phyllene (%)	α-Caryo phyllene (%)
1	325	110	81.93	11.03	6.30	0.84
2	343	164	86.55	9.51	3.94	–
3	353	190	86.70	13.30	–	–
4	416	190	87.41	8.50	4.09	–

oil from clove bud in order to obtain less contaminated product.

The main discovery of the present study is, minimizing the number of extracts to two main compounds (eugenol and eugenyl acetate) under an optimum condition. In addition, SFE was compared with both conventional steam liquid solvent extraction processes and microwave-assisted extraction.

Results and Discussion

Effects of SFE parameters on oil yield and composition

The objective of this study was to establish appropriate conditions for the selective isolation of eugenol and eugenyl acetate using SCF-CO_2. To optimize the extraction, different temperatures and pressures were tested in the ranges of 110 – 190 bar and 325 – 416 K. The effect of time on the composition of clove bud oil was reported to be unimportant [16].

The analytical results are shown in Table 1 and Scheme 1. The essential oils of clove bud show eugenol, eugenyl acetate and caryophyllene as major constituents; although the presence of other constituents has been observed in all samples studied but in trace amounts. The extraction yields were 12 – 13%. In Table 1 and Fig. 1, it can be seen that, while pressure and temperature were increasing, the yield of eugenol increased, while at the same time, the relative percentage of caryophyllene in the extracts shows the opposite trend. In the case of eugenyl acetate, there is no direct correlation between its yield and the extraction conditions.

Optimization of supercritical fluid extraction

The optimum processing conditions were chosen by considering both quantity and quality of the oil. It was observed also a significant increase in the relative proportion of eugenol contents and a large reduction in the amount of caryophyllene which once optimized led to the disappearance of this compound in the case of SFE extraction.

Entry	Method[a]	Extraction time	Eugenol (%)	Eugenyl acetate (%)	Caryo-phyllene (%)	α-Caryo-phyllene (%)	Total yields (%)
a	SD[b]	6 h	79.08	4.13	14.51	1.67	17.0
b	SE[c]	24 h	78.88	9.67	10.14	1.31	11.4
c	MAE[d]	90 sec	81.47	8.11	9.10	1.32	14.2
d	MAE	360 sec	79.57	9.08	10.03	1.30	17.1

Table 2. Effects of traditional and microwave methods on clove bud oil yield and composition.

a) See the experimental section. b) SD: Steam Distillation. c) SE: Solvent Extraction. d) MAE: Microwave-Assisted Extraction.

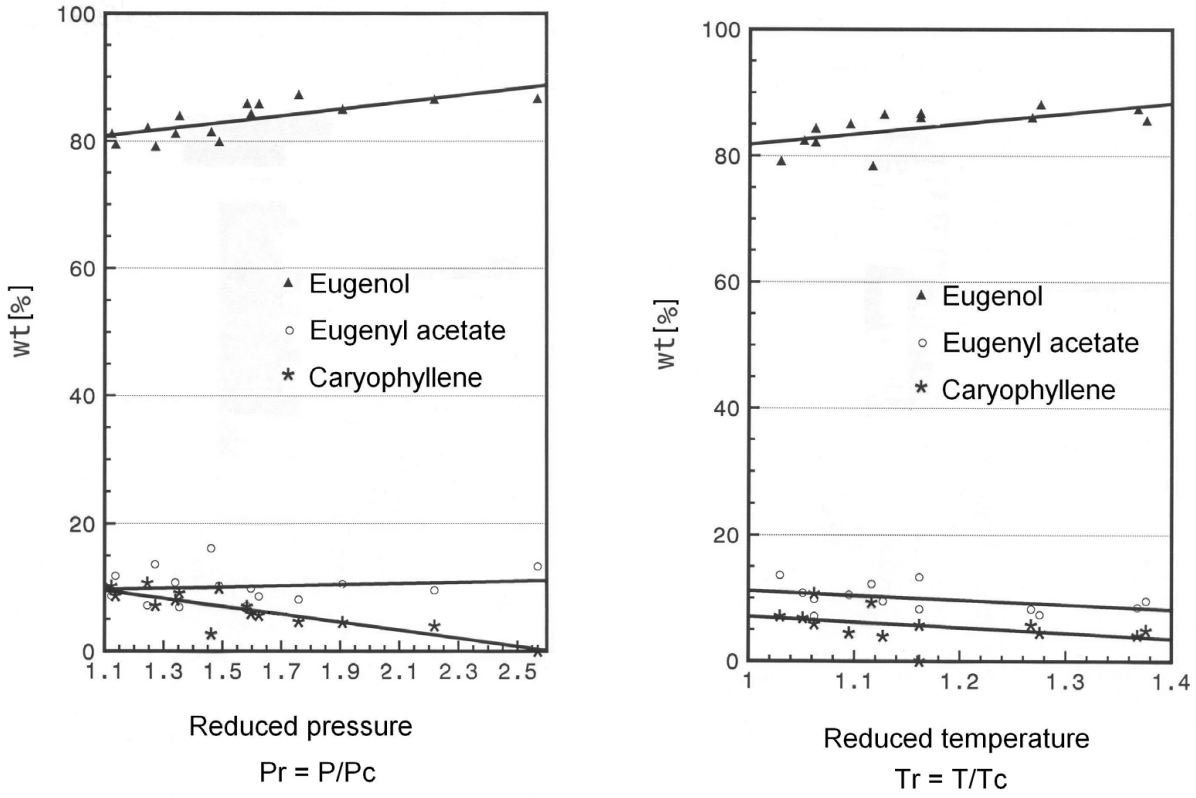

Fig. 1. Relative percentage of eugenol in the clove bud essential oil obtained with SC-CO_2 at different reduced pressures (left) and temperatures (right); P_c= critical pressure, T_c= critical temperature.

Effects of temperature and pressure on the eugenol content can be seen in Table 1. Entry 3 shows a remarkable selective extraction of eugenol and eugenyl acetate with no caryophyllene.

Comparison of the SFE extract and the essential oil obtained by steam distillation and microwave-assisted extraction

Steam distillation is typically considered the best way to obtain essential oils for use in aroma therapy. However, this method produces varying oil qualities dependant upon the temperature, pressure and time used for distillation. An important point regarding steam distillation of essential oils is that the temperature involved in the process changes the molecular composition of the plant matter. However, in practice most essential oils are obtained by distillation at rather elevated temperature, in order to optimize the yield.

The extraction solvent most commonly employed in the industry is hexane. Generally for complete extraction it is necessary to repeat the process several times in order to obtain an acceptable yield. The main disadvantage of this method is that, after the extraction, the solvent needs to be evaporated, which causes heat degradation of eugenyl acetate. At the end of the process the residual solvent concentration has to be lowered below legal limits, requiring even more drastic conditions. The extraction of clove bud oil was car-

ried out by traditional and microwave-assisted extraction (MAE) methods in hexane.

The other extraction methods and the main compositions of extracts are shown in Table 2. Entry **a** and entries **b** – **d** show the results of the steam and hexane extraction, respectively. The oil extraction produced after 360 s of microwave irradiation has a 17.1% yield. As shown in Table 2, the higher the total extraction yields, the lower the amount of eugenol. Thus, the irradiation for 90 s gives a better result for the eugenol concentration even if the total extraction yields are inferior (14.2%). Therefore, the microwave irradiation method has the advantage that the content of the flower is extracted in a short period of time. Moreover, as shown in Table 2 (entry **a**), the SD technique shows the lowest yield of eugenyl acetate, perhaps due to the hydrolysis of eugenyl acetate to eugenol.

Under the optimized SFE conditions, all the minor products obtained by the SD and MAE methods have completely disappeared or only a small trace of them is left.

Experimental Section

Materials

All the samples of Indian clove buds were obtained by sieving to 100 μm sized particles. Thus, the same particle size of all samples was available for the various extracting methods. All samples were first dried at room temperature before undergoing extraction.

The carbon dioxide used in SFE was 99.5% (w/w) pure. As a collection solvent *n*-hexane (99%) was used. The dried Indian clove bud was stored in dark bags. The maximum duration for storage was 3 months.

Steam distillation

A sample of 100 g of sieved material (100 μm sized) was extracted by steam distillation (SD). A sample was placed on a grill in a stainless steel container. Steam at atmospheric pressure was supplied from the bottom by a steam distributor with sparge holes. The distillation was conducted until no more essential oil was obtained and the essential oil collected at the exit of the glass condenser was separated by the action of gravity.

Solvent extraction

A sample of 5 g of sieved plant material was extracted with 100 ml of hexane for 24 h at 50 °C.

The microwave oven used for this study was a domestic National model NN-6755 with 7 power settings (90 – 900 W). In a typical experiment, 5 g of sieved sample (100 μm sized) in 100 ml of hexane in an open pyrex glass flask was exposed to microwave irradiation at 650 W for 90 s. The same experiment was also done for 360 s. During every 30 seconds irradiation, an interval of 5 min was carried out. The irradiated samples were filtered and the solvent was evaporated.

Supercritical CO_2 extraction

Supercritical fluid extractions were conducted using a jacketed (SS 1.4301) stainless steel vessel (SS 1.4571) of 1 l (Büchiglasuster, Switzerland) with maximum vessel working pressure of 200 bar and working temperature of 250 °C. Maximum pressure and temperature of the jacket were 20 bar and 250 °C, respectively.

Before each set of yield determinations at given extraction conditions, the extractor was filled with a weighed quantity of sieved sample (about 50 g) and a sufficient amount of CO_2, which was at least 10 times higher than the sample weight. After closing the vessel head and ensuring that there was no leak in the equipment, the condition of the extraction was arranged at predetermined pressure and temperature values. After a sufficient period (about 30 min) of equilibrium condition in the extraction cell, a very small amount of fluid phase was taken out *via* a needle valve and a narrow and short line which entered in *n*-hexane solvent. After closing the valve, the line was washed with the solvent and the sample was taken to analysis. Then, for further pressure and temperature conditions and equilibrium establishment, a new sample was taken out. At the end, by cooling the cell to below critical temperature and then releasing the pressure, the weight of the extract deposited in the cell was determined.

Oil analysis

Gas chromatography (GC)

A Fisons GC 8000 system was used for GC analysis, fitted with a 15% Apiezon L on Chromosorb WNAW (2 m × 1/8”). The oven temperature was isothermal at 190 °C. Injection was performed at 210 °C; 0.1 μl of sample was injected. A flow of 20 ml/min carrier gas (He) was used. Thermal conductivity detection (TCD) was performed at 210 °C.

Gas chromatography – mass spectrometry (GC – MS)

Analyses were carried out in a Fisons 8000 gas chromatograph fitted with a fused SE 54 column (25 m × 0.32 mm i.d., 0.4 μm film thickness), coupled to a Trio 1000 mass detector. Column temperature was programmed from 40 to 200 °C at 5 °C/min. Injection was performed at 220 °C. Helium was used as carrier gas (1.5 ml/min). Mass spectra were recorded in the scan mode at 70 eV (35 – 350 U); 0.5 μl of the sample in pentane, 1 mg/ml was injected in the split ratio (1 : 180).

Qualitative and quantitative analyses

Most constituents were identified by comparison of their GC retention indices with those of authentic standards available in the authors' laboratory or with GC data previously published. Identification was confirmed when possible by comparison of their mass spectra with those stored in the MS database (National Institute of Standards and Technology, NIST, and Wiley libraries) and with literature data for mass spectra. Relative concentrations of the components were obtained directly from GC peak areas.

Conclusion

This study presents a novel supercritical fluid extraction condition for the selective extraction of eugenol and eugenyl acetate (which gives a characteristic note to cloves fragrance) from clove buds. The extractions at the studied pressures and temperatures gave 12 – 13% of extracts to the dry weight of the raw material, and under optimum conditions the extract contained 86.7% and 13.3% eugenol and eugenyl acetate, respectively.

The percentage of eugenol was higher than in the extracts obtained by steam distillation (SD) and microwave-assisted extraction (MAE). The results show that the eugenol content is the major product and its extracts have been 86.70, 81.47 and 79.08% for SFE, MAE and SD, respectively. The extraction recovery from the dry flower material was 12 – 13, 14.2 and 17% for SFE, MAE and SD, respectively. The results demonstrated that SFE is an effective and selective method for recovery of eugenol and eugenyl acetate.

For commercial application the SFE is recommended because it reduces or eliminates health and environmental risks.

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