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Study on Salting out-Steam Distillation Extraction Technology and Antibacterial Activities of Essential Oil from Cumin Seeds

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Abstract - The effects of different factors on the yield of cumin essential oil extraction by salting out-steam distillation were analysed by single-factor test and orthogonal experiment. In addition, antibacterial activities of cumin essential oil on several common food spoilage bacteria were also assessed in this study. The results showed that, the impact order of the influence factors was liquid/solid ratio > distilling time > NaCl. The optimum extraction condition was liquid to material ratio 15:1, soaking time 1 h, 4% NaCl and steam distilling time 3 h. The yield of essential oil was up to 4.45% under this condition. The results of antibacterial activity assays showed that the essential oil from cumin seeds exhibited the different antibacterial activities against some food borne pathogens, especially it presented the best inhibitory effect against *Bacillus subtilis* ─ with the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of 6.25 and 12.5 mg/mL, respectively ─ followed by *Staphylococcus albus* and *Staphylococcus aureus*. The lowest antibacterial activity was for *Pseudomonas aeruginosa* and *Shigella dysenteriae*.
Keywords: Cumin; Essential oil; Extraction; Antibacterial activity

Introduction

Cumin (*Cuminum cyminum* L.) is cultivated mainly in Egypt, Ethiopia, India, Iran, Russia, the Mediterranean Sea, North America and China (Thippeswamy and Naidu, 2005). Cumin is annual or biennial herbs, its seeds possess an aromatic odour and have a spicy and bitter taste. It is used as an essential ingredient in mixed soups, curry powder sausages, and a lot of delicious flavors (Hu *et al*., 2005). In traditional Chinese medicine, cumin has long been considered as a digestion promoting agent appetizer and is used for therapeutic purposes. It is also used in stimulant, carminative medicines for its sweet cumin flavor and non-toxic. Cumin oil contains aldehyde, alcohols, alkenes, hydrocarbon and other volatile substances, which are the major source of cumin flavor (Hu *et al*., 2005; Rebey *et al*., 2012; Kiralana *et al*., 2014; Chen *et al*., 2014), and it possessed antioxidant (Allahghadri *et al*., 2010; Rebey *et al*., 2012), antibacterial (Nostro *et al*., 2005; Allahghadri *et al*., 2010; Kedia *et al*., 2014), insecticidal activities (Tunc *et al*., 2000) and anti-cancer activities (Nalini *et al*., 1998). It is extensively used in the food, pharmaceutical and healthcare industries (Rebey *et al*., 2012;;Chen *et al*., 2014; Kiralana *et al*., 2014).

According to some reports in the literature, there are several extraction methods of essential oil from cumin seeds including the traditional steam distillation (Korbanjhon, 2011), solvent extraction (Kiralana *et al*., 2014), microwave assisted extraction (Yang *et al*., 2009), and enzyme assisted extraction (Sowbhagya *et al*., 2011), ultrasonic assisted extraction (Zhang *et al*., 2011) and supercritical fluid extraction technology (Hu *et al*., 2010). However, these methods have own shortcomings due to differences in extraction conditions and mechanism of different methods. For instance, extraction time is longer, which easy to cause the decomposition heat sensitive compounds in essential oil for the traditional steam distillation; supercritical extraction need high pressure, resulting in large equipment investment and high energy consumption; other extraction methods require a large amount of solvent and easy to cause the residual organic solvents. In this study, extraction method of volatile oil from cumin seeds by salting out-steam

distillation was studied based on orthogonal design. In addition, the antibacterial activities of cumin essential oil on common food spoilage bacteria were investigated. The results of this study are expected to provide technical support for industrial production and comprehensive development and utilization.

Materials and Methods

Extraction of essential oil and determination of the yield

Dried cumin seeds were grounded and passed through a 40 mesh screen. The powder (5.0 g) was extracted in a Clevenger-type apparatus with NaCl solutions. The single factor experiment was performed in a designed ratio of NaCl solutions to material (range from 5 to 25 mL/g), NaCl concentration (range from 0 to 9 %), soaking time (range from 1 to 5 h) and distillation time (range from 1 to 6 h). The oily layer obtained on top of the aqueous distillate was separated and dried with anhydrous sodium sulfate. The yield of essential oil was calculated using the following equation: Yield (%) =100×We/Wm, where We is the weight (g) of essential oil, and Wm is the weight (g) of material.

Optimization of ASSP ultrasound extraction conditions

On the basis of the single factor experiment, an orthogonal experiment (L9(3)4) was used to evaluate the combination effects of the three parameters on the extraction yield of essential oil. The independent variables, including liquid/material ratio (A), distilling time (B) and NaCl concentration (C) at three levels in the extraction process were shown in Table 1.

Microbial strains and culture

The antimicrobial activity of the essential oil was tested against eight different microorganisms including *Staphylococcus aureus*, *Staphylococcus albus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Shigella dysenteriae*, *Salmonella* Typhimurium and *Listeria monocytogenes*. The strains were provided by the College of Life Science, Shanxi Normal University, and cultured at 37 °C on nutrient agar (NA) and nutrient broth (NB) mediums.

The antibacterial activity test

According to the method described by Diao *et al.* (2014), the essential oil was dissolved in DMSO and sterilized by filtration through 0.22 μm Millipore filters. Antimicrobial tests were then carried out by the Oxford cup method using 100 μL of suspension containing 1×10^7 colony forming units (CFU)/mL of bacteria spread on nutrient agar (NA) medium. Oxford cups (6 mm in diameter) were placed on the inoculated agar, and then 100 μL of essential oil was added with a micropipette. The diameter of inhibition zone (DIZ) was measured after 24h of incubation at 37 °C, and DMSO was used as a negative control. Tests were performed in triplicate.

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) determination

MIC and MBC were determined according to the method described by Diao *et al.* (2014) with minor modifications. Briefly, stock solution of essential oil was prepared in DMSO. Two fold serial dilutions of essential oil were filtered through 0.22 μm Millipore filters and prepared in sterile NB medium. To each tube, 100 μL of the inoculum containing approximately 1×10^7 CFU/mL microorganisms was added. A control test containing inoculated broth supplemented with only DMSO was also performed. The tubes were then incubated at 37 °C for 24 h and examined for evidence of the growth. The MIC was determined as the lowest concentration of the essential oil that demonstrated no visible growth. The MBC was the lowest concentration of the test essential oil that showed no visible growth in the culture incubating at 37 °C for 48 h. All experiments were performed in triplicate.

Results and Discussion

The effects of liquid/material ratio on the yield of essential oil

As shown in Figure 1A, with the increase of the liquid/material ratio, the yield of essential oil increased significantly. The yield reached the maximum when the liquid/material ratio was 15:1. Thereafter the yield of essential oil decreased with the increase of the liquid/material ratio. The reason may be that larger ratio of liquid could help oil diffusion and dissolution (Lin and Su, 2012). However, when the liquid/material ratio is too high, the yield of essential oil reduced at the same time because of the longer heating time. The higher liquid/material ratio may lead to the increase in solubility of essential oil in solutions, which also resulted in the decreases in extraction rate (Wu *et al.*, 2012). Therefore, the best choice of liquid/material ratio was 15:1.

The effects of NaCl concentration and soaking time on the yield of essential oil

The yield of essential oil under different concentrations of NaCl is showed in Figure 1B. When the NaCl concentrations were in the 1% ~ 5%, the yield of essential oil increased with the increase of NaCl concentrations, which can be caused by the decrease solubility of oil in water because of the existence of NaCl. Then with further increasing the mass fraction of NaCl, the essential oil yield began to decrease, the reason may be that the essential oil in the external osmotic pressure is not easy to get steamed out. Therefore, the choice of the concentration of NaCl was 5%. The yield of essential oil under different soaking times is showed in Figure 1C. The yield of essential oil had no significant change within the test ranges, which indicates that soaking time had little influence on the yield of the essential oil. However the longer soaking time may result in the decrease in yield of oils. The reason may be that longer time immersion lead to dissolution or emulsification of protein, pectin components, thereby inhibiting the volatile composition of distillate oil, and eventually leading to a decline in oil yield . Considering factors including the production cycle and economic benefits, the choice of the soaking time was 1 h.

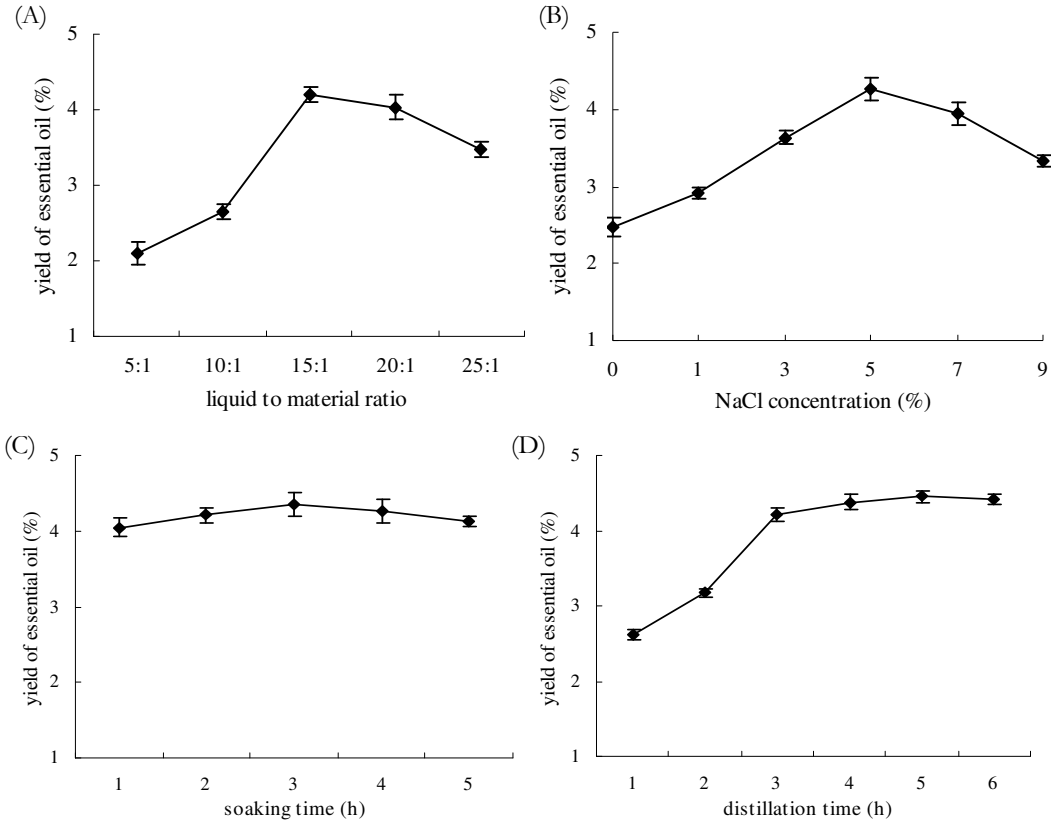

Figure 1. (A) Effects of liquid to material ratio on the yield of essential oil, (B) Effects of NaCl concentration on the yield of essential oil, (C) Effects of soaking time on the yield of essential oil, (D) effects of steam distilling time on the yield of essential oil

The effect of distillation time on the yield of essential oil and orthogonal test

The yield of essential oil under different distillation time is showed in Figure 1D. The yield of essential oil gradually increased with extended distillation time, but the yield of essential oil had no obvious change after 3 h of distillation. The longer distillation time could increase costs, at same time cause the degradation and impurities of oil. Therefore, appropriate distillation time was 3~4h. According to the test results of single factors, the best conditions were investigated through an orthogonal experiment (L_9 (3^4)). As shown in Table 1, the parameters used in the present study on the extraction yields of essential oil could be classified in the following decreasing order: liquid/material ratio > distilling time > NaCl concentration.

Table 1. Results of orthogonal experiment extracting essential oil from cumin seeds

No.	A Liquid/material ratio	B Distilling time/h	C NaCl concentration/%	D Null column	Yield /%
1	1(10:1)	1(2h)	1(4%)	1	3.96
2	1	2(3h)	2(5%)	2	4.20
3	1	3(4h)	3(6%)	3	4.05
4	2(15:1)	1	2	3	4.31
5	2	2	3	1	4.45
6	2	3	1	2	4.34
7	3(20:1)	1	3	2	3.94
8	3	2	1	3	4.26
9	3	3	2	1	4.11
k_1	4.0700	4.0700	4.1867	4.1733	
k_2	4.3667	4.3033	4.2067	4.1600	T=37.62
k_3	4.1033	4.1667	4.1467	4.2067	
R	0.2967	0.2333	0.0600	0.0467	

Variance analysis of orthogonal experiment

The results of variance analysis showed that the factor A and B had significant difference ($p<0.05$), indicating that there were significant influences of the ratio of liquid to material and distillation time on the yield of the essential oil. No significant differences was found for factor C, indicating that the concentration of NaCl in the three level of orthogonal design had no significant effect on the yield of essential oil; therefore, the levels can be selected according to the actual situation (Table 2). Based on this analysis, extraction efficiency, the cost of energy and the feasibility of experiment, the optimal conditions of salting out-steam distillation extraction were determined the combination of $A_2B_2C_1$: liquid/material ratio 15:1, soaking time 1 h, 4% NaCl, steam distilling time 3 h. Under this condition, the yield of essential oil from cumin seeds was 4.45%, which was much higher than that of essential oil extracted by water steam distillation under the same extraction conditions (2.85%).

Table 2. Variance analysis of orthogonal experiment extracting essential oil from cumin seeds

Sources of variation	Quadratic sum	Degree of freedom	Mean square	F value	p value
A	0.1585	2	0.0792	45.71	0.0214
B	0.0825	2	0.0412	23.79	0.0403
C	0.0056	2	0.0028	1.62	0.3824
D	0.0035	2	0.0017		
Error	0.0035	2	0.0017		
Total	0.2500				

Antibacterial activities of essential oil on several food spoilage bacteria

The antibacterial activities **of** cumin essential oil on several common food spoilage bacteria are shown in Table 3. Essential oil had varing degrees of antibacterial activity on eight food spoilage bacteria. With respect to inhibition zone, its bacteriostatic effect on food spoilage bacteria from high to low were as follows: *B. subtilis* >*S. albus* > *S. aureus* > *E. coli* > *S.* Typhimurium > *L. monocytogenes* > *S. dysenteriae* > *P. aeruginosa*. The essential oil from cumin seeds had the lowest MIC and MBC values on *B. subtilis*, indicating it was the most sensitive to cumin essential oil, followed by *S. albus*, *S. aureus*, while *P. aeruginosa* and *S. dysenteriae* had the highest tolerance to cumin essential oil.

Table 3. The diameter of inhibition zone (DIZ), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the essential oil from cumin seeds

Bacteria	DIZ/mm	MIC/mg·mL^{-1}	MBC/mg·mL^{-1}
S. aureus	12.5	12.5	25
S. albus	13.6	12.5	12.5
B. subtilis	15.2	6.25	12.5
E. coli	11.0	25	50
L. monocytogenes	9.4	50	100
S. Typhimurium	10.1	50	100
P. aeruginosa	8.4	100	Not detected
S. dysenteriae	8.9	100	Not detected

Conclusions

Based on the single factor experiment and orthogonal design, the important factors on the yield of essential oil by salting out-steam distillation extraction were liquid/material ratio>steam distilling time>NaCl concentration. The optimum conditions of salting out-steam distillation extraction was liquid/material ratio 15:1, soaking time 1 h, 4% NaCl and steam distilling time 3 h. The yield of essential oil was up to 4.45% under this condition. Essential oil from cumin seeds had vary degrees of antibacterial activities on different food spoilage bacteria. *B. subtilis* was the most sensitive to cumin essential oil followed by *S. albus* and *S. aureus*, while *P. aeruginosa* and *S. dysenteriae* had the highest tolerance to cumin essential oil.

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