THE USE OF ESSENTIAL OIL OF CLOVE (*Syzygium aromaticum)* AS TOFU'S NATURAL PRESERVATIVE

Minyak Atsiri Cengkih (*Syzygium aromaticum*) Sebagai Pengaw*e*t Alami Tahu

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Naskah diterima tanggal 25 September 2015

ABSTRACT

The essential oil of the leaves of cloves (*Syzygium aromaticum*) had been widely known for its antimicrobial activity. This research was aimed to analyze chemical constituents and the potency of clove essential oil as natural preservative of tofu. The chemical constituents were analyzed by gas chromatography/mass spectrometry (GC/MS) and its potency as natural preservative was determined by its activity inhibiting bacterial growth on tofu. The major constituents of essential oil of *S. aromaticum* were p-eugenol (75.190%) and β-caryophyllene (18.364%). Essential oil of *S. aromaticum* at concentration of 250 µg/ml can be used as tofu preservative and capable of prolonging tofu's shelf life up to 2 days at room temperature.
Keywords: chemical constituents, clove, essential oil, natural food preservative, tofu.

ABSTRAK

Minyak atsiri cengkih (*S. aromaticum*) telah dikenal memiliki aktivitas sebagai antimikroba. Penelitian ini bertujuan untuk mengetahui kandungan kimia dan potensi minyak atsiri cengkih sebagai pengaw*et al*ami tahu. Kandungan kimia minyak atsiri cengkih dianalisis dengan menggunakan kromatografi gas/spektrometri massa (KG/SM) dan potensinya sebagai pengawet makanan dianalisis berdasarkan aktivitasnya dalam menghambat pertumbuhan bakteri pada tahu yang disimpan di suhu ruang. Kandungan kimia utama dari minyak atsiri cengkih adalah p-eugenol (75.190%) dan β- karyofilen (18.364%). Minyak atsiri cengkih pada konsentrasi 250 µg/ml dapat memperpanjang masa simpan tahu hingga 2 hari pada suhu ruang.
Kata Kunci: cengkih, kandungan kimia, minyak atsiri, pengawet makanan alami, tahu.

INTRODUCTION

Food-borne illness is a growing public health problem in many countries (Jahan, 2012; Newell *et al*., 2010). In Indonesia, *Vibrio cholera* and *Salmonella typhimurium* are the common causes of food-borne illness along with food poisoning caused by *Escherichia coli*, *Bacillus* spp*.,* and *Staphylococcus aureus* (Agustina *et al*., 2013; Lesmana *et al*., 2002; Vollaard *et al*., 2004). Plant derived products are often considered to be safer and more effective against the pathogens by functioning on multi-targeted sites and thus be useful to control of food-borne illness (Han and Bhat, 2014; Voon *et al*., 2011). Spices have been reported possessing antimicrobial activity and popular in the scope of searching novel antimicrobial agents for food and pharmaceutical applications (Babu *et al*., 2011; Panpatil *et al.,* 2013; Rahman *et al*., 2010; Sethi *et al*., 2013).

Clove (*Syzygium aromaticum)* essential oil had been known widely as a potent antimicrobial against various bacteria and fungi, with eugenol as the active ingredient (Hossain *et al*., 2014; Joshi *et al*., 2010; Naveed *et al*., 2013; Oliveira *et al*., 2013; Pandey and Singh, 2011; Park *et al*., 2007; Pinto *et al*., 2009). Based on those explanations, clove essential oil was possibly used as food preservative. We evaluated its potency as tofu's natural preservative based on

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its activity inhibiting bacterial growth on tofu using spectrophotometric method. We also determine the chemical constituents of clove essential oil using gas chromatography/mass spectrometry (GC/MS), particularly to analyze its eugenol concentration.

MATERIALS AND METHODS

General

The materials used in this study were leaves of clove, tofu, and sterilized water. We used Clevenger apparatus to distill clove essential oil, GC-MS to analyze the chemical constituents of clove essential oil, spectrophotometer to estimate the microbial growth on tofu during storage, and glassware commonly used in laboratory.

Plant Materials

The leaves of clove were collected from Purwokerto, Central Java, Indonesia. The leaves were dried at room temperature for 7 days and ground into a fine powder using grinding machine.

Hydrodistillation of essential oils

Powder of dried leaves (2 kg) were subjected to hydro-distillation using a Clevenger apparatus for 4 hrs for the isolation of essential oils according to the method recommended (Guenther, 1961). We obtained 15,0 mL essential oil from this process.

Analysis of chemical constituents of essential oil

The volatile composition of clove essential oil were analyzed using GC-MS system (Agilent 6980N GC System coupled to Agilent 5973 inert MSD detector), equipped with a ZB-5 capillary column (30 m x 0.25 mm x 0.25 µm). The carrier gas was helium at flow rate of 1.3 mL/min, and 2 µL of sample was injected. The electron impact technique (70 eV) was used. The injector and detector temperatures were 250 °C and 230 °C.

Preservative potency of essential oil on tofu.

The white tofu used in this study was obtained from local market at Tambak Sogra, Purwokerto. The tofu was cut in size of 1 x 1 x 1 cm. Each piece was immersed in boiling water for 2 min, in order to reduce the number of the microorganisms attached to the surface of the tofu. Tofus were soaked in 100 ml of broth containing 3 different concentrations of clove essential oil under sterile condition, they were 250, 1250, and 6250 µg/ml, respectively. The tofus in the broths were stored at room temprature. On day 2,4,6,8, and 10, the optical densities of broths were observed at the wavelength of 600 nm (Andayani *et al*., 2014).

Statistical Analysis

Means separation of MIC was accomplished by Duncan's multiple range tests. Significance was evaluated at $p < 0.05$. Statistical analysis was conducted by the general procedures of SPSS Statistics v.13 (SPSS Inc.).

RESULTS AND DISCUSSIONS

In this study, the rendement of essential oil of *S. aromaticum* was 0.75 %. The chemical constituents of essential oil of *S. aromaticum* are shown in Table 1.

The major constituents of clove essential oil are p-eugenol (75.190%), β-caryophyllene (18.364%), α-humulene (2.729%) and caryophyllene oxide (1.130%) (Figure 1). The composition of essential oils from a particular species of plant can differ between harvesting seasons and geographical sources (Burt and Reinders, 2003). Typically, clove essential oil contained p-eugenol, eugenyl acetate and β-caryophyllene as the major constituents. Somehow, the quality of clove essential oil is determined by its eugenol concentration

Table 1. Chemical constituents of clove essential oil

No	Compound name	Retention time (min)	Percentage (%)
1	α-cubebene	29,367	0.800
2	p-eugenol	30.436	75.190
3	eugenyl acetate	30.551	0.427
4	β-caryophyllene	32.301	18.364
5	α-humulene	33.409	2.729
6	1(5).6-guaiadiene	34.120	0.090
7	α-farnesene	35.358	0.230
8	δ-cadinene	35.671	0.696
9	cadina-1,4-diene	35.908	0.087
10	2-hydroxy-4,6-cyclooctadien-1-one	36,435	0.135
11	caryophyllene oxide	37.261	1.130
12	naphthalene	37.956	0.121

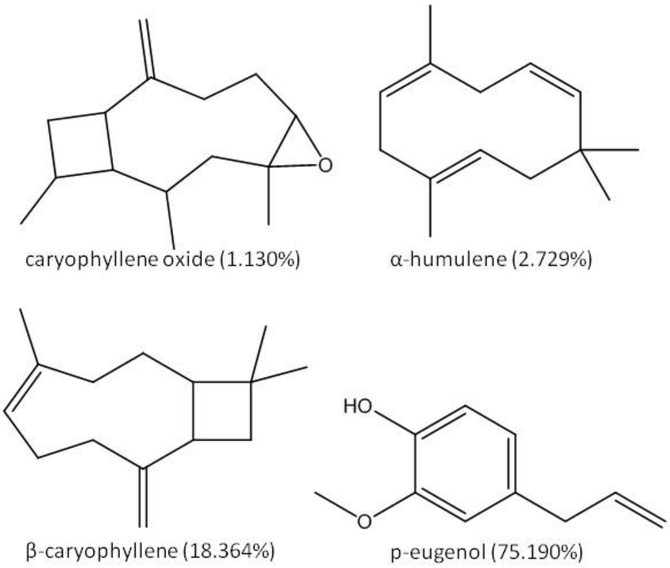

Figure 1. The major constituents of clove essential oil

(Bhuiyan *et al*., 2010). The previously reported data showed that eugenol concentration in essential oils obtained from clove's leaves varied 74.28-87.00% (Bhuiyan *et al*., 2010; Pino *et al*., 2001; Razafimamonjison *et al*., 2014; Srivastava *et al*., 2005). The chemical constituents of clove essential oil distilled from buds are different from that of leaves, as shown by earlier data (Fayemiwo *et al*., 2014; Hossain *et al*., 2014; Razafimamonjison *et al*., 2014). The highest concentration of eugenol is 96.65%, recorded from the essential oil from stems of *S. aromaticum* (Razafimamonjison *et al*., 2014).

In our previous study, the MIC of essential oil of *S. aromaticum* against *Staphylococcus aureus, Salmonela typhimurium* and *Vibrio cholera* was 250 µg/ml (Hartanti *et al*., 2014). In this study, we use that data to provide 3 different concentrations of clove essential oil to preserve tofu, they are 250, 1250, and 6250 µg/ml, respectively. The optical density at the wavelength of 600 nm of the broth used to soak the tofu was used as the indirect method to estimate the number of bacteria grown in tofu. The measured optical density on day 2, 4, 6, 8, and 10 are presented in Table 2.

The result showed that clove essential oil at all given concentrations were active inhibiting the growth of bacteria on tofu until day 2, shown by significantly different OD 600 nm of all concentrations of clove essential oil from that of negative control ($p<0.05$). After day 2, the number of bacteria grown on tofus' broths increased and showed no significantly different from that of negative control. On day 8, clove essential oil at concentration of 6250 µg/ml also possessed inhibitory effect on the growth of tofu's bacteria, but this effect did not observed on the lower concentration of essential oil. This suggested that clove essential oil could be used as natural preservative of tofu and capable of prolonging its shelf life at the room temperature up to 2 days. The trends of bacterial growth on tofu at every given concentration are shown in Figure 2.

The potency of clove essential oil as tofu's preservative is related to its chemical constituents. It has been reported that essential oils containing aldehydes or phenols showed the highest antibacterial activity, followed by essential oils containing terpene alcohols. Other essential oils, containing ketones or esters and acetate had much weaker activity, while volatile oils containing terpene hydrocarbons were

Table 2. The optical density of soaking broth of tofu

Concentration (µg/ml)	OD 600 nm				
	Day-2	Day-4	Day-6	Day- 8	Day-10
(-) control	0.158±0.023	0.070±0.043	0.228±0.021	0.408±0.084	0.187±0.057
250	0.077±0.032*	0.051±0.027	0.214±0.037	0.347±0.035	0.223±0.085
1250	0.035±0.011*	0.019±0.001	0.220±0.106	0.321±0.038	0.083±0.058
6250	0.003±0.003*	0.024±0.015	0.154±0.021	0.194±0.080*	0.042±0.015

* Mean value of MIC (n=3) was significantly different ($p<0.05$) from that of negative control

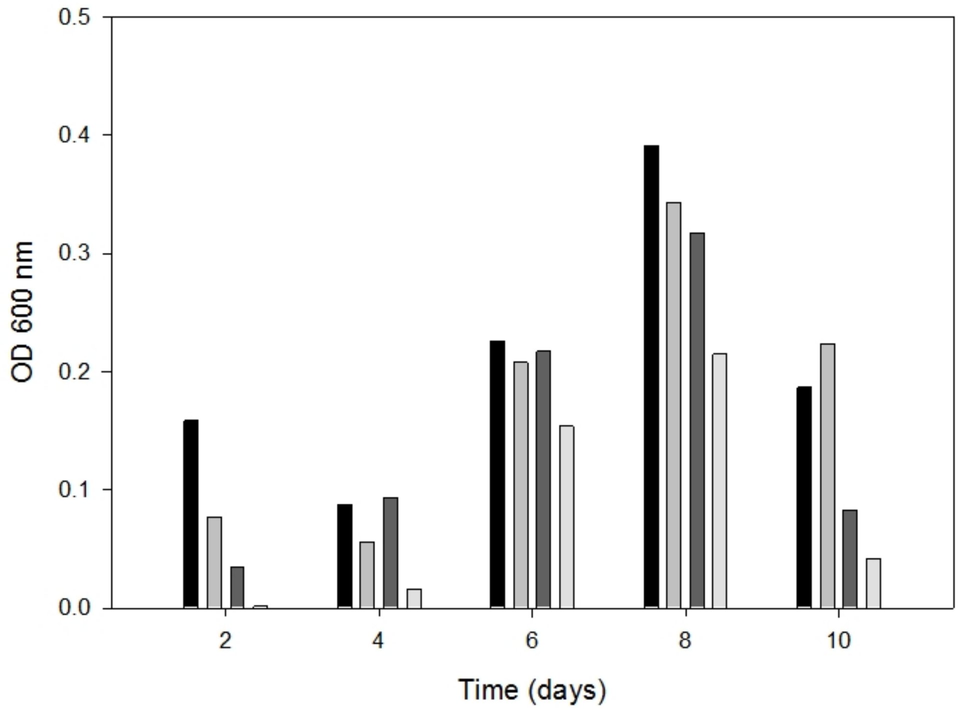

Figure 2. The trends of bacterial growth on tofu preserved with clove essensial oil : control, 250 µg/ml, 1250 µg/ml, 6250 µg/ml.

usually inactive (Bassolé and Juliani, 2012). The clove essential oil used in this study contained high level (75.190%) of p-eugenol, a volatile phenolic compound.

Essential oils are immiscible to water. It was possible that the preservation of tofu was not optimal because the clove essential oil was only deposited on the upper part of the broth. The use of an emulsifier, such as lecithin, might enhance the preservative activity of clove essential oil, as previously reported that the use of eugenol and carvacrol together with lecithin could enhance their activity against *Escherichia coli* on microbiological media and food (Li, 2011).

CONCLUSIONS

The essential oil of *S. aromaticum* at the concentration of 250 µg/ml can be used as food preservative and capable of prolonging tofu's shelf life up to 2 days at room temperature. This activity was related to its major phenolic constituent, eugenol.

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