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Ohmic heating assisted hydrodistillation of clove essential oil

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ABSTRACT

Ohmic heating assisted hydrodistillation is a novel green technology for the extraction of essential oils. In this study, clove bud essential oils (CEO) were extracted by ohmic heating assisted hydrodistillation (OAHD) and compared with conventional hydrodistillation (HD) process, in terms of extraction yield, energy consumption and chemical properties. Ohmic heating assisted hydrodistillation process conditions were optimized by response surface methodology. Voltage gradient (7.5–12.5 V/cm, A), process time (30–120 minutes, B) and mass of clove bud (20–40 g, C) were varied in order to maximize CEO yield and to minimize energy consumption. Optimized extraction conditions were determined as, 12.5 V/cm voltage gradient, 97.967 min for process time and 40 g of mass of clove bud. Yield of CEO and energy consumption were found 13.18 ± 1.50% and 0.214 ± 0.030 kW h/g CEO at optimum extraction conditions, respectively. Under optimum conditions, both OAHD and HD methods were used for CEO extraction. Yields of CEO obtained by OAHD, HD-D (distilled water) and HD-S (salted water) were 13.18 ± 1.50, 8.23 ± 0.35 and 7.88 ± 0.60 respectively. Energy consumptions of OAHD and HD methods were not significantly different ($P > 0.05$). The main chemical component of CEO was eugenol and the yield of eugenol (g/100 g clove bud) was found higher with OAHD (6.95 ± 0.6) method than HD-D (5.16 ± 0.20) and HD-S (5.13 ± 0.28). Antioxidant and antimicrobial properties of CEOs were investigated by FRAP, DPPH and disk diffusion method. Antioxidant properties of CEOs obtained by different extraction methods were not significantly different ($P > 0.05$) according to FRAP and DPPH analysis. Although inhibition zones of CEOs extracted with different methods were not significantly different ($P > 0.05$) for gram positive bacteria's, CEO obtained by OAHD and HD-D have significantly higher ($P < 0.05$) inhibition zones for *K. pneumonia* and *E. coli*, respectively. The results of this study showed that OAHD method is applicable for CEO production.

1. Introduction

Clove (*Syzygium aramaticum* (L.)) which belongs to the *Myrtaceae* family is a commercially cultivated tree in tropical and sub-tropical countries (Hossain et al., 2014). Clove buds, leaves and stems are used for different purpose in medicine, pharmacy, food, flavor and fragrancy industries (Cetin Babaoglu et al., 2017). Clove bud contains approximately 15 to 20% essential oil by weight and the main component of clove essential oil is eugenol (Nurdjannah and Bermawie, 2001). Clove bud essential oil has antibacterial, antifungal, insecticidal, antioxidant and flavoring properties (Guan et al., 2007). Clove bud essential oil has been commercially used for pain control during dental work and in the gums to reduced toothache (Hossain et al., 2014). Essential oils have been extracted from aromatic herbs and spices by different methods. These methods could be classified as (1) classical/conventional methods and (2) advanced/innovative methods (Asbahani et al., 2015). Hydrodistillation, steam distillation, solvent extraction and cold pressing could be classified as conventional methods (Asbahani et al., 2015; Guan et al., 2007). Ultrasound assisted extraction (Asbahani et al., 2015; Tavakolpour et al., 2017), supercritical fluid extraction (Asbahani et al., 2015; Guan et al., 2007), microwave assisted extraction (Gavahian et al., 2015a) and ohmic heating assisted extraction (Seidi Damyeh et al., 2016; Taban et al., 2018) could be classified as advanced methods.

Some studies about extraction of CEO with different extraction methods were done by various researchers. Yang et al. (2014) evaluated the ultrasound-assisted supercritical carbon dioxide ($USC-CO_2$) extraction of CEO. They reported that the $USC-CO_2$ extraction method increased the extraction yield 13.5% when compared to $SC-CO_2$ extraction method. Kapadiya et al. (2018) investgated the extraction of CEO by microwave assisted extraction (MAE) and they optimized MAE parameters for maximize yield, eugenol content and bacterial inhibition

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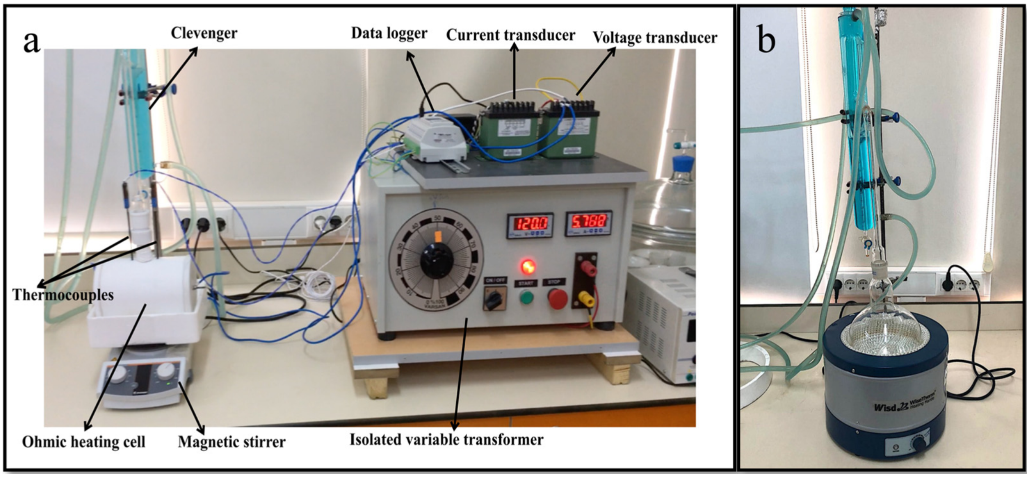

Fig. 1. Ohmic assisted hydrodistillation system (a) and classis hydrodistillation system (b).

of CEO. They reported that the MAE method reduced water usage, extraction time, energy consumption and CO_2 emission, while improved the quantity and quality of essential oil along with a higher ZOI compared to conventional hydrodistillation method. Golmakani et al. (2017) and Gonzalez-Rivera et al., (2016) used microwave assisted hydrodistillation (MAHD), Wei et al. (2016) used $USC-CO_2$ method for extraction of CEO.

Heat energy is transferred to the food by conduction, convection and radiation with conventional heating methods (Varghese et al., 2012). Ohmic heating is defined as a process whereby electrical resistance of the food itself generates heat as electric current is passed through foods (Knirsch et al., 2010; Sarkis et al., 2013). Therefore, heat generated by instantly inside the food with electric current. Ohmic heating system could be designed in many different applications. Power supply, electrodes and ohmic heating cell are main parts of an ohmic heating system. Ohmic heating is related to voltage gradient and electric conductivity (Icier, 2012). Electric conductivity is affected by food properties such as ionic strength, free water and food microstructure. Ionic substances such as acids and salts increases the electric conductivity, while non polar constituents such as fats and lipids decreased conductivity (Varghese et al., 2014). Distilled water is an excellent electrical insulator, so in many studies on ohmic heating, salted water is used as the liquid phase for ohmic treatments (Gavahian et al., 2015b). Heat generation rate during ohmic heating is proportional to the electrical conductivity at a constant voltage gradient (Varghese et al., 2014).

Uniformly heating food, improving food quality, decreasing energy consumption and cost, increasing energy efficiencies, enables to heat foods rapid rates are the main advantages of ohmic heating method (Knirsch et al., 2010; Varghese et al., 2014). Different applications of ohmic heating including blanching (Bhat et al., 2017), evaporation (Sabanci and Icier, 2017), dehydration (Moreno et al., 2016), fermentation (Schoina et al., 2018), extraction (Aamir and Jittanit, 2017), sterilization (Choi et al., 2015), pasteurization (Achir et al., 2016) and thawing (Liu et al., 2017) were successfully done by various researchers. Commercial utilization of ohmic heating technology has increased in the world, since USDA (United States Department of Agriculture) and FDA (Food and Drug Administration) suggested it for pumpable foods (Yildiz-Turp et al., 2013). Ohmic hydrodistillation which is combination of ohmic heating and hyrodistillation has increasing interest over last decade (Gavahian and Farahnaky, 2018). Ohmic assisted hydrodistillation (OAHD) has shorter extraction time, consumed less energy and has higher yield than conventional hydrodistillation methods. There are some studies about the extraction of essential oils from aromatic herbs and species by OAHD such as oregano (Hashemi et al., 2017), damask rose (Manouchehri et al., 2018), sweet bay (Taban et al., 2018), peppermint (Gavahian et al., 2015b).

However, to the best of our knowledge, there is no study about extracting CEO by ohmic heating assisted hydrodistillation method in literature. Thus, the aim of this study was optimizing the OAHD of CEO process and comparing the effects of conventional hydrodistillation and OAHD methods on the clove essential oil.

2. Material and methods

2.1. Material

Clove bud (Bağdat Baharat, Ankara, Turkey) were obtained from a local market. Analytical standard of eugenol for GC analysis obtained from Sigma Aldrich (Germany).

2.2. Extraction methods

2.2.1. Conventional hydrodistillation

Conventional hydrodistillation was carried out with an all glass Clevenger-type apparatus, a heating mantle and a circulating water bath. The extraction was performed with 40 g clove bud with 97.53 min extraction time. The extraction parameters determined according to results of optimization of OAHD method. Conventional HD method was applied both distilled water and 0.5% NaCl solution. 240 mL distilled water or 0.5% NaCl solution and 0.05 g antifoaming agent were used for hydrodistillation. The 0.5% NaCl solution were used for compare OAHD system. The essential oils were stored in amber vials at −18 °C until further analysis.

2.2.2. Ohmic heating assisted hydrodistillation

Ohmic heating assisted hydrodistillation system consisted of a power supply, an isolated variable transformer (Varsan Elektrik, 0–360 V, 5kVA, İstanbul, Turkey), a data logger (Novus, Fieldlogger, Porto Alegre, Brazil) connected to a computer, a voltage transducer (Ohio Semitronics, VT8-10D, Ohio, USA), a current transducer (Ohio Semitronics, CTRS-050D, Ohio, USA), thermocouples (T type, PTFE coated, Sigma Aldrich Inc., Missouri, USA), a glass Clevenger, a magnetic stirrer (MR Hei-Standard, Heidolph, Schwabach, Germany), a circulating water bath (SRC-13C (L), Korea) and an ohmic heating cell (Fig. 1). Ohmic heating cell was a cylindrical PTFE with an inner diameter of 0.1 m and a length of 0.13 m and titanium type electrodes. Cross-sectional area of electrodes was 0.000785 m^2. The distance between electrodes was 0.135 m. Temperature, voltage and current data were monitored for 5 s intervals via data logger. 240 mL 0.5% NaCl solution and 0.05 g antifoaming agent were used for hydrodistillation. The NaCl solution was provided sufficient electric conductivity during

Table 1
Central composite design for OAHD of clove bud experiment responses and process data.

Run	Voltage gradient (V/cm)	Time (min)	Mass of clove bud (g)	Yield (%)	Energy consumption (kWh/g CEO)
1	7.5	30	20	3.899	0.1573
2	7.5	120	20	14.983	0.1958
3	12.5	30	20	5.943	0.2981
4	12.5	120	20	17.395	0.3762
5	7.5	30	40	1.278	0.2326
6	7.5	120	40	12.810	0.1148
7	12.5	30	40	5.547	0.1446
8	12.5	120	40	16.817	0.2169
9	10.0	30	30	3.686	0.2037
10	10.0	120	30	16.445	0.1982
11	7.5	75	30	8.639	0.1338
12	12.5	75	30	14.387	0.2082
13	10.0	75	20	12.185	0.2608
14	10.0	75	40	12.169	0.1236
15	10.0	75	30	12.698	0.1525
16	10.0	75	30	12.468	0.1705
17	10.0	75	30	12.001	0.1790
18	10.0	75	30	12.132	0.1607

CEO; Clove essential oil.

Table 2
ANOVA table showing the CEO variables as linear, quadratic and interaction terms on each response variable.

	Yield (%)		Energy consumption (kWh/g CEO)	
Independent variable	Sum of squares	P-value	Sum of squares	P-value
Model	396.62	< 0.0001	0.067	0.0001
A	34.15	< 0.0001	0.017	0.0004
B	337.52	< 0.0001	4.299E-004	0.4236
C	3.35	0.0309	0.021	0.0002
AB			6.598E-003	0.0085
AC			0.012	0.0014
BC			3.282E-003	0.0439
B^2	21.60	< 0.0001	7.173E-003	0.0067
Residual	7.43		6.177E-003	
Lack of fit	7.13	0.0670	5.779E-003	0.0807
Pure error	0.30		3.985E-004	
Total	404.05		0.073	
R^2	0.9816		0.9154	

A: Voltage gradient, B: process time and C: amount of mass.
P-value < 0.05 is significant at $\alpha < 0.05$ and lack of fit is not significant at *P*-value > 0.05.
CEO; Clove essential oil.

the hydrodistillation process. Magnetic stirrer was operated at 1000 rpm and circulating water bath was setted 0 °C. The essential oils were stored in amber vials at −18 °C until further analysis.

2.3. Optimization of ohmic heating assisted hydrodistillation

A Central Composite Design with three factors and three levels was carried out to determine the optimal levels of voltage gradient (7.5–12.5 V/cm, A), process time (30–120 minutes, B) and mass of clove bud (20–40 g, C) to maximize CEO yield and to minimize energy consumption (Table 1). Analysis of variance (ANOVA), determination of regression coefficients and generation of three-dimensional response surface graphs were carried out using the Design Expert software, version 10.0 (Statease Inc., Minneapolis, MN, USA).

2.4. Analytical methods

2.4.1. Physical properties

Specific gravity of CEO was determined with a pycnometer at 20 °C (Food Chemical Codex) (FCC, 1996).

2.4.2. Determination of essential oil yield

Extraction yield of CEO was determined by Eq. (1) where m_{EO} is the mass of essential oil and m_C is mass of clove bud.

$$Yield\ (\%) = \frac{m_{EO}}{m_C} \tag{1}$$

2.4.3. Energy consumption

Energy consumption of OAHD method for each run was calculated as kWh/g. Eq. (2) indicate that Q_{ohmic} and Eq. (3) indicate that energy consumption;

$$Q_{Ohmic}\ (j) = \int V \bullet I \bullet dt \tag{2}$$

$$Energy\ consumption \left(\frac{kWh}{g\ CEO}\right) = \frac{Q_{ohmic}}{m_{EO}} \bullet \frac{1\ hour}{3600\ s} \tag{3}$$

Energy consumption of Conventional HD method was determined with a Watt-meter (TT T-ECHNI-C, China) and it was calculated with Eq. (4).

$$Energy\ consumption \left(\frac{kWh}{g\ CEO}\right) = \frac{Q_{heater}\ (kWh)}{m_{EO}\ (g)} \tag{4}$$

2.4.4. Gas chromatography-mass spectrometry

Essential oils were analysed by GC (Agilent GC 7890A) coupled with MSD detector (Agilent 5975C VL) and equipped with HP-5MS capillary column (30 m length, 0.25 mm diameter and 0.25 μm film thickness). Helium was used as a carrier gas at a constant flow rate of 1 mL/min. The injector and detector temperatures were maintained at 250 °C. The temperature gradient of the oven was programmed accordingly; the initial temperature was set to be 45 °C and was then allowed to increase to 230 °C (at a rate of 3 °C/min). A volume of 1 μL of the sample was injected into the GC/MS with the injector being in the split mode (split ratio of 1/50). Eugenol and the other components in the clove oil were identified by matching their mass spectra with those of pure compounds. Essential oils diluted with methanol (1:100 CEO: methanol) before GC analyses. The quantity of the eugenol was calculated by comparing their peak area to that of the standards. Eugenol content was estimated using gas chromatography with eugenol standard (calibration curve having $R^2 = 0.9999$).

2.4.5. Antioxidant properties of essential oil

The FRAP assay was carried out according to the procedure of Benzie and Szeto (1999) with some modifications. 50 μL of diluted essential oil were mixed with 0.95 mL of FRAP reagent (prepared by mixing 300 mM acetate buffer, pH 3.6, 10 mM TPTZ in 40 mM HCl, and 20 mM $FeCl_3$ in proportions of 10:1:1). The absorbances of mixtures were measured at 593 nm after 5 min of incubation. $FeSO_4$ was used as a standard and antioxidant power expressed as mmol Fe(II)/mL essential oil.

Determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical scavenging activity of CEOs were done according to the method described by Odabaş and Koca (2016). 50 μL of the diluted extract (1:1000) was added to 1 mL of the 100 μM DPPH solution prepared in absolute ethanol. The mixture was shaken and allowed to stand at room temperature in the dark for 30 min, and the absorbance was recorded at 515 nm. DPPH solution was used as control. Reduction rate of DPPH was calculated with following equation;

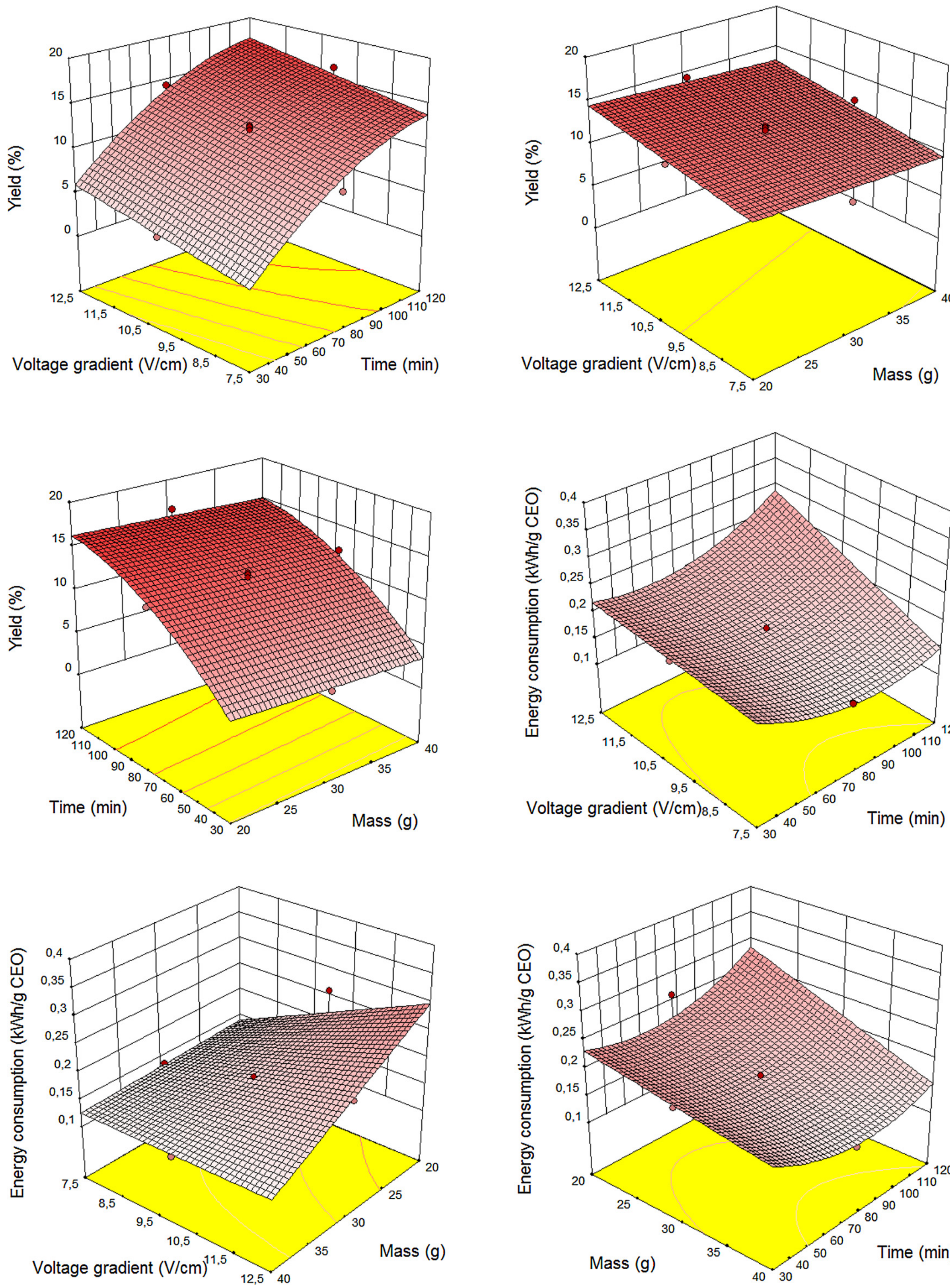

Fig. 2. The change in yield and energy consumption of OAHD method versus independent variables (CEO; Clove essential oil).

$$Reduction\ (\%) = \frac{A_c - A_s}{A_c} \tag{5}$$

where A_c is the absorbance of control and A_s is the absorbance of extract. The sample amount (μL) necessary to decrease the initial DPPH concentration by 50% (EC_{50}) was determined by an exponential curve. DPPH radical scavenging activity is defined as $1/EC_{50}$ (mL/ μL CEO).

2.4.6. Antimicrobial properties of essential oil

2.4.6.1. Microorganisms. The antimicrobial activity of clove essential oils was investigated using three Gram-positive bacteria: *Bacillus cereus* (ATCC 33019), *Listeria monocytogenes* (ATCC 7644), *Staphylococcus aureus* (ATCC 25923) and two Gram-negative bacteria: *Escherichia coli* O157:H7 (ATCC 33150), *Klebsiella pneumoniae* (ATCC 13883). These were maintained on nutrient agar.

2.4.6.2. Disc-diffusion assay. In order to evaluate the antimicrobial activity of essential oils, agar disc diffusion assay was performed based on the method described by Seidi Damyeh and Niakousari

Table 3
Optimum condition values of CEO according to desirability function.

Run	Voltage gradient (V/cm)	Time (min)	Mass of clove bud (g)	Yield (%)	Energy consumption (kWh/g CEO)	Desirability
1	12.5	97.967	40	15.745	0.149	0.884
2	12.5	97.433	40	15.703	0.148	0.884
3	12.5	98.610	40	15.796	0.150	0.884
Measured values	12.5	97.967	40	13.183 ± 1.50	0.214 ± 0.03	
% Differences				16.27	43.62	

CEO; Clove essential oil.

(2017). Bacterial suspension was adjusted to 0.5 McFarland; approximately 10^8 CFU/mL. Then, sterile filter discs (diameter 4 mm, Whatman Paper No. 1) were soaked with 5 µL essential oil solution (1:1; CEO: methanol). After incubation at 37 °C for 24 h, the distinct zone of growth inhibition around the wells was measured using a digital caliper. The diameter of zones of inhibition around each of the discs was measured in mm and recorded as antibacterial activity.

2.5. Statistical analysis

All analyses were performed with tree replicates. Data were subjected to statistical analysis using SPSS software (version 24, SPSS inc) for the analysis of variance (ANOVA). The differences between means of dependent variables were compared with post hoc-Duncan tests with 95% confidence level.

3. Results and discussion

3.1. Extraction parameters, yield, energy consumption and physical properties

Experimental responses and process parameters for OAHD method are presented in Table 1. Yield and energy consumption values were changed in a range 3.686–17.395% and 0.1148 - 0.3762 kW h/g EO, respectively (Table 1). Hydrodistillation yield is significantly ($P < 0.05$) affected by voltage gradient, process time and mass of clove bud. Table 2 indicated that the validities of the linear, quadratic and interaction term models for yield and energy consumption according to their P-values. Models were improved by excluding insignificant terms from model equations. Lack of fit values for all the responses were not significant ($P > 0.05$) indicating that the models were adequately fitted the experimental data. Voltage gradient, process time and mass of clove bud have quadratic effect on both yield and energy consumption. The regression coefficients of the models for yield and energy consumption were 0.9816 and 0.9154, respectively. R^2 values implied that a high degree of fitness of the models and closer conformity between experimental and predicted values. The differences in yield and energy consumption of OAHD method versus independent variables shown in Fig. 2. Increasing voltage gradient and process time increased the yield of CEO. Increasing mass of clove bud was decreased energy consumption. But increasing voltage gradient was increased energy consumption.

Ohmic heating assisted hydrodistillation process conditions were optimized for maximum yield and minimum energy consumption. Optimum OAHD of CEO process conditions were found as 12.5 V/cm voltage gradient, 97.9 min for process time and 40 g for mass of clove bud (Table 3). The desirability value was found 0.884. Under these optimum conditions, estimated and measured dependent variables are given in Table 3. Differences between estimated and measured values were found 16.17% and 43.62% for yield and energy consumption, respectively.

3.2. Yield

Optimum time and mass of clove bud values were used for production CEO with conventional HD method. Comparison of different hydrodistillation methods for production CEO is given in Table 4. Yield values of OAHD method are significantly higher ($P < 0.05$) than HD methods. Similarly reaching to boiling point time and oil accumulation starting time are significantly lower than HD methods. Although process time of OADH and HD methods are same at 97.53 min, OAHD method boiled earlier and this effect the yield of CEO. At the OAHD method the boiling starts earlier, and by this means extraction time was became longer than HD.

Gavahian et al. (2015b) reported that electric energy which is more severe and effective, caused eruption of approximately all samples and because of this samples cannot survive from destruction. This may explain higher yield level of OAHD. Electroporation is defined that under the influence of electric filed, cell walls and membranes become permeable below the temperature at which cell membranes are normally permeable (Seidi Damyeh and Niakousari, 2016). Because of electroporation diffusion of essential oil enhanced at OAHD system and this proved much easier extraction of essential oil compared with HD (Seidi Damyeh and Niakousari, 2016). Using distilled water or salted water at HD was not significantly ($P > 0.05$) affect yield. Similarly reaching to boiling point and extraction rate were not affected from water type (Table 4). Other researchers reported similar result (Gavahian et al., 2015a; Gavahian et al., 2011; Gavahian et al., 2015b). They declared that no significant differences between HD-D and ND-S methods in terms of yield and extraction parameters.

Kapadiya et al. (2018) were used microwave radiation as a green technology for extraction of essential oil from clove buds. They optimized process conditions and their results showed that yield of CEO and yield of eugenol was changed by changing process parameters between 4.92–12.67 % and 4.44–11.80%, respectively. At the optimum point, they found yield of CEO 13.11%, yield of eugenol 11.93%, eugenol in oil 90.10% (Kapadiya et al., 2018). Golmakani et al. (2017) studied about eugenol enrichment of CEO with MAHD and they used both hydrodistillation and steam distillation. They reported that highest CEO yield was 13.94% by MAHD. These results are similar with our study. Green technologies provide higher EO yield than conventional hydrodistillation.

3.3. Chemical composition

Chemical composition of CEOs obtained by OAHD and HD methods are given at Table 5. Ten components have been identified which represented more than 98% of total essential oil and the main components of CEOs were eugenol (82.68–87.12%) and isoeugenol (11.11–15.78%). According to Golmakani et al. (2017) clove EOs contain high amounts of phenolic monoterpene alcohol, eugenol (82.65–88.80%). Similar main components are presented CEOs by extracted by OAHD and HD methods. Although CEO obtained by OAHD method contained lowest percentage of eugenol, it contained highest percentage of isoeugenol. It is understood that the application of ohmic heating significantly affects the chemical composition of CEOs (Table 5). Application of electric current was significantly increased ($P < 0.05$) isoeugenol peak area of CEO. Isoeugenol is an isomer of eugenol. Electric current might accelerate isomerization of eugenol to isoeugenol. Tong et al. (2008) reported that low static electric field can

Table 4
Comparison of different hyrodistillation methods.

	Yield (%)	Reach to boiling point (min)	Starting time to oil accumulation (min)	Extraction rate (g/min)	Consumed energy (kWh/g CEO)	Emitted CO_2 (g/mL CEO)	Density (g/m^3)	FRAP (mmol Fe(II)/mL CEO)	$1/EC_{50}$ (mL/µL CEO)
OAHD	13.18 ± 1.50^{b}	3.66 ± 0.23^{a}	6.28 ± 0.12^{a}	0.050 ± 0.00^{b}	0.2140 ± 0.03^{a}	171.19 ± 23.61^{a}	1.052 ± 0.00^{a}	4.00 ± 0.90^{a}	172.28 ± 8.79^{a}
HD-D	8.23 ± 0.35^{a}	8.60 ± 0.51^{b}	12.70 ± 0.77^{b}	0.034 ± 0.00^{a}	0.1881 ± 0.01^{a}	150.48 ± 6.30^{a}	1.051 ± 0.00^{a}	3.34 ± 0.69^{a}	177.62 ± 8.09^{a}
HD-S	7.88 ± 0.60^{a}	9.00 ± 0.43^{b}	13.89 ± 0.61^{c}	0.032 ± 0.00^{a}	0.2152 ± 0.01^{a}	172.12 ± 6.75^{a}	1.052 ± 0.00^{a}	3.29 ± 0.65^{a}	168.66 ± 6.88^{a}

OAHD; Ohmic heating assisted hydrodistillation, HD-D; Hydrodistillation with distilled water, HD-S; Hydrodistillation with salted water.
Clove essential oil.
In each column, means with different letters are significantly different ($P < 0.05$).

dramatically increase the rate of cis-trans isomerization of an azobenzene derivative. Bouchachia et al. (2017) were reported that different percentages of chemical compositions were probably occurred during the extraction notably thermal and hydrolysis reactions, isomerization, oxidation dehydrogenation, polymerization and thermal rearrangements.

Eugenol content of clove buds were calculated 553.88 ± 49.94–682.82 ± 37.27 mg eugenol/ ml CEO. Although eugenol content of CEO obtained by OAHD was significantly lower than CEOs extracted by HD methods. The yield of eugenol was found higher with OAHD (6.95 ± 0.6 g/100 g clove bud) method than HD-D (5.16 ± 0.20 g/100 g clove bud) and HD-S (5.13 ± 0.28 g/100 g clove bud). The reason of these results is that OAHD had provides highest extraction yield of CEO.

Golmakani et al. (2017) informed that eugenol yield (g/g clove) ranged from 9.53 to 12.37%. Yang et al. (2014) indicated that eugenol yield (weight of eugenol/weight of CEO) ranged from 34.03 ± 1.11–59.18 ± 2.02% and extraction methods were affected eugenol yield of CEOs.

There are no significant differences ($P > 0.05$) between HD-D and ND-S methods in terms of chemical components of CEOs. These results indicate that using 0.5% NaCl solution did not affect the chemical composition of CEOs.

3.4. Energy consumption

Energy consumptions of OAHD and HD methods are given in Table 4. There are not significantly differences ($P > 0.05$) between OAHD and HD methods. It is mostly because of time of extraction methods were all same and higher voltage gradient was chosen as optimum conditions. As described by Seidi Damyeh and Niakousari (2017), 800 g CO_2 will be emitted to the atmosphere to generate 1 kW h energy through the combustion of fossil fuels. CO_2 emitted values of different methods were not significantly different ($P > 0.05$). Ohmic heating assisted hydrodistillation convert the entire input energy from electrical to thermal (Gavahian et al., 2018). Damyeh and Niakousari (2016) were extracted EOs from *Prangos ferulacea* Lindle., with HD and OAHD methods. They reported that 0.50 kW h and 0.83 kW h energy required for OAHD (45 min) and HD (159 min) extraction, respectively. These energy requirements caused 0.33 kg CO_2 /g EO and 0.52 kg CO_2/g EO carbon dioxide emission for OAHD and HD, respectively. Ohmic heating assisted hydrodistillation method has lower energy consumption and carbon dioxide emission because of it generated heat directly within the salted water and energy conversation rate of about 100% (Gavahian and Chu, 2018). When comparing MAHD and OAHD methods, Gavahian et al. (2015a) indicated that energy consumption values of OAHD method 64% of that consumed in MAHD and 20% of that consumed in HD process.

3.5. Antioxidant properties

Antioxidant properties of CEOs extracted by HD and OAHD methods were investigated FRAP and DPPH analysis. Ferric reducing ability and $1/EC_{50}$ values of CEOs are given in Table 4. The results indicate that CEOs have high antioxidant activity. Similarly; Gülçin et al. (2004) and Wang et al. (2017) CEO has powerful antioxidant activity compared to various antioxidant materials. The antioxidant activity of essential oils mostly correlates with terpenes, phenols and ketones (Seidi Damyeh et al., 2016). High antioxidant properties of CEOs associated with high content of eugenol (González-Rivera et al., 2016). On the other hand, isoeugenol also possess antioxidant activity. The phenoxyl radicals of eugenol-related compounds are the products of scavenging of free radicals during the inhibition of lipid peroxidation (Atsumi et al., 2005). Although CEO extracted from OAHD method ferric reducing value (4.03 ± 0.90^{a}) were higher than CEO extracted from HD methods (3.34 ± 0.69 for HD-D and 3.29 ± 0.65 for HD-S), there was not

Table 5
Chemical compositions and eugenol contents of CEOs.

			Peak areas (%)		
No.	Compound	RT (min)	ODHD	HD-D	HD-S
1	Linalol	20.703	nd[a]	nd[a]	0.05 ± 0.06[a]
2	Methylsalicylate	25.293	nd[a]	0.09 ± 0.01[b]	0.09 ± 0.01[b]
3	2,4-Dimethylbenzaldehyde	28.116	0.34 ± 0.06[a]	0.33 ± 0.05[a]	0.35 ± 0.03[a]
4	Copaene	32.442	nd[a]	0.05 ± 0.06[a]	0.05 ± 0.06[a]
5	Eugenol	33.605	82.68 ± 0.59[a]	87.12 ± 0.31[b]	86.54 ± 0.62[b]
6	beta-Caryophyllene	35.638	0.81 ± 0.23[a]	1.01 ± 0.42[a]	1.11 ± 0.61[a]
7	Linalyl butyrate	37.025	0.11 ± 0.03[a]	0.13 ± 0.03[a]	0.05 ± 0.06[a]
8	Isoeugenol	40.203	15.78 ± 0.84[b]	11.11 ± 0.18[a]	11.37 ± 0.49[a]
9	Caryophyllene oxide	42.292	0.15 ± 0.03[a]	0.15 ± 0.02[a]	0.14 ± 0.02[a]
10	2',3',4' Trimethoxyacetophenone	46.212	0.14 ± 0.05[a]	0.05 ± 0.06[a]	nd[a]
	mg eugenol / ml CEO		553.88 ± 49.94[a]	658.16 ± 24.94[ab]	682.82 ± 37.27[b]
	g eugenol / 100 g CB		6.95 ± 0.63[b]	5.16 ± 0.20[a]	5.13 ± 0.28[a]

CEO; Clove essential oil, CB; Clove bud.
OAHD; Ohmic assisted hydrodistillation, HD-D; Hydrodistillation with distilled water, HD-S; Hydrodistillation with salted water.
In each row, means with different letters are significantly different ($P < 0.05$).
nd; not detected.

Table 6
Diameter of inhibition zones (mm) of clove essential oils (5 µL/disc).

Bacteria	OAHD	HD-D	HD-S
K. pneumoniae	15.92 ± 3.18[b]	12.17 ± 0.43[a]	16.67 ± 1.84[b]
E. coli	10.78 ± 1.24[a]	12.23 ± 0.83[b]	9.67 ± 0.25[a]
L. monocytogenes	13.52 ± 1.01[a]	12.88 ± 0.73[a]	13.85 ± 1.05[a]
B. cereus	13.07 ± 3.40[a]	13.55 ± 1.78[a]	11.22 ± 0.52[a]
S. aureus	12.34 ± 0.82[a]	13.17 ± 0.12[a]	13.14 ± 0.15[a]

OAHD; Ohmic assisted hydrodistillation, HD-D; Hydrodistillation with distilled water, HD-S; Hydrodistillation with salted water.
In each row, means with different letters are significantly different ($P < 0.05$).

significant difference ($P > 0.05$) between results. Similarly, $1/EC_{50}$ values of CEOs extracted by different methods were not significantly different ($P > 0.05$). These results showed that extraction methods have no effect on antioxidant properties of CEOs. Similar findings are reported by Damyeh et al., Seidi Damyeh et al. (2016). They reported that the essential oil obtained by OAHD showed a slightly higher antiradical activity than the essential oils obtained through HD and MAHD, which can be attributed to the higher amount of linalool (22.38%) in OAHD essential oil (Seidi Damyeh et al., 2016). Also, Tavakolpour et al. (2017) were extracted essential oil from *Thymua danesis* subsp. *Lancifolius* by HD, OAHD, UAHD (Ultrasonic assisted hydrodistillation), UAOE (ultrasonic assisted ohmic extraction), they were found no differences between IC_{50} values of EOs extracted by HD and OAHD.

3.6. Antimicrobial properties

The bacteria used in this study are known as food-borne infection or food intoxication. The antibacterial activities of the CEOs against three Gram-positive and two Gram-negative bacteria were evaluated using disc diffusion method. CEO has a strong antimicrobial activity because of presences of eugenol (Yang et al., 2014). The diameters of inhibition zones are shown in Table 6. According to results, Gram-positive bacterias were not significantly affected HD methods, although Gram-negative bacterias were significantly affected CEOs extracted by different methods. This could be changed because of chemical composition differences of CEOs. The most sensitive bacteria to the CEOs obtained by OAHD method was *K. pneumoniae* with largest inhibition zone. According to the results there was no differences ($P > 0.05$) between CEOs obtained by OAHD method and HD methods against *B. cereus, L. monocytogenes and S. aureus* which are Gram-positive bacteria. Although, CEOs obtained by the HD-S method was higher inhibition zone than the essential oil obtained by OAHD against to *K. pneumoniae*., there was no significant difference between OAHD method and HD-S method. Minimum inhibition zones were obtained against *E. coli* at all HD methods. Similar results reported by Hashemi et al. (2017). They are extracted oregano essential oil by HD and OAHD method and their result showed that different methods were not affected antimicrobial properties of oregano essential oil towards *S. Typhimurium, E. coli, P. aeruginosa, S. aureus and B. cereus*. Antimicrobial activity of EO's is affected both by the presence of multiple components and by the major component. For this reason, it is expected that a differences of compounds in the essential oil caused by the extraction method will affect its antimicrobial activity (Reyes-Jurado et al., 2015).

4. Conclusion

Optimization results showed that voltage gradient, time and mass has significant effects on yield of essential oil. Also, energy consumption was affected by voltage gradient and mass. There was no significant difference between energy consumption of hydrodistillation methods, however, yield of CEO extracted by OAHD was significantly higher than CEO obtained with HD method. Essential oils extracted by HD and OAHD were similar in chemical compositions. Eugenol and isoeugenol were found as main components of CEO. Ohmic heating assisted hydrodistillation method can also developed with regard to scale up, electrode type or salt concentration of sample solution.

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