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Comparison of essential oils of clove buds extracted with supercritical carbon dioxide and other three traditional extraction methods

Guan Wenqiang [a], Li Shufen [a,*], Yan Ruixiang [b], Tang Shaokun [a], Quan Can [a]

[a] *Key Laboratory for Green Chemical Technology of State Education Ministry, School of Chemical Engineering and Technology, Tianjin University, Tianjin 300072, PR China*

[b] *National Engineering and Technology Research Center for Preservation of Agricultural Products, Tianjin 300384, PR China*

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Abstract

Supercritical fluid extraction (SFE) of essential oil from clove buds with CO_2 was explored. The effect of different parameters, such as temperature (30 °C, 40 °C, 50 °C), pressure(10 MPa, 20 MPa, 30 MPa) and particle size (three degree index), on the extraction yield and the content of eugenol in extracts was investigated using three-level orthogonal array design. The experimental results show that the temperature has the largest effect on the eugenol content of the extracts, and particle size has the maximum effect on the oil yield. The essential oil of 19.56% yield, in which the maximum content of eugenol in extracts is 58.77%, can be extracted from clove buds at pressure of 10 MPa and temperature of 50 °C. Essential oil of clove buds obtained by SFE, hydrodistillation, steam distillation and Soxhlet extraction were further analyzed by gas chromatography/mass spectrometric detection to compare the extraction methods. Twenty three compounds in the clove oils have been identified, showing that the composition of the clove oil extracted by different methods is mostly similar, whereas relative concentration of the identified compounds is apparently different. General characteristics of the clove oils obtained by different methods were further compared, and SFE is considered as the optimum process among the four processes for obtaining clove oil with high quality.

Keywords: Supercritical fluid extraction; Carbon dioxide; Essential oil; Clove bud; Steam distillation; Hydrodistillation; Soxhlet extraction

1. Introduction

Clove (*Eugenia caryophyllata* Thunb.) is widely cultivated in Madagascar, Sri Lanka, Indonesia and the south of China (Bureau of Drug Administration of China, 1989). Clove bud oils have biological activities, such as antibacterial, antifungal, insecticidal and antioxidant properties, and are used traditionally as flavoring agent and antimicrobial material in food (Huang, Ho, Lee, & Yap, 2002; Lee & Shibamoto, 2001; Velluti, Sanchis, Ramos, & Marín, 2003). For example, clove oil was effective against *L. monocytogenes* and *S. Enteritidis* in tryptone soya broth (TSB) and cheese (Smith-Palmer, Stewart, & Fyfe, 1998, 2001). The high levels of eugenol contained in clove essential oil give it strong biological activity and antimicrobial activity. This phenolic compound can denature proteins and reacts with cell membrane phospholipids changing their permeability (Briozzo, 1989; Deans & Ritchie, 1987). Clove oil also has several therapeutic effects, including antiphlogistic, antivomiting, analgesic, antispasmodic, anticarminative, kidney reinforcement, antiseptic, HCMV extracorporeal restraining effect (Liu, Mao, & Hong, 1997; National Pharmacopoeia Committee of China, 2000). In Korea, clove oil has been successfully used for asthma and various allergic disorders by oral administration (Kim, Lee, & Hong, 1998).

The essential oil of aromatic herbs is traditionally obtained by hydrodistillation, steam distillation, or solvent

* Corresponding author. Tel.: +86 22 27407388; fax: +86 22 27402720.
E-mail addresses: guanwenqiang1@hotmail.com (W. Guan), shfli@tju.edu.cn (S. Li).

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extraction. These processes cost not expensive but can induce thermal degradation, hydrolysis and water solubilization of some fragrance constituents. Extracts obtained by solvents contain residues that pollute the foods and fragrances to which they are added. These disadvantages can be avoided by using the supercritical fluid extraction (SFE) process (Mostafa, Yadollah, Fatemeh, & Naader, 2004; Reverchon, 1997). It is well known and SFE has been established as an environmentally benign technique for separating essential oils from the vegetable substrates. Moreover, extracts obtained by SFE can retain the organoleptic characteristics of the starting spice material (Biljana, Zika, Vladimir, & Aleksandar, 2005).

Supercritical CO_2 extraction of cloves oil has been performed previously by some authors (Della Porta, Taddeo, D'Urso, & Reverchon, 1998; Liu, Chen, Chen, & Chang, 2003; Reverchon & Marrone, 1997; Rodrigues et al., 2002). The conditions explored were at pressures from 10 to 50 MPa and temperatures ranging from 30 to 40 °C. Compounds with high molecular weight and coloring materials, such as chlorophyll, were recovered together with the fragrance constituents owing to the high pressures utilized. For example, in the extraction performed by Gopalakrishnan, Shanti, and Narayanan (1990), increasing the extraction pressure from 10 to 50 MPa at a temperature of 40 °C resulted in an increase of the chlorophyll content in the extract (Gopalakrishnan et al., 1990). Comparison of some essential oils obtained by SFE with conventional methods and GC analysis of clove oil in ethanol had been studied by some authors (Myint, Wan, Mohamad, & Kadhum, 1996; Reverchon & Senatore, 1992; Scalia & Giuffreda, 1999), however, none of investigations has examined in detail the comparison of essential oil composition between clove buds oil obtained by SFE and the conventional extraction techniques such as hydrodistillation, steam distillation, and solvent extraction.

Since the clove oil has been used widely as pharmaceuticals, flavoring and antimicrobial agents in food industry, it is necessary to find the most suitable method for the improvement of the quality of clove oil. The aim of this work is to compare clove oils obtained by the supercritical CO_2 extraction with other three traditional methods, in which extraction of clove oil from clove buds with supercritical CO_2 was first investigated intensively. Compositions of clove oil were analyzed by gas chromatography (GC) and gas chromatography–mass spectrometry (GC–MS).

2. Materials and methods

2.1. Materials

The buds of clove (*E. caryophyllata* Thunb.) were purchased from Tianjin factory for Chinese herbs. The samples were dried at 30 °C in a ventilated drying oven and stored in plastic bags at ambient temperature and protected from light. The samples were ground by a FW80 Sample Mill (Taisite CO., Tianjin, China) in different periods to get the different particle distribution, which was measured by mechanical sieving after extraction and calculated by weight of different size of clove bud particle. Grades of particle size was classified on the following scale: $1 > 10$ mesh; $2 = 10$–20 mesh; $3 = 20$–40 mesh; $4 = 40$–60 mesh; $5 = 60$–80 mesh; $6 = 80$–100 mesh; $7 = 100$–120 mesh; $8 < 120$ mesh. Particle size index was calculated by following formula: Particle size index $= \sum$(weight of each grade $\times$ grade)/(total weight $\times$ highest grade). The particle size index of material in this experiment was 0.7944, 0.6430, 0.5223, and named as 1#, 2# and 3#, respectively.

The solvents and chemicals were obtained from following sources: carbon dioxide, 99.99% purity, from Tianjin Anxing gas factory; China. Dichloromethane, purity > 99.9%, from Tianjin chemical Co., China; Eugenol, Purity $\geqslant$ 99.0% (GC), from Fluka Co., Germany; Eugenol acetate, Purity > 90.0% (GC), TCI America Co., Japan.

2.2. Experimental apparatus and methods

2.2.1. Supercritical CO_2 extraction

Extraction of essential oil from clove buds was experimentally determined using the Speed SFE instrument (Applied Separations Inc., Allenton, PA, USA). Liquid CO_2 was pressurized with a high-pressure pump and then charged into the extraction column to desired pressure. The pressure was controlled to an accuracy of about 1% over the measuring range. The extraction column was 32 ml with 14.40 mm inner diameter and 195 mm length, being packed with powdered raw materials and glass beads. The extraction column was heated with an oven and its temperature was indicated and controlled by a thermocouple to within ± 1 °C. The supercritical CO_2 with dissolved compounds passed through a heated micrometer valve, and was subsequently expanded to ambient pressure. The extract was precipitated in a collect vial at ambient pressure and temperature. A calibrated wet-test meter at known temperature and pressure measured the total amount of CO_2.

For each extraction test, the extractor was charged with about 15 g of ground clove bud powder. CO_2 flow rates ranging about 2 l/min were used. The oil weight was measured by precision balance until no oil was extracted out from the clove bud powder.

2.2.2. Hydro and steam distillation

The plant (100 g of dried and ground clove buds) in 500 ml flask was submitted to hydrodistillation for 4–6 h and steam distillation for 8–10 h.

The volatile distillate was collected until no oil drop out. The distillate was saturated with sodium chloride and added with some ether. Then, the ether layer and hydro layer were separated by funnel. After dehydrated by anhydrous sodium sulphate, the ether layer was further heated in 60 °C water bath to make oil to be concentrated and the ether to be recovered. The oil was weighed and refrigerated prior to analysis.

2.2.3. Solvent extraction

The ground clove bud samples (30 g) were weighed and quantitatively transferred into a filter paper extraction thimble and inserted into a 500-ml reflux flask, then extracted with 250 ml absolute ethanol for about 6 h in a Soxhlet apparatus (Quan et al., 2004). After Soxhlet extraction, extracts were concentrated using rotary vacuum evaporator at 50 °C.

2.2.4. GC and GC/MS analysis

GC analyses were performed using a Shimadzu GC-2010 gas chromatograph equipped with a FID and a DB-5 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm, Agilent). Oven temperature was 80 °C for 2 min, then programmed heating from 80 to 230 °C at a rate of 6 °C/min, and at 230 °C for 2 min. Injector and detector temperatures were 230 °C. The carrier gas, nitrogen, was adjusted to a linear velocity of 24 ml/min. The samples (0.1% in absolute ethanol) were injected into the GC by split mode with a split ratio of 1/20. Methyl salicylate was used as internal standard.

GC/MS analyses were performed using Finnigan Trace GC/DSQ equipped with a BTX.5ms fused-silica capillary column (15 m × 0.25 mm i.d., film thickness 0.25 μm, RESTEK CORP.). Oven temperature conditions were: 40 °C for 1 min, then programmed heating from 40 to 200 °C at a rate of 6 °C/min and from 200 to 280 °C at a rate of 30 °C/min. Injector temperature was 250 °C. The carrier gas, helium, was adjusted to a linear velocity of 1 ml/min. Ion source temperature was 250 °C;The ionization energy was 70 eV with a scan time of 1 s and mass range of 20–500 AMU. Samples were run in dichloromethane with a dilution of 0.1% (V/V). Compounds were identified by matching their mass spectra and retention times with those of pure compounds whenever possible. NIST (National Institute of Standards and Technologies) Mass Spectra Library was also used as a reference (see Table 1).

3. Results and discussion

3.1. Yield and eugenol content of the oil extracted by SFE

The following parameters was used: temperature, 30 °C, 40 °C and 50 °C; Pressure, 10 MPa, 20 MPa and 30 MPa; particle size, 1#(0.7944), 2#(0.6430) and 3#(0.5223). All the selected factors were examined by using a three-level orthogonal array design with an OA9 (3^3) matrix. Table 2 shows the experimental conditions for each of the SFE runs. It can be seen from the order of the maximum differences that the particle size had the most influence on the oil yield, then the temperature and pressure. However, the sequence of the influences of the parameters on the eugenol content in the oils was temperature, particle size, and pressure. That is, the factor of temperature has the maximum influence on the eugenol content in the oils.

Fig. 1 shows the effect of temperature, pressure and particle size on the yield and eugenol content of clove

Table 1
Three-level orthogonal design and experimental results for extraction of clove oil with SC–CO_2

	Factor A (*T*/°C)	Factor B (*P*/MPa)	Factor C (particle size/#)	Yield (kg extract/kg feed)	Eugenol content (%)
Run No.					
1	1(30)	1(10)	1(1#)	0.2056	53.69
2	1(30)	2(20)	2(2#)	0.1943	54.22
3	1(30)	3(30)	3(3#)	0.1830	55.64
4	2(40)	1(10)	3(3#)	0.1910	56.20
5	2(40)	2(20)	1(1#)	0.2224	54.52
6	2(40)	3(30)	2(2#)	0.2043	55.30
7	3(50)	1(10)	2(2#)	0.1956	58.77
8	3(50)	2(20)	3(3#)	0.1827	57.83
9	3(50)	3(30)	1(1#)	0.2395	56.97
Yield (%)					
K1	0.5830	0.5923	0.6676	∑=1.8186	
K2	0.6178	0.5995	0.5942		
K3	0.6179	0.6269	0.5568		
K1/3	0.1943	0.1974	0.2225		
K2/3	0.2059	0.1998	0.1981		
K3/3	0.2060	0.2090	0.1856		
R	0.0117	0.0116	0.0369		
Eugenol content (%)					
K1	163.55	168.66	165.18	∑=503.14	
K2	166.02	166.57	168.29		
K3	173.57	167.91	169.67		
K1/3	54.52	56.22	55.06		
K2/3	55.34	55.52	56.10		
K3/3	57.86	55.97	56.56		
R	3.34	0.25	1.04		

Table 2
Composition of clove oil obtained by different methods

		RT[a]	Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	SD[b]	HD[c]	SO[d]
1	4-(2-propenyl)-Phenol	10.91	0.38	0.48	0.49	0.45	0.47	0.46	0.46	0.45	0.44	0.35	0.26	0.52
2	α-Cubebene	12.79	0.53	0.51	0.49	0.45	0.48	0.43	0.32	0.4	0.4	0.47	0.85	
3	Eugenol	13.21	53.8	54.58	55.07	55.18	54.79	55.14	57.36	56.81	55.94	58.2	48.82	57.24
4	α-Copaene	13.34	0.95	0.63	0.86	0.5	0.57	0.51	0.65	0.72	0.76	0.81	1.93	0.93
5	Caryophyllene	14.23	17.77	17.32	16.78	16.52	16.62	15.52	13.99	15.73	15.71	20.59	36.94	17.5
6	α-Humulene	14.88	2.32	2.26	2.15	2.15	2.13	2.02	1.9	2.08	1.95	2.61	4.41	2.03
7	Isoledene	15.32	0.08	–	–	0.11	–	–	–	–	–	0.41	0.55	–
8	γ-Muurolene	15.44	0.37	0.4	0.37	0.35	0.36	0.36	0.65	0.42	0.32		0.19	
9	α-Muurolene	15.79	0.28	0.27	0.28	0.22	0.22	0.22	–	–	–	–	–	–
10	α-Farnesene	16.1	0.25	0.23	0.29	0.32	0.22	0.2	0.2	0.27	0.22	0.27	0.37	0.16
11	Unidentified	16.17	0.06	0.07	–	–	0.06	0.07	–	–	–	–	–	0.5
12	Cadinene	16.31	0.71	0.68	0.67	0.64	0.65	0.64	0.68	0.66	0.62	1.05	1.46	0.72
13	Eugenol acetate	16.6	20.91	20.55	20.49	20.89	20.78	20.32	22.34	21.5	21.75	13.84	3.89	19.37
14	Cubenol	16.91	–	–	–	–	–	–	–	–	–	0.52	–	–
15	unidentified	17.06	–	–	–	–	–	–	–	–	–	0.41	–	–
16	Caryophyllene oxide	17.41	0.8	0.88	0.93	0.69	0.84	0.93	0.89	0.6	1.05	0.49	0.32	0.49
17	Diethyl Phthalate	17.8	0.12	0.35	0.33	1.1	0.74	1.43	–	–	–	–	–	–
18	1,4-Methanoazulen-7(1H)-one, octahydro-4,8,	17.92	0.11	0.13	0.12	–	0.12	0.15	–	–	–	–	–	–
19	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	18.35	0.11	0.14	0.13	–	0.14	0.14	–	–	–	–	–	–
20	Tetracyclo[6.3.2.0(2,5).0(1,8)]tridecan-9-ol, 4,4-dimethyl-	18.42	0.21	0.27	0.27	0.3	0.26	0.25	0.37	0.36	0.56	–	–	0.54
21	Isoaromadendrene epoxide	19.07	0.14	0.25	0.15	0.12	0.15	0.25	–	–	0.28	–	–	–
22	2,2,4-Trimethyl-3-(3,8,12,16)-tetramethyl-heptadeca-3,7	30.6	–	–	–	–	0.24	0.73	–	–	–	–	–	–
23	Tetratetracontane	30.82	–	–	0.15	–	0.14	0.22	–	–	–	–	–	–

– = undetectable.
[a] Retention time on BTX.5ms fused-silica capillary column.
[b] Steam distillation.
[c] Hydrodistillation.
[d] Soxhlet extraction.

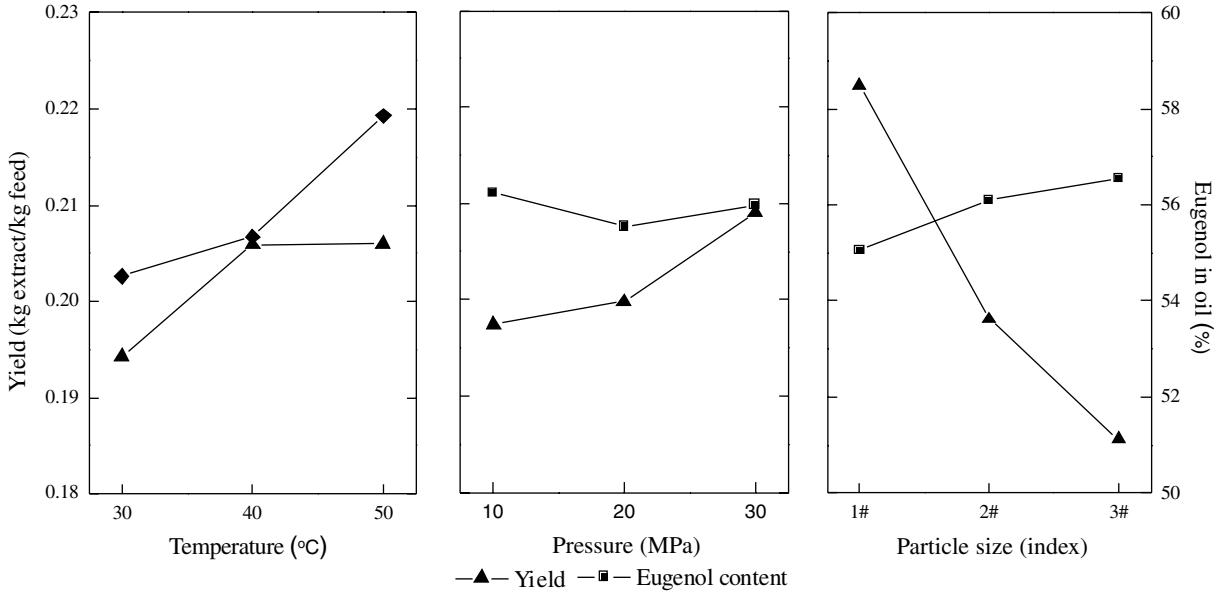

Fig. 1. Effect of temperature, pressure and particle size on yield and eugenol content of clove oil extracted by supercritical CO_2.

oil extracted by supercritical CO_2. It can be observed that the increase of temperature from 30 °C to 40 °C results in the increase of the extraction yield and high eugenol content in the oils, while the increase of temperature from 40 °C to 50 °C does not result in the increase of the oil yield, and there is increase of eugenol content in clove oil. This is ascribed to the decrease of the CO_2 density with increasing temperature, which dominates over the increase of the solute vapor pressure at this certain pressure (Lou, Folas, Voutasa, & Magoulas, 2003). As it was expected, the extraction yield enhanced significantly with increase of pressure, due to the increase of the solubility of the oil components. This is attributed to the increase of the CO_2 density, which results in the increase of its dissolving ability. However, as the high-molecular weight compounds in clove buds (fatty acids, fatty acids methyl esters, sterols, etc.) were also co-extracted with increase of the pressure, the eugenol content of the clove oil does not change obviously. Therefore, the highest eugenol content of the clove oil is extracted at 50 °C and 10 MPa.

The extraction yield increases by decreasing the particle size of the comminuted clove buds. It is due to the higher amount of oil released as the bud cells are destroyed by milling (Mostafa et al., 2004; Reverchon, 1997), and this amount of oil is easily extracted for direct exposure to the supercritical CO_2. Moreover, shorter diffusion paths in the milled solid matrix result in a smaller intraparticle resistance to diffusion. However, the eugenol content in the clove oil increases as the particle size increase. Therefore, the particle size should not be too small if the aim is to extract the volatile ingredients and to avoid more compounds with high-weight molecules to be co-extracted. It is better for the particle size index to be controlled at about 0.6430(2#).

3.2. Composition of clove oil obtained by different methods

Table 2 lists the composition of clove oil obtained by different methods according to the results of GC–MS. It can be seen that, twenty three compounds in the clove oils have been identified, in which eugenol, β-caryophyllene and eugenol acetate are the main components of clove oil. The composition of the clove oil extracted by different methods is mostly similar, whereas relative concentration of the identified compounds is apparently different. Clove oil obtained by steam distillation contained highest percentage of eugenol (58.2%), then did oil obtained by SFE (53.8–55.9%). Clove oil obtained by hydrodistillation had the lowest percentage of eugenol (48.82%). However, clove oil obtained by SFE contained the highest percentage of eugenol acetate (20.32–21.75%), which is also the main antioxidant ingredients in clove oil (Lee & Shibamoto, 2001). The highest percentage of active antioxidant ingredients, eugenol together with eugenol acetate, in the extracted clove oil by SFE was therefore obtained. The content of eugenol and eugenol acetate in the extracted clove oil by Soxhlet method was also higher than those by the two kinds of distillation methods. Thermal degradation of eugenol acetate may take place during hydro and steam distillation as eugenol acetate was only 13.84%, 3.89%, respectively.

Besides eugenol, and eugenol acetate in clove oil, relative content of β-caryophyllene in the clove oil extracted by steam distillation, hydrodistillation, SFE and Soxhlet method was as high as 20.59%, 36.94%, 13.99–17.77% and 17.5%, respectively. It was reported that β-caryophyllene showed anti-inflammatory activity in several experiments (Ghelardini, Galeotti, Di Cesare Mannelli, Mazzanti, & Bartolini, 2001). If the aim was to obtain high content of β-caryophyllene in the clove oil, it maybe necessary to choose hydrodistillation.

Table 3
Characteristic of the clove oils obtained by different methods

Clove oil	Yield (%)	Eugenol plus eugenol acetate (%)	Extraction period (h)	Color and texture	Organic solvent used
SFE (50 °C, 10 MPa)	19.6	58.8 + 19.6	2	Pale yellow oil	No
Steam distillation	10.1	61.2 + 10.2	8–10	Pale yellow oil	Yes
Hydrodistillation	11.5	50.3 + 3.2	4–6	Brown yellow oil	Yes
Soxhlet extraction	41.8	30.8 + 9.3	6	Brown ointment	Yes

It can also be seen that the oil obtained by SFE contains small amount of co-extracted cuticular waxes. This result is similar to other author's work (Mostafa et al., 2004; Myint et al., 1996).

3.3. Comprehensive comparison of the clove oils obtained by different methods

Color and texture are the prime characteristics and quality factors of essential oil, and extraction yield and extraction time are the important factor for the industrialization. Therefore, comprehensive comparisons of the clove oils obtained by different methods were further listed in Table 3. In Table 3, the content of eugenol and eugenol acetate was determined by GC.

It can be seen from Table 3 that, the content of the main biological ingredients of eugenol plus eugenol acetate in the clove oil by Soxhlet extraction is the lowest although its yield of the clove oils is the highest among the four extraction methods. Furthermore, the extracts by Soxhlet method is brown ointment, which means more undesired impurities and organic solvent residue may existed. SFE offers the most important advantages over other methods. Extraction yield of SFE was about two times as high as that obtained by steam and hydrodistillation. The highest content of eugenol plus eugenol acetate in the extracted oil was obtained. Pale yellow oil is desired and shortest extraction time is needed for SFE compared with other three extraction methods. Additionally, using supercritical CO_2 instead of some harmful organic solvents would result in ''greener'' processes.

4. Conclusions

In the investigation of the effect of the three tested parameters (pressure, temperature and particle size) on the SFE extraction of essential oils from clove bud within the experimental domain. Particle size had the most apparent effect on the yield of essential oil; temperature was the most important factor for determining the content of eugenol of the clove oil. The possibility of manipulating the euenol content of the clove bud oil by changing the parameters of the extraction is attainable in SFE.

The composition and some character of clove oil obtained by SFE and other methods were compared. Although compositions of the oils obtained by SFE and steam, hydrodistillation are similar, they do differ quantitatively. Extraction yield of SFE was about two times as high as that obtained by steam and hydrodistillation. SFE offers many important advantages over other three traditional methods, including higher extraction yield, the highest percentage of active antioxidant ingredients of eugenol together with eugenol acetate in the extracted clove, shorter extraction time, and so on. Therefore, SFE is considered as the optimum process for obtaining clove oil with high quality.

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