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USE OF TARRAGON (*ARTEMISIA DRACUNCULUS*) ESSENTIAL OIL AS A NATURAL PRESERVATIVE IN BEEF BURGER

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ABSTRACT

Nowdays, adding natural food preservatives is one of the methods for increasing shelf-life. The aim of this study was evaluation of antioxidant activity and antibacterial effects Tarragon (*Artemisia dracunculus*) essential oil (TEO) in beef burger product.

In this experimental study, essential oil of the Tarragon was isolated by hydrodistillation. Then, TEO was analyzed by gas chromatography-flame ionization detector (GC-FID) and gas chromatography/mass spectrometry (GC-MS). The effect of different concentrations of Tarragon essential oil (0.00, 0.062, 0.125, and 0.25%) in 4±1°C temperature and storage time up to 12 days was evaluated on lipid oxidation, anti *Staphylococcus aureus* activity and organoleptic effects in beef burger.

The monoterpenes hydrocarbons constitute the major fraction of the TEO (95.91%) and the sesquiterpene hydrocarbons were the minor fraction (0.46%). No significant differences were observed after adding of different concentrations of essential oil on lipid oxidation value in raw beef burger (P>0.05). The Tarragon essential oil 0.25% in storage temperature (4±1°C) decreased growth rate of *S. aureus* in beef burger (p<0.05). Also overall acceptance rate in beef burger containing Tarragon essential oil 0.125% created a better sense in product (p<0.05).

Therefore, this essential oil might be used as an antibacterial agent and flavor enhancer in meat products such as beef burger.

- Keywords: antibacterial effect, antioxidant activity, *Artemisia dracunculus*, beef burger -

INTRODUCTION

In recent years, the food industries researchers search for superseded sources of antibacterial and chemical preservatives against inroad of bacteria and lipid oxidation in foods (GUIMARÃES *et al.*, 2010). Meat and its products such as beef burger are widely consumed all over the world. During storage time shelf life of these products is reduced. Oxidation of lipids and degradation of organoleptic agents change the flavor, color and texture of meat products (SALEM *et al.*, 2010). Also, food spoilage and pathogenic bacteria could contaminate meat products and lead to public health hazard and economic losses (SALEM *et al.*, 2010). On the other hand, the use of the chemical antioxidants with high activity, such as TBHQ (tertiary butyl hydroquinone), can threaten human health (RAEISI *et al.*, 2012; KHOSRAVI-BOROJENI *et al.*, 2012). Natural antioxidants other than possessing protective activities against food spoilage they have therapeutic and protective effects against a wide variety of different diseases (RAFIEIAN-KOPAEI *et al.*, 2013; KHOSRAVI-BOROJENI *et al.*, 2012). The presence of limonene, carvacrol and eugenol as bioactive substances in essential oils may exert inhibitory effect on microorganisms growth and reduce deterioration due to lipid oxidation in foods (SENGUL *et al.*, 2011; GUIMARÃES *et al.*, 2010).

The *Artemisia dracunculus* is commonly consumed in the most food as smell and flavor enhancer in barbecues, salads and soup. The *Artemisia dracunculus* is a small shrub from the *Asteraceae* family and is called "Tarkhon" in Iran, Tarragon, dragon wormwood, dragon sage-wort, estragon. In traditional medicine its used for the treatment of collywobbles, fever, diabetes and bacterial or parasitic infections (RAEISI *et al.*, 2012; AYOUGHI *et al.*, 2011). Previous studies have shown the antioxidant and antibacterial activities of essential oil of *Artemisia dracunculus* on kind of bacteria in vitro (EREL *et al.*, 2012; AYOUGHI *et al.*, 2011; SEN-GUL *et al.*, 2011; KORDALI *et al.*, 2005).

The aim of this study was evaluation of antioxidant and antibacterial effects of Tarragon (*Artemisia dracunculus* L.) essential oil in beef burger.

MATERIALS AND METHODS

Preparation of the essential oil

The Tarragon was purchased from the local grocery of Shahrekord city and identified by the standard botanic work in Medical Plants Research Center in Shahrekord University of Medical Sciences, Iran. The essential oil of Tarragon leaves (TEO) was extracted by steam distillation (1:5 herb/water, in w/v ratio) for 3 h using a Clevenger type apparatus and dried by adding anhydrous sodium sulphate as well as

stored at 4°C before being used for assay (RAEISI *et al.*, 2012).

Analysis of essential oil

The essential oil was analyzed using a Younglin Acme 6000 GC-FID with a HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm). Helium as carrier gas was used at a flow rate of 0.8 ml/min. The essential oil was diluted in n-pentane (1/1000, v/v) and 1.0 µL injected in the splitless mode. The primary oven temperature was maintained at 50°C for 5 min and then increased to 240°C at the rate of 3°C/min. Temperatures of injector and detector were 290°C and 300°C, respectively. Quantitative data were obtained from GC peaks area percent.

Then GC/MS essential oil analysis was performed on a HP-5MS capillary column (30 m × 0.25 mm, film thickness 0.25 µm), using a Gas Chromatograph Agilent 6890, interfaced with a Mass Spectrometer Agilent HP- 5973. The electron ionization (EI) system with ionization energy of 70 eV and temperature of ion source 220°C was used for GC-MS detection. Other stages were under similar conditions as GC. Mass spectra were scanned between 50 and 550 a.m.u range. The essential oil compounds were identified by retention indices (RI) and compared their RI with data reported in the articles, references books as well as standard libraries (Wiley275.L and Wiley7n.L) (ADAMS, 1995).

Preparation of beef burger

Five kg beef burger was taken from a batch production of a meat products factory. For microbiological analysis, samples (100 g) of beef burgers were placed in the stomacher bags and transported to the Atomic Energy Organization of Tehran, Iran for sterilization with Gamma irradiation (60 Cobalt emitting gamma rays, period time 26 minutes for 5.5 KGy). Also microbial culture was carried out for confirmation of any growth bacteria. The samples were divided into treated and untreated samples (control). The treated groups were added 0.062, 0.125 and 0.25% concentrations of Tarragon essential oil. All samples were labeled and stored at 4 °C. Then, the samples were analyzed on days 0, 1, 3, 6, 9 and 12 for chemical and microbiological factors (NOORI *et al.*, 2012).

Determination of lipid oxidation

Thiobarbituric acid (TBA) assay was conducted as described by MARASCHIELLO *et al.* (1999) with some modifications. 0.5 g of raw beef burger samples was added to 10 mL of deionized water and mixed vigorously for 1 min and then 2.5 mL of 25 % TCA (Trichloroacetic acid) (Sigma-Aldrich Corporation, St. Louis, MO., USA) was added. The samples were stored for 15 min at 4 °C

Table 1 - Composition of Tarragon essential oil.

Name	Concentration (%)
α -pinene	0.57
β -pinene	0.11
β -myrcene	0.10
Limonene	1.79
z- β -ocimene	3.42
Trans-ocimene	3.86
Terpinene	0.08
Linalool	0.16
Ocimene (allo)	0.16
Methyl chavicol	84.83
Geranial	0.17
Iso bornyl acetate	0.10
Eugenol	0.12
Iso safrole (E)	0.37
Methyl eugenol	0.07
Valencene	0.1
β -sesquiphellandrene	0.07
Cinnamaldehyde (para-methoxy)	0.20
Spathulenol	0.09
Monoterpenes	10.09
Oxygenated monoterpenes	85.82
Sesquiterpenes	0.17
Oxygenated sesquiterpenes	0.29
Total	96.37

and centrifuged for 5 min (4000 rpm, at 4 °C). The 3.5 mL of supernatant was mixed with 1.5 mL of 0.6 % TBA (Sigma-Aldrich Corporation, St. Louis, MO., USA) and placed in water bath (70 °C) for 30 min. The absorbance of the solutions was measured at 532 nm with spectrophotometer (Unico UV-2100, USA) against a blank containing of 2.5 mL of deionized water, 1 mL 25% TCA and 1.5 mL 0.6% TBA. The standard curve was prepared by standard solutions of 1, 1, 3, 3-tetraethoxypropane (TEP) (Sigma-Aldrich Corporation, St. Louis, MO., USA). The amount of TBA was expressed as mg of malonaldehyde (MDA) per kg of meat.

Preparation of inocula and enumeration of *S. aureus*

Staphylococcus aureus with PTCC 1189 (Persian Type Culture Collection) was obtained from the Iranian Research Organization for Science

and Technology (IROST), Iran. The lyophilized bacterium was moved to Brain heart infusion (BHI) (Merck Ink. Darmstadt, Germany) and was incubated at 37°C for 18 h.

For the test, final inoculum of pathogen cells of 10^3 colony forming unit (CFU)/g of *S. aureus* to beef burgers samples were used by spectrophotometer (absorbance 600 nm) and surface cultivation, simultaneously (SHEKARFOROUSH *et al.*, 2007).

Sensory evaluation

A hedonic test was used for sensory evaluation described by AMANY *et al.* (2012). Cooked samples were served warm to 6 members of household trained panel without care of age or sex. A nine-point hedonic scoring scale (1 =dislike extremely, 2= dislike very much, 3= dislike moderately, 4= dislike slightly, 5= neither like nor dislike, 6= like slightly, 7= like moderately, 8, like very much, 9= like extremely) was used for overall acceptability.

Statistical analysis

The data concerning this present study were framed as means \pm standard deviation of triplicates. The significance of difference was performed by Kruskal- Wallis test and Friedman test using INSTAT software. $P<0.05$ was considered to be significant.

RESULTS

The composition of the TEO is demonstrated in Table 1. Nineteen compounds were identified in the *Artemisia dracunculus* essential oil samples, representing the 96.37% of the total oil. The main compound of TEO were methyl chavicol (84.83%), trans-ocimene (3.86%) and z- β -ocimene (3.42%). The monoterpenes hydrocarbons constitute the major fraction of the oil (95.91%) and Sesquiterpene hydrocarbons amounted to 0.46%.

Table 2 shows the mean TBA values for raw beef burgers with different concentrations of

Table 2 - Mean TBA values (mg of malonaldehyde/kg) for raw beef burger with different concentration of Tarragon essential oil (TEO) during refrigerated storage (4 \pm 1°C) for 12 days.

Days Treatment	0	1	3	6	9	12
Control	0.95 \pm 0.02 ^a	1.04 \pm 0.04 ^a	1.36 \pm 0.01 ^a	1.92 \pm 0.02 ^a	2.57 \pm 0.02 ^a	3.22 \pm 0.02 ^a
BHT 0.25%	0.95 \pm 0.02 ^a	0.97 \pm 0.01 ^a	1.12 \pm 0.02 ^b	1.51 \pm 0.02 ^b	2.14 \pm 0.01 ^b	2.86 \pm 0.01 ^b
TEO 0.0625%	0.95 \pm 0.02 ^a	0.97 \pm 0.01 ^a	1.35 \pm 0.01 ^a	1.91 \pm 0.01 ^a	2.54 \pm 0.01 ^a	3.22 \pm 0.01 ^a
TEO 0.125%	0.95 \pm 0.02 ^a	0.95 \pm 0.01 ^b	1.33 \pm 0.02 ^a	1.86 \pm 0.01 ^a	2.49 \pm 0.01 ^a	3.18 \pm 0.01 ^a
TEO 0.25%	0.95 \pm 0.02 ^a	0.95 \pm 0.01 ^b	1.28 \pm 0.02 ^a	1.76 \pm 0.01 ^a	2.23 \pm 0.01 ^a	3.13 \pm 0.01 ^a

The different superscripts within the same column are significantly different ($p < 0.05$).

Table 3 - Effect of different concentration of Tarragon essential oil (TEO) on *Staphylococcus aureus* in raw beef burger during refrigerated storage ($4\pm 1^{\circ}\text{C}$) for 12 days.

Days Treatment	0	1	3	6	9	12
Control	$1.5 \times 10^3 \pm 48^a$	$1.2 \times 10^3 \pm 57^a$	$1.7 \times 10^3 \pm 57^a$	$<100^a$	$<100^a$	$<100^a$
TEO 0.0625%	$1.5 \times 10^3 \pm 48^a$	$5.3 \times 10^2 \pm 152^a$	$<100^b$	$<100^a$	$<100^a$	$<100^a$
TEO 0.125%	$1.5 \times 10^3 \pm 48^a$	$1 \times 10^2 \pm 1^a$	$<100^b$	$<100^a$	$<100^a$	$<100^a$
TEO 0.25%	$1.5 \times 10^3 \pm 48^a$	$<100^b$	$<100^b$	$<100^a$	$<100^a$	$<100^a$

The different superscripts within the same column are significantly different ($p < 0.05$).

Tarragon essential oil (TEO) during refrigerated storage ($4\pm 1^{\circ}\text{C}$) for 12 days. Control sample showed the highest TBA values and in the TEO 0.25% treatment after day 1 lower than the control in both raw beef burgers ($p < 0.05$). On other days BHT 0.25% showed lower oxidation values than control group ($p < 0.05$) and no statistically significant differences ($p > 0.05$) were observed between the TBA values TEO treatment samples and the control sample.

The time-related survival of *S. aureus* following treatment with different concentrations of TEO is demonstrated in Table 3. The Tarragon essential oil in storage temperature ($4\pm 1^{\circ}\text{C}$) decreased growth rate of *S. aureus* till third storage day in beef burger than control group ($p < 0.05$).

Fig. 1 show overall acceptability values for all cooked beef burgers treated to TEO up to 12 days. The results of organoleptic evaluation demonstrated that the best overall acceptability related to 0.125% TEO treated samples ($p < 0.05$).

Furthermore, after third day of storage all sensory attributes were declined for control samples while TEO treated samples were scored between 5.5 and 7.5. After sixth day of storage all

of the samples revealed reduction of overall acceptability values.

DISCUSSION

It is obvious that different plant essential oils have antioxidant and antibacterial compounds (RAEISI *et al.*, 2012). On based results obtained in this study, essential oil of Tarragon had antioxidant and antibacterial properties. Nineteen compounds were identified, accounting for 96.36% of the essential oil for *Artemisia dracunculus*. The main constituents of essential oil were Methyl chavicol (84.83%), Trans-ocimene (3.86%), *z*- β -ocimene (3.42%), Limonene (1.79%) and α -pinene (0.57%). In previous studies *Artemisia dracunculus* essential oil contained (*Z*)-anethole (81.0%), *z*- β -ocimene (6.5%), (*E*)- β -ocimene (3.1%), limonene (3.1%) from Turkey (KORDALI *et al.*, 2005), and (*Z*)-anethole (51.72%), *z*- β -ocimene (8.32%), methyl-eugenol (8.06%) from Iran (AYOUGHI *et al.*, 2012). There is some similarity between the compound of TEO of this study and the above studies. The

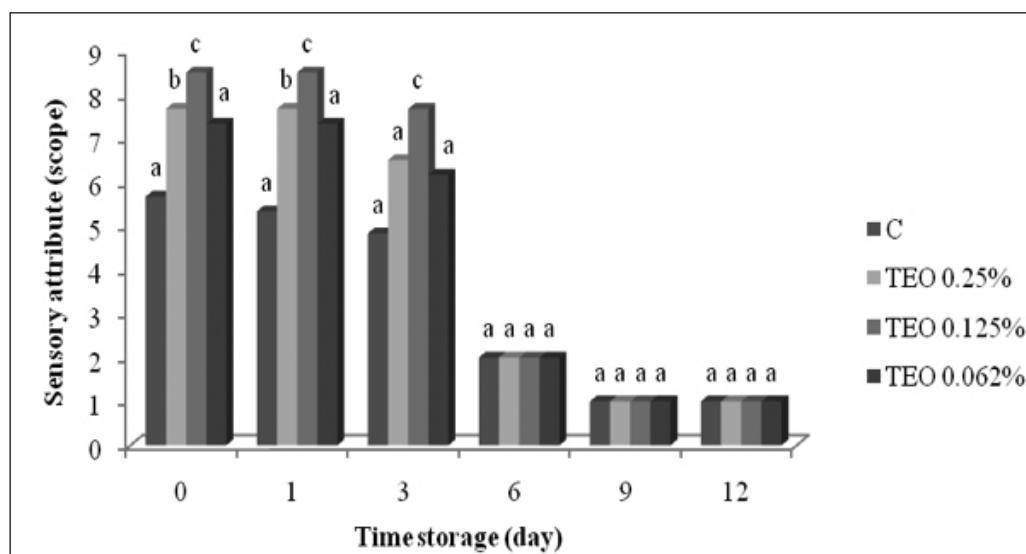


Fig. 1 - Overall acceptability evaluation of cooked beef burger mixed with different concentration of Tarragon essential oil (TEO) (Different letters have significant difference ($p < 0.05$)).

some variations chemical compositions might be related to different locations of sampling. Also, weather conditions can change year to year in essential oil compound.

There was seen in table 1 that lipid oxidation increased over time in all samples. Increase of TBA values may be caused auto-oxidation of meat lipids, bacteriological and oxidative rancidity (SALEM *et al.*, 2010). Nevertheless, the antioxidant activity of TEO were significant in 0.25 and 0.125% concentration of essential oil in first storage day of beef burgers ($p < 0.05$) but in other days up to twelfth day was not observed significant difference in all of TEO treatment samples with control group ($p > 0.05$). These low antioxidant effects of TEO can be caused by low levels of phenolic and flavonoid compounds. Although, Previous studies are demonstrated the antioxidant activity of medicinal plants and spice essential oils such as *Artemisia dracunculus in vitro* (AYOUGHFI *et al.*, 2011; JAZANI *et al.*, 2011; SHARAFATI CHALESHTORI *et al.*, 2011). Activity of EOs is due to their composition as phenolic and flavonoid compounds that can donate hydrogen in oxidation reactions, scavenge free radicals and chelate to metallic ions (VIUDA-MARTOS *et al.*, 2010).

Staphylococcus aureus is a food borne pathogen and can cause a transmissible disease by inappropriate handling and storage of food contaminated with staphylococci as well as in many countries it is as the third most pathogen responsible for outbreaks of food poisoning (DE SOUZA *et al.*, 2009). These results showed that TEO has an antibacterial activity against *S. aureus*. The counted number of bacteria showed a decrease in the count of *S. aureus* with increasing essential oil concentration. Indeed, 0.25% TEO and 0.125% TEO in beef burgers after 24 hours reduced the viable cell counts by a 2 log CFU/g at 4°C though the number of bacteria in the control was approximately constant (3 log CFU/g). After six days of storage, the control samples revealed decreasing on *S. aureus* counts. The reason for the decrease in the counts of *S. aureus* may be due to the sensitive of this bacterium against temperature of refrigerator (4±1°C). Previous works have shown similar results about effects of medicinal plant extracts and essential oils on *S. aureus* in several food models (JAGADEESH BABU *et al.*, 2012; CHOOKAR *et al.*, 2010) MAHDAVIAN MEHR *et al.* (2010) evaluated Nowroozak leaf extract on growth of *S. aureus* in hamburger and then stored at -12°C. Its results demonstrated that the number of *S. aureus* in all samples with different concentrations of extract reduced during storage. The presence of antibacterial compound as polyphenols and carvacrol in essential oil of plants is more effective on the gram-positive bacteria than gram-negative bacteria due to their influence on membrane fluidity (MAHDAVIAN MEHR *et al.*, 2010; ROMANO *et al.*, 2009). The results

obtained in this study are also consistent with above studies.

The results showed that the best overall acceptability related to 0.125% TEO. The significant improvement of TEO treated samples compared to control samples is due to addition of Tarragon essential oil and this could be related to its aromatic compounds (KASSEM *et al.*, 2011). However, at the used TEO 0.25% exhibited more antibacterial activity against *S. aureus*, its addition in beef burgers had lower overall acceptability than 0.125% TEO. Therefore, high concentration of essential oils, their practical consume is limited due to a negative smell-taste effect in foods. We are suggested that TEO may be used as a part of combination with other preservation systems to decrease the amount of antimicrobial required and unfavorable sensorial effects (HAYOUNI *et al.*, 2008).

CONCLUSIONS

With mentioned to the dose-related antibacterial activity of Tarragon essential oil in this work is suggested that TEO can be used as a food natural preservative and food flavouring in beef burger products and meat industries.

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