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Research Article

Influence of drying technology on chemical composition and antimicrobial activity of essential oil from star anise (*Illicium verum* Hook. f.) fruit

Fang Zhao[1], Yu Liu[2] and Mei-Ping Quan[3]

[1]Laboratory & Equipment Management Department, Shanxi Normal University, Linfen, China
[2]School of Food Science, Shanxi Normal University, Linfen, China
[3]College of Chemistry and Life Science,Weinan Normal University, Weinan, China

*Corresponding Author
Yu Liu

Abstract: Four kinds of drying treatment process (air-dried, de-enzyming, sulfuring, oven-dried) were applied in this paper to investigate the influences of drying technology on the chemical composition and antimicrobial activity of star anise essential oil. There were some differences on chemical composition and antimicrobial activity of essential oil derived from four drying processes. A total of 36 compounds were identified by Gas Chromatography-Mass Spectrometer (GC-MS) from EOA and trans-anethole (83.09%), estragole (5.28%), limonene (2.18%) and linalool (1.67%) were found to be the majority of components. By contrast, oven-drying could significantly reduce the content and composition of star anise essential oil while the other two drying processes had a slight change on the profile of star anise essential oil. As for the antimicrobial activity, the essential oil from air-dried and oven-dried samples showed stronger antimicrobial activity while E. coli was the most resistant strain according to the diameter of inhibition zone (DIZ), minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) detection. Results revealed that the star anise essential oil had potential for exploitation and application as antibacterial agent in food industries.

Keywords: Star anise, essential oil, chemical compositon, antimicrobial activity, drying.

1. INTRODUCTION

Enterprises, researchers and regulatory agencies in food field are continuously concerned about the high and growing number of disease occurrence caused by some pathogenic and spoilage microorganisms in foods. It is well known that chemical preservatives often used in food processing to control the occurrence of this phenomenon (Diao *et al*., 2013). However, many studies about food safety revealed there were side effects of synthetic antimicrobials including hypersensitivity, toxicity, carcinogenicity and immunity suppression. Nowadays, consumers are increasingly concerned about the safety of foods containing synthetic antimicrobials and they hope the food additives such as antimicrobials and antioxidants are from nature (Wu *et al*., 2007; Ma *et al*., 2014). Essential oils of natural plants have shown strong antimicrobial activity and exhibited important prospect in exploitation of natural bactericides. Therefore, plant essential oils have wide range of food applications (Shan *et al*., 2007).

Chinese star anise (*Illicium verum* Hook. f.) is an aromatic evergreen tree of the family Illiciaceae, which is indigenous to the south eastern part of the China and Vietnam. Its fruit has strong fragrance and it is an important traditional Chinese medicine as well as a commonly used spice. Traditionally, the oil of star anise is used topically for rheumatism and otalgia, and it has been reported to possess antibacterial, anti-inflammatory, and anticancer properties (Chang *et al*., 2001; Itoigawa *et al*., 2004). There are many reports in the literature on the chemistry (Cu *et al*., 1990; Cook et al., 1996), antimicrobial (Gurdip *et al*., 2006) and insecticidal (Chang *et al*., 2001) behavior of star anise essential oil. Currently, star anise essential oil has been widely used in many food categories, and as a fragrance component in flavouring alcoholic drinks, candies,

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liquers, confectionery and stewed meat (Sun *et al.*, 2014; Gurdip *et al.*, 2006). A survey of the literature available showed that star anise essential oil was mainly composed of trans-anethole, estragole, and limonene (Porta *et al.*, 1998) and anethole, the main component of star anise oil, has been reported to possess antifungal activity (Huang *et al.*, 2010). In fact, star anise oil extracted from the fruits of *Illicium verum* is more popular than the fruit itself (Wang *et al.*, 2011). However, the researches on the essential oil are mostly restricted to different extraction method, extraction solvent as well as vegetable origin. Little information is available about the influence by different drying process in former literatures. The most noteworthy is the process of drying technology is indispensable in the course of extracting and analyzing plant essential oils. In addition, the constituent and yield of essential oil vary depending on the origin and preparation of raw material. It would have important guiding significance to study the changes of chemical composition and antimicrobial effect of star anise essential oil by different drying processes. Therefore, the aim of the present study was to investigate the changes of chemical composition of star anise essential oil under the different drying technologies, mainly referring to air-dried, de-enzyming, sulfuring, oven-dried method. Furthermore, its antibacterial activity on food spoilage microbes was also discussed.

2. MATERIALS AND METHODS

2.1 Plant material and chemicals

The fresh plant materials were collected in autumn and from Fangchenggang city, Guangxi, China. After harvesting, the fresh fruits were sent to the laboratory under shady and cool conditions. The materials stored in clean packaging bags and temperature was about 4°C before processing. The main laboratory chemicals such as dimethyl sulfoxide (DMSO), nutrient agar (NA), potato dextrose agar (PDA) and nutrient broth (NB) mediums were respectively purchased from Sigma (USA) and Beijing-Aoboxing Corporation (China). Other chemicals used were all of analytical grade.

2.2 Microbial Strains and Culture

The antimicrobial activity of the star anise essential oil with different treatments was tested against 9 selected food-related microorganisms. The three Gram-positive (G+) strains were *Staphylococcus aureus* ATCC 25923, *Bacillus subtilis* ATCC 6051 and *Bacillus cereus* A018. The three Gram-negative (G-) strains were *Salmonella typhimurium* ATCC 19430, *Shigella dysenteriae* CMCC (B) 51252 and *Escherichia coli* ATCC 25922. *Aspergillus niger* ATCC 02512, *Penicillium citrinum* ATCC 1109 and beer yeast ATCC 9763 were the three eukaryocytes studied. All tested strains were provided by the School of Food Science, Shanxi Normal University.

2.3 Different Treatments

The raw material star anise was selected to insure no impurities and good quality. The materials were randomly separated into 4 batches and then subjected the following processing to get a water content (≤ 15% dry base). (1) Air-dried treatment: spread the fresh materials on the clean mat in the sun. The materials were placed under the sun for drying and final products were gathered 5 days later. (2) De-enzyming treatment: put the fresh materials into hot-water at the temperature 90-100 °C. Maintain the heat and cook, stirring for about 5 min until the material turned light yellow from green and remove the materials from the hot water. Then the de-enzymed materials were subjected to air-dried treatment as above. (3) Sulfuring treatment: spread the fresh materials on home-made shelf and place a container with 0.2% burning sulphur (the ratio of sulpfur to materials) under it. The process of smoking with sulphur continued for 60 h until sulphur was used up. (4) Oven-dried treatment: put the fresh materials into baking oven at the temperature 90-100 °C and keep 15 min. Adjust the temperature of baking oven to 55-60 °C and keep 10 h. All the samples after different treatments were stored at 4 °C and the storage time was less than 10 days before next step.

2.4 Extraction of the Essential Oil

The dried materials were ground with a micro plant grinding machine to a powder. The 60 g powder with different treatments was soaked for 1 h and then hydrodistilled for 4 h at room temperature with a Clevenger-type apparatus. The essential oil obtained on glass tube of essential oil receiver was collected, dried with anhydrous sodium sulfate and stored in a tightly closed dark vial at 4°C until chemical composition analysis.

2.5 Determination of Relevant Indicators

Moisture content in dry product was measured by oven drying at 105 °C. The ratio of dry to fresh fruit weights and yield of the essential oil are determined by Chinese standard. The qualities such as colour & odourof the dry product were compared with sensory analysis by trained panelists. All above indicators were repeated thrice.

2.6 GC and GC-MS Analysis

The essential oil from star anise was analyzed using gas chromatography with flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS). The GC 6890N/MS5973N apparatus was equipped with a FID detector. The fused silica capillary column (30 m×0.25 mm; film thickness 0.25 μm) was silica capillary Agilent Technology HP-5ms (5% phenyl methyl siloxane) and temperature for injector and detector were maintained at 230°C. Oven temperature was programed as: 80-250 °C (5°C /min) and isotherm at 250°C for 14 min. The carrier gas was nitrogen at a flow rate of 1.0 mL/min. Sample was

dissolved in ethyl acetate at 2 mg/mL; injection volume and split ratio was separately 2.0 µL and 1:50.

GC-MS was conducted with the equipment from SHIMADZU (Japan). A separated column (30 m×0.25 mm; film thickness 0.25 µm) was silica capillary SHIMADZU HP-5 MS (5% phenyl methyl siloxane). Injector and ion source temperature were set at 230 and 250 °C, respectively. The 2.0 µL diluted oil sample was injected into the column using a 1:10 split ratio. Carrier gas was Helium at 1.3 mL/min flow rate. The energy operated in electronimpact ionization (EI) for mass spectrometer was 70 eV and scan range was 40-350 amu and the scan rate was 0.5 s per scan. The linear retention indices for all the compounds were determined by co-injection of the sample with a solution containing the homologous series of C_8-C_{20} n-alkanes. The essential oil constituents were verdicted by comparing their retention indices, mass spectra with published data and National Institute of Standards and Technology mass spectra library data provided by the software of GC-MS system.

2.7 Antimicrobial Activity

The essential oil was dissolved in DMSO and sterilized by filtration through 0.22 µm Millipore filters. The antimicrobial activity of the essential oil was evaluated using the standardized filter paper disk diffusion method as described previously (Abdullah *et al*., 2011). The 100 µL organism suspension including bacteria and fungus were spread on nutrient agar (NA) and potato dextrose agar (PDA), respectively. Proper concentration of suspension was determined by turbidimetry and 1×10^7 colony-forming units (CFU)/mL for bacteria and 1×10^6 spore/mL for fungus were employed in the study. Sterilized filter paper (6 mm in diameter) was placed on the inoculated agar, and then 100 µL of essential oil 8 mg/mL was added with a micropipette. The diameter of inhibition zones (DIZ) was measured after 24 h of incubation at 37°C for bacteria and after 48 h of incubation at 28°C for fungus. At the same time, positive and negative control was respectively performed with Gentamicin (4 mg/mL) and DMSO solvent instead of essential oil in the same way.

2.8 Determination of MIC and MBC

The MIC values and the MBC values were determined for the microorganisms that were sensitive to the essential oil in the filter paper disk diffusion assay. MIC and MBC were determined according to the method described by previous reports (Gao *et al*., 2011; Joshi *et al*., 2010) with minor modifications. Briefly, Proper concentration of essential oil was prepared in DMSO as stock solutions. Two-fold serial dilutions of essential oil were prepared in sterile NB medium ranging from 0.0625 to 8 mg/mL. To each tube, 50 µL of the suspension containing approximately 1×10^7 CFU/mL or 1×10^6 spore/mL microorganisms was added. Positive control was also performed containing inoculated broth supplemented with Gentamicin (4 mg/mL) under identical conditions. The tubes were then incubated at 37°C for 24 h or 28°C for 24 h and examined for evidence of the growth of microorganisms. The MIC was defined to the minimum concentration of the essential oil that showed no visible growth during the whole incubation period. The MBC values were determined according to the method as reported previously (Zhu *et al*., 2005; Shan *et al*., 2008). After MIC assay, 50 µL samples were taken out from the tubes of the MIC assays where there was no visible growth observed. And then the 50 µL samples were supplemented with freshly prepared nutrient agar plates and were incubated additional culture of 24 h at 37°C. The MBC value was the least concentration of the essential oil that showed no visible growth on the agar plates. Triplicate samples were performed for each test concentration.

2.9 Statistical Analysis

All the experiments were performed in triplicates and the experimental data were expressed as mean ± standard deviation (SD). Data obtained were analysed using one-way analysis of variance (ANOVA) and Duncan's multiple range tests. P-values of <0.05 were considered to be statistically significant (SPSS, version 16.0).

3. RESULTS AND DISCUSSION

3.1 Influence of Drying Treatments on the Quality Of Star Anise

Viewed from appearance properties, smell and color of 4 kinds of essential oil with different treatments were all light yellow transparent liquid and all of them had characteristic odour and sharp taste and 4 kinds of essential oil were similar. Results of the differences were presented in Table 1. As far as sensory state of star anise dry fruit was concerned, Table 1 showed there was a certain difference on air-dried and de-enzyming treaments. The final product with sulfuring and oven-dried treatment respectively showed pale brown red and brown appearance. There was a difference on smell with different treatments and sulfuring treatment gave the dry fruit a slightly SO_2 odour. In terms of physical parameter, the content of moisture content directly affected the quality and conservation of star anise dry fruit. Moisture of dry fruit in descending order was as follows: air-dried > de-enzyming > sulfuring > oven-dried. There was a good agreement between the 2 parameters and dry weight/fresh weight in descending order was also as follows: air-dried > de-enzyming > sulfuring > oven-dried. Yield of essential oil with different treatments was as follows in descending order: oven-dried > sulfuring > air-dried > de-enzyming.

Air dried and de-enzyming methods are frequently-used in star anise production. As can be seen from the results, the final product with the two methods had good aroma and color but higher moisture. Critical value of moisture content in dry star anise is limited to 13.50% according to national standard, our result

showed moisture content with air-dried treatment exceeded the limit and this easily led to possible damage and deterioration because of mold growth during the storage of dry star anise, further processing was needed to lower water content after air-dried treatment. Sulphur fuming technology is often adopted to deactivation of enzymes, desiccation, fungi-proofing and insect prevention in the processing process of Chinese medical herbs. Furthermore sulfur dioxide produced by sulfur burning had bleaching efficiency and our products via the treatment proved it. The lowest water content and highest yield of essential oil was obtained by oven-dried treatment perhaps due to shortest drying time. Maybe oven-dried treatment is conducive to the preservation and processing of the materials.

Table.1 Influence of drying treatments on relevant indicators of star anise and yield of essential oil

Treatments	Color	Odour	Moisture (%)	Dry/fresh weight	Yield (%)
Air-dried	Brown red	Fragrant	14.60±0.61 a	25.79±0.50 a	10.34±0.16 b
De-enzyming	Brown red	Fragrant	12.43±0.19 b	23.27±0.40 b	9.30±0.46 c
Sulfuring	Pale brown red	with a slight SO_2 odour	12.25±0.09 b	22.82±0.28 bc	11.08±0.22 a
Oven-dried	Brown	Fragrant	11.74±0.56 b	22.42±0.63 c	11.33±0.06 a

Numbers represent mean values of three independent replicates ± SD. Mean values within a column with different lowercase letters for drying technologies are significantly different at $p < 0.05$.

3.2 Influence of Drying Treatments on the Composition of Essential Oil

The chemical composition of star anise essential oil with different drying processes was analyzed by GC-MS, and the results were presented in Table 2. The 36 components were identified in the essential oil with air-dried treatment. Padmashree et al. (2007) reported that 25 components, representing 94% of the total content, were identified from star-anise essential oil (the material from India) obtained by hydrodistillation. Gurdip et al. (2006) reported that 25 components were also identified from the essential oil of star-anise (the material from India) obtained by hydrodistillation but they represented 99.9% of the total content. Table 2 showed that trans-anethole (83.09%), estragole (5.28%), limonene (2.18%), and linalool (1.67%) were found to be the major compounds in the essential oil of air-dried technology (EOA), with minor amounts of 1,8-cineole (0.53%), p-anisaldehyde (0.47%) and the rest were found in less quantity. Compared with the profile of star-anise essential oil, the result of our study was very similar to the previous results (Padmashree *et al*., 2007). Main components of the essential oil were trans-anethole, estragole and limonene in spite of different proportion of each component. There was also a little difference between our result and the previous result (Gurdip, et al., 2006). Methyl chavicol (1.82%) in their report was absent from our study. Such disparities could be mainly caused by the variety and regional difference of the plant materials. In addition, operating conditions before extraction of essential oil can make a big difference (Ma *et al*., 2014).

Table.2 Chemical composition (%) of essential oil from star anise with different treatments

RI[a]	Compounds	Air-dried	De-enzyming	Sulfuring	Oven-dried
802	Hexanal	Trace	Trace	-	-
920	α-Thujene	0.03	0.02	-	-
928	α-Pinene	0.25	0.26	0.21	0.13
973	β-Pinene	0.07	0.04	0.03	0.06
992	β-Myrcene	0.04	0.08	0.03	0.02
1002	α-Phellandrene	0.07	0.10	0.05	0.11
1011	3-Carene	0.12	0.11	0.04	0.09
1019	α-Terpinene	0.02	0.01	-	-
1025	p-Cymene	0.03	0.05	-	0.02
1032	Limonene	2.18	1.95	1.78	1.27
1035	1,8-Cineole	0.53	0.42	0.36	0.38
1061	γ-Terpinene	0.15	0.06	0.05	-
1090	Terpinolene	0.03	0.02	-	-
1099	Linalool	1.67	1.4	0.93	1.28
1170	4-Terpineol	0.10	0.14	0.08	0.09
1183	α-Terpineol	0.10	0.12	0.07	0.05
1208	Estragole	5.28	5.36	4.56	4.86
1255	p-Anisaldehyde	0.47	0.49	0.38	0.35
1260	cis-Anethole	0.21	0.16	0.15	0.36
1290	Trans-anethole	83.09	82.30	78.29	85.58
1383	α-Copaene	0.22	0.25	0.16	0.21
1396	β-Elemene	0.04	0.04	-	0.03
1432	Caryophyllene	0.06	0.05	0.02	0.08
1443	Trans-α-bergamotene	0.07	0.09	-	0.08
1455	α-Humulene	0.01	-	-	0.03
1496	β-cis-Farnesene	0.06	0.05	0.03	0.03
1499	Methyl isoeugenol	0.15	0.10	0.06	0.16
1511	β-Bisabolene	0.19	0.18	0.15	0.19
1528	α-Cadinene	0.05	0.05	-	0.03
1529	δ-Cadinene	0.08	0.10	-	0.05
1551	Elemol	0.03	0.03	-	0.02
1566	(E)-Nerolidol	0.1	0.2	-	0.1
1572	Caryophyllene oxide	0.08	0.05	-	-
1641	α-Cadinol	0.03	0.03	-	-
1672	Foeniculin	1.32	1.03	2.21	0.86
1958	Hexadecanoic acid	0.03	0.04	0.02	0.02
	Total identified (%)	96.96	95.38	89.66	96.54

[a] Linear retention index on a HP-5 MS column.

The 35 components were identified in the essential oil of de-enzyming technology (EOD). Table 2 showed de-enzyming technology did not cause significantly change in terms of the essential oil components without regard to the content and proportion of each component. While 22 and 29 components were identified in the essential oil of sulfuring technology (EOS) and the essential oil of oven-dried technology (EOO) respectively, which showed significant difference compared with EOA and EOD. In general, sulfuring and oven-dried could decrease components and the proportion of most components of essential oil to a much greater extent. Sulfuring and oven-dried maybe promoted decomposition or transformation of specific components, which led to the deficiency, decrease or increase of the proportion of part of essential oil in EOS, EOO, such as β-elemene, estragole and trans-anethole. Previous study (Wang *et al*., 2007) showed that terpene compounds such as limonene, phellandrene

and pinene assumed a definite proportion and this conclusion was well-supported by our results. It must be said that some terpene compounds suffered different degrees of lost under the condition of oven-dried especially sulfuring treatment. One reason for that is most likely that longtime treatment at high temperature for oven-dried and sulfuring, which suggested that the temperature was one of the main factors influencing the aroma components of star anise during pre-treatment of plant materials, and the effects of sulfuring treatment with high temperature, could be more severe. Ma et al. (2014) indicated that series of reactions and changes, the loss of flavour components, maillard and caramelization reaction could be caused by heat treatment (Anese et al., 1999; Julie 2002).

3.3 Influence of Drying Treatments on Antimicrobial Activity of Essential Oil

The antimicrobial activity of 4 kinds of essential oil with different treatments against the microorganisms was qualitatively and quantitatively assessed by means of DIZ, MIC values and MBC values. Control group (DMSO) did not form inhibition zones, which indicated that the solvent show no inhibitory effect on any of the tested microorganisms while gentamicin (4 mg/mL), positive control, showed strong antibacterial effect for bacteria. Table 3 indicated that 4 kinds of oil exhibited antimicrobial activity against almost all microorganisms except *E. coli* at the concentration of 8 mg/mL, which suggested the essential oil from star anise is a potential antibacterial agent.

EOA and EOO exhibited stronger ability of inhibitory effect for all microorganisms in comparison with EOD and EOS. The EOO exhibited the most effective inhibitory effect to bacteria and fungus, with the DIZ of 15.62 for *S. aureus* and 22.65 for *A. niger* respectively, followed by the EOA, EOD and EOS. Fungus was the most sensitive strain to 4 kinds of oil with the maximum value of DIZ (>20.69mm), whereas *E. coli* were the most insensitive strain. On the whole, star anise essential oil with different treatments exhibited stronger inhibitory effect against eukaryote than bacteria. Aly et al. (2016) reported that star anise essential oil reduced by 83.2%, 72.8% and 65.11% at the concentration of 100 ppm for *A. flavus*, *A. parasiticus* and *F. verticillioides* respectively while it can completely inhibited growth at 200 ppm for *A. flavus* and *A. parasiticus*. Another study (Gurdip et al., 2006) indicated that star anise essential oil was found to be effective for controlling growth of fungus, *F. moniliforme*, *F. moniliforme*, *A. flavus*, *A. niger*, with two methods of inverted petriplate technique and food poison technique. In this study, 4 kinds of essential oil exhibited stronger inhibitory activity for the tested gram-positive bacteria than gram-negative bacteria except for *S. dysenteriae*. Some previous studies indicated that Gram-positive bacteria appear to be more sensitive than Gram-negative to essential oil. But relevant literatures have also revealed (Dorman *et al.*, 2000) showed sensitivity of the bacteria depended on the type of different plant essential oil and had little relationship with the type of gram reaction. Therefore, further investigations will be required to explore the mechanism of antimicrobial activity of star anise essential oil.

Table.3 DIZ (mm) of the essential oil from star anise fruit with different treatments

	Air-dried	De-enzyming	Sulfuring	Oven-dried	Gentamicin
Gram-positive bacteria					
Staphylococcus aureus	15.33±0.3 b	13.53±0.7 c	11.55±0.6 d	15.62±0.6 b	29.15±0.91 a
Bacillus subtilis	11.83±0.5 b	11.25±0.6 bc	10.31±0.5 c	11.60±0.8 b	18.23±0.31 a
Bacillus cereus	12.53±1.6 b	13.06±1.0 b	11.28±0.4 b	12.58±0.5 b	25.52±0.62 a
Gram-negative bacteria					
Salmonella typhimurium	10.86±1.0 b	9.66±0.5 b	9.53±0.3 b	9.95±1.1 b	23.58±1.1 a
Shigella dysenteriae	15.39±0.8 b	15.28±1.0 b	13.68±0.3 b	14.33±0.5 b	28.31±1.5 a
Escherichia coli	7.60±0.5 b	no activity	no activity	7.90±0.6 b	25.02±0.9 a
Fungus					
Aspergillus niger	21.18±1.0 a	20.69±1.3 a	21.03±1.1 a	22.65±0.9 a	8.65±0.62 b
Penicillium citrinum	16.20±0.6 a	15.73±0.8 a	15.38±0.6 a	15.93±0.6 a	8.16±0.52 b
yeast	16.35±0.6 a	16.18±2.3 a	15.66±1.8 a	17.18±0.5 a	8.02±0.39 b

Values represent means of three independent replicates ± SD.

MIC and MBC of 4 kinds of essential oil were showed in Tables 4 and 5, the oil could inhibit or almost completely kill the tested microorganism within a certain range of concentrations except for *E. coli*, which also exhibited the most resistant under DIZ detection. The MIC and MBC values of essential oil were in the range of 0.0625-1.0 mg/mL and 0.0625-2.0 mg/mL. In conclusion, star anise essential oil with different treatments performed antimicrobial activity and had characteristics of both a maximum DIZ and minimum MIC & MBC value against the specific strain. Results of DIZ, MIC and MBC suggested that pretreatment of plant materials, namely drying technologies of the study, do have a significant impact

on antibacterial activity. Performance of antimicrobial activity of star anise essential oil can vary with tested bacterial strains, even to the point of the pretreatment methods of plant material, extraction conditions and process (Ma *et al*., 2014).

Table.4 Values of MIC (mg/mL) for essential oil from star anise fruit with different treatments

	Air-dried	De-enzyming	Sulfuring	Oven-dried	Gentamicin
Gram-positive bacteria					
Staphylococcus aureus	0.125	0.25	0.25	0.125	≤0.0625
Bacillus subtilis	0.25	0.5	1.0	0.25	≤0.0625
Bacillus cereus	0.5	0.5	0.5	0.5	≤0.0625
Gram-negative bacteria					
Salmonella typhimurium	0.25	0.5	1.0	0.5	≤0.0625
Shigella dysenteriae	0.125	0.125	0.25	0.125	≤0.0625
Escherichia coli	8	Not detect	Not detect	8	≤0.0625
Fungus					
Aspergillus niger	0.0625	0.0625	0.0625	0.0625	Not detect
Penicillium citrinum	0.125	0.25	0.25	0.125	Not detect
yeast	0.125	0.25	0.25	0.125	Not detect

Table.5 Values of MBC (mg/mL) for essential oil from star anise fruit with different treatments

	Air-dried	De-enzyming	Sulfuring	Oven-dried	Gentamicin
Gram-positive bacteria					
Staphylococcus aureus	0.125	0.5	0.5	0.125	≤0.0625
Bacillus subtilis	1.0	2.0	2.0	1.0	≤0.0625
Bacillus cereus	0.5	1.0	1.0	1.0	≤0.0625
Gram-negative bacteria					
Salmonella typhimurium	0.5	2.0	2.0	1.0	≤0.0625
Shigella dysenteriae	0.25	0.25	0.5	0.25	≤0.0625
Escherichia coli	8	Not detect	Not detect	8	≤0.0625
Fungus					
Aspergillus niger	0.0625	0.0625	0.0625	0.0625	Not detect
Penicillium citrinum	0.125	0.5	0.5	0.125	Not detect
Beer yeast	0.125	0.25	0.25	0.125	Not detect

We mentioned above high temperature treatment has great impact on the components of star anise essential oil, especially the sulfuring process. Sulphur treatment was carried out under the condition of sulfur burning and this process may have a greater influence on the major antibacterial ingredients of star anise essential oil. On the one hand, the absence of chemical composition had taken place. On the other hand, contents of the main elements of star anise essential oil decreased. What is surprising is that EOS showed a similar and weak effect as far as antimicrobial activity. This may be due to components decomposition and lowest anethole content. Researches revealed anethole present in the dried fruit was responsible for a major portion of star anise essential oil antimicrobial property. Furthermore, other fractions except anethole also indicated some minor microbial activities (Aly *et al*., 2016). Synergy action of key component and else trace elements also took charge of the antimicrobial activity. That is why EOO with highest anethole content and the less chemical composition, EOA with upper anethole content and the most abundant chemical composition, did exhibit relatively stronger antimicrobial activity. By contrast, EOD exhibited common inhibitory effect as a whole while it performed the worst for *E. coli*. Undoubtedly, de-enzyming treatment also affected the composition and antimicrobial activity of essential oil. Specific mechanism of antibacterial action of essential oil with different treatments needed our further study.

4. CONCLUSION

Based on the present research, quality of star anise fruit as well as chemical composition and bioactivity of its essential oil were influenced by different drying technologies. From the research we can draw the conclusion that we can choose the right process according to our specific requirements. Star anise essential oil with different treatments was rich in trans- anethole, estragole and limonene in spite of their

different chemical composition and proportion. And they also showed antibacterial activity of different degrees against selected food-borne pathogens especially for fungus in our study. Hence, the essential oil could be considered as natural antimicrobial agent which may be used for prevention foods from microbe pollution. To the best of our knowledge, this is the first report to study the effect of four preprocessing (air-dried, de-enzyming, sulfuring and oven-dried) on chemical composition and antimicrobial activity of star anise essential oil. Further research, particularly on other biological activities and antibacterial mechanism of key monomeric compound in anise essential oil, is still necessary.

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